

APPENDIX

- 1 ***Section 1 is amended by repealing the definition of "cigarette equivalent" and substituting the following:***

"cigarette equivalent" means a cigarette prepared from

- (a) leaf tobacco in accordance with the method set out in Schedule C, or
- (b) other cured and processed tobacco by incorporation into cigarette tubes in accordance with the method set out in the Canadian General Standards Board standard CAN/CGSB-176.1-92, entitled *Preparation of Cigarettes from Cigarette Tobacco for Testing*.

- 2 ***Section 2 (4) is amended by adding "and must be accompanied by an electronic copy of the information in the report, and that electronic copy must be in a comma-delimited, non-encrypted format" after "of Schedule B".***

- 3 ***Section 3 is amended***

(a) by adding the following subsection:

(2.1) Despite subsection (2), any testing and analyzing for the purpose of reports under subsection (1) may be carried out in accordance with a test method that differs from the one required under subsection (2) if

- (a) the use of the different test method results in information that is at least as accurate and precise as the information that would be produced if the test method required under subsection (2) was used, and
- (b) the manufacturer submits with the report of analysis under subsection (7)
 - (i) a description of the different test method, and
 - (ii) data that demonstrate that the requirements in paragraph (a) have been met.

(b) in subsection (3) (b) (i) by striking out "35 milliliters to 56 milliliters" and substituting "35 millilitres to 55 millilitres",

(c) in subsection (3) (b) (ii) by striking out "26 seconds" and substituting "30 seconds",

(d) in subsection (6) by adding "and must be accompanied by an electronic copy of the information in the report, and that electronic copy must be in a comma-delimited, non-encrypted format" after "of Schedule B", and

(d) by adding the following subsections:

- (7) A manufacturer must submit with each report under subsection (1) a separate report of analysis that does all of the following:

- (a) identifies and lists, by brand and for each smoke constituent, the mean, standard deviation, 95% confidence limit and coefficient of variation, all of which must be based on at least 20 observations of a sample;
 - (b) identifies and lists, by brand, the limits of quantitation and detection for each smoke constituent;
 - (c) sets out the criteria used for evaluation of the acceptability of the data and the evidence of acceptability;
 - (d) sets out the criteria used for the detection and treatment of outliers as defined in section 2.64 of the International Organization for Standardization standard ISO 3534-1:1993 (E/F) entitled *Statistics – Vocabulary and Symbols*;
 - (e) identifies and lists the yield ratings for all smoke constituents, the pH of whole tobacco smoke and the filter efficiency for each of the following, to be tested concurrently with the brands included in the report under subsection (1):
 - (i) at least one Canadian monitor cigarette;
 - (ii) at least one Kentucky Reference cigarette;
 - (iii) at least one duplicate test of a brand included in the report under subsection (1);
 - (f) includes any other information necessary to meet the requirements for reporting test results to clients as set out in section 5.10 of the International Organization for Standardization draft standard ISO/IEC FDIS 17025:1999 (E) entitled, *General requirements for the competence of testing and calibration laboratories*.
- (8) A report of analysis under subsection (7) must be accompanied by an electronic copy of the information in the report, and that electronic copy must be in a comma-delimited, non-encrypted format.

4 Section 5 (2.3) is repealed and the following substituted:

- (2.3) Despite section 3 (1), reports under that section for 1999 are not required for the following:
- (a) all brands of cigarettes that are not listed in Schedule D;
 - (b) all brands of cigarette tobacco.

5 Schedule C is repealed and the attached Schedule C is substituted.

6 The attached Schedule D is added.

SCHEDULE C
TEST METHODS

No.: T-101
Date: December 31, 1999
Page: 1 of 9

1 SCOPE

- 1.1 Applicable to the isolation and quantitation of ammonia in mainstream tobacco smoke by ion chromatography.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specifications for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 10 cigarettes* are smoked on a rotary smoking machine through a 92 mm glass fiber filter disc (pad) with two impingers containing 15 mL of 0.1N H₂SO₄ placed in series between the pad and the drawing syringe. The pad, after being weighed for total particulate matter (TPM) is placed into a 125 mL Erlenmeyer flask to which the contents of the impingers are also added.

- 4.2 Smoking may also be done on a linear type smoking machine. In this case 10 cigarettes* are smoked using 44 mm glass fiber filter discs (pads) with single impingers containing 30 mL of 0.1N H₂SO₄ placed between the pads and the drawing syringes. In this case the pad must be changed after five cigarettes in order to prevent overloading of the pad (breakthrough), as evidenced by brown stains on the back of the pad.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The adequacy of the required impingers must first be evaluated by creation of a smoke train with three impingers in series. All components of the smoke train must be analyzed separately in order to determine trapping efficiencies. If breakthrough to the second and third impinger is >3% of the first impinger, these must be included in the smoke train. Trapping efficiencies may differ slightly dependent on impinger design.

- 4.3 The pad (or pads) is extracted with the diluted impinger solution on a wrist action shaker for 30 minutes. This mixture is then filtered through a 0.45 µm syringe filter into a scintillation vial where the sample may be stored at 4 °C for up to 48 hours.
- 4.4 An aliquot of the filtered sample is then transferred to an autosampler vial and is analyzed by cation exchange chromatography.

- 4.5 A 35 μL volume of sample is injected onto a cation exchange analytical column that uses a Carboxylic acid/Phosphonic acid functional group to achieve separation of ammonium and monovalent cations. In order to adequately resolve sodium from the ammonium cation for quantitation, a 0.003N (Normal) methane sulfonic acid solution is used as the mobile phase. After the ammonium ion has eluted, a gradient using concentrations of 0.2N H_2SO_4 to a 0.05N H_2SO_4 is used to remove any divalent cations and quaternary amines that may be present in the sample.
- 4.6 Detection of cations is achieved using a suppressed conductivity detector in external water mode. This method of detection reduces background conductivity from the mobile phase, thus increasing the sensitivity of the detector for the analyte.
- 4.7 Quantitation is obtained from a five point external standard calibration using the peak height response of ammonium sulphate. The amount of ammonia per cigarette is determined by calculating the amount of ammonia present in the analytical solution, then multiplying by the appropriate multiplier (impinger volume X dilutions) and divisor (# of cigarettes).

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Analytical balance measuring to at least four decimal places.
- 5.5 Tweezers and gloves for transferring pads.
- 5.6 125 mL polymethylpentene (PMP) Erlenmeyer Flasks with screw – caps.
- 5.7 Wrist-action shaker.
- 5.8 Syringe filter: glass fibre (25 mm X 0.45 μm).
- 5.9 70 mL impingers without frits.
- 5.10 25, 50, and 100 mL volumetric flasks.
- 5.11 Disposable 5 cc syringe .
- 5.12 7 mL screw top vials with aluminium lined cap.
- 5.13 Autosampler vials, caps and Teflon-lined septa.
- 5.14 High Performance Liquid Chromatograph (HPLC) consisting of:
- 5.14.1 Refrigerated autosampler with 100 μL partial fill loop.
 - 5.14.2 Tertiary gradient system.
 - 5.14.3 Column heater.
 - 5.14.4 Dionex ED-40 conductivity detector or equivalent.
 - 5.14.5 Dionex CTC-1 cation trap or equivalent.
 - 5.14.6 Dionex CSRS-II conductivity suppresser in external water mode or equivalent.
 - 5.14.7 Data collection system.
 - 5.14.8 Dionex IonPac CS12A cation exchange analytical column (250 mm X 4 mm) or equivalent.

5.14.9 Dionex IonPac CG12A cation exchange guard column (50 mm X 4 mm) or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Ammonium Sulphate > 99 %.
- 6.2 Sulphuric Acid 96 %.
- 6.3 Methanesulphonic Acid (MSA) 100 %.
- 6.4 Type I water (Meets ASTM D 1193-77 specifications).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.
- 7.2 Immediately prior to use, all impingers are rinsed two times with 0.1N H₂SO₄, then three times with Type I water.

8 PREPARATION OF SOLUTIONS

8.1 Sulphuric Acid, 0.10N - Impinger Solution

- 8.1.1 Carefully add 5.108 g of H₂SO₄ (96 % w/w) to 900 mL of Type I water.
- 8.1.2 Mix and dilute to 1 L with Type I water.

8.2 Sulphuric Acid, 0.20N - Solution C (Ion Chromatography)

- 8.2.1 Carefully add 10.216 g of H₂SO₄ (96 % w/w) to 900 mL of Type I water.
- 8.2.2 Mix and dilute to 1 L with Type I water.

8.3 MSA 0.003N - Solution A (Ion Chromatography)

- 8.3.1 Carefully add 0.2883g of Methanesulphonic Acid (MSA) to 900 mL of Type I water.
- 8.3.2 Mix and dilute to 1 L with Type I water.

9 PREPARATION OF STANDARDS

9.1 Primary (1°) Ammonium Stock:

- 9.1.1 Accurately weigh 0.20 g of ammonium sulphate into a 50 mL volumetric flask.
- 9.1.2 Dissolve in 0.10N H₂SO₄.
- 9.1.3 Mix and dilute to 50 mL with Type I water.
- 9.1.4 Prepare fresh every 10 working days.

Note: This corresponds to a 1.0898 mg/mL NH₄⁺ ion stock solution.

9.2 Working Standards:

Standard # [µg/mL]	Volume of 1° Standard (µL)	Final Volume (mL)	Concentration
0	0	25	0.000
1	250	25	10.898
2	175	25	7.6283
3	75	25	3.2693
4	75	50	1.6346
5	50	100	0.5449
6	20	100	0.2180

Note: All working standards are made to volume in order to achieve a concentration of 50mN H₂SO₄. Prepare fresh every five working days.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115 noting the following:

12.2.2 Specific to Smoking on a Rotary Machine:

- 12.2.2.1** Connect two 70 mL impingers, each containing 15 mL of 0.1N H₂SO₄, between the 92 mm filter and the back-up glass fiber filter cassette on the pneumatics panel using Tygon tubing.
- 12.2.2.2** The pneumatic panel for smoking machine, is adjusted for a 35 mL (+/- 0.2 mL) puff volume (with the Filter and impingers in place) and 1.85 seconds sweep-time, using the supplied timer to measure the adjusted sweep-time.

12.2.3 Specific to Smoking on a Linear Machine:

12.2.3.1 Connect one 70 mL impinger, containing 30 mL of 0.1N H₂SO₄, between the 44 mm filter holder and the solenoid of the smoking machine.

12.2.3.2 The smoking machine, is adjusted for a 35 mL (+/- 0.2 mL) puff volume (with the filter and impinger in place).

13 SAMPLE GENERATION

13.1 Cigarettes shall be smoked and TPM collected as specified in T-115.

13.1.1 After smoking is complete, the 92 mm cassette is weighed and recorded, and the TPM yield per cigarette is calculated using the formula found in the Calculations section.

Note: Smoking on a linear machine requires the appropriate changes previously mentioned in 12.2.3. In addition to these changes, the glass fiber filter pad must be changed after five cigarettes in order to prevent breakthrough. A single clearing puff is taken at the end of smoking each individual cigarette plus an additional two clearing puffs must be taken before removing the cassette holder (at the end of the fifth and 10th cigarette).

14 SAMPLE ANALYSIS**14.1 Extraction of Filter Pads**

14.1.1 After smoking, remove the 92 mm (or 44 mm - linear) filter and fold into quarters.

14.1.2 Place the pad (or pads - linear) into a 125 mL Erlenmeyer flask.

14.1.3 Add the solution from the impinger(s).

14.1.4 Rinse the impinger(s) with an equal volume of water (i.e. 30 mL impinger volume uses 30 mL of Type I water), and add to the Erlenmeyer flask.

14.1.5 Close the flask, and shake on the wrist action shaker for 30 minutes.

14.2 Sample Clean-up

14.2.1 Filter the solution through a syringe filter into a storage vial noting to rinse the vial initially with approximately 1 mL of sample.

14.2.2 Transfer the filtered extract to an autosampler vial.

14.2.3 Samples can be stored at 4 °C for up to 48 hours prior to analysis.

14.3 Instrument Analysis - HPLC Analysis**14.3.1 Dionex ED-40 Conditions**

Suppresser Conductivity (SRS):	100 mA
Scale:	20 uS
Output:	Offset
Offset:	1 % of Full Scale

14.3.2 Autosampler : Injection Volume

14.3.2.1 Analyse using a 100 µL sample loop with the parameter for injection volume in the sample list at 35 µL with a 60 µL wash.

14.3.3 Column Temperature: 30 °C.

14.3.4 Mobile Phase / Gradient Conditions (Tertiary Gradient System)

Solvent A: 0.003N MSA
 Solvent B: Type I water
 Solvent C: 0.2N H₂SO₄
 Flow: 1.5 mL/minute
 Gradient: Minor adjustments may be required depending on column conditions and resolution of analyte.

Time (minutes)	Composition		
	% A	% B	% C
0.00	100	0	0
13.00	100	0	0
13.01	0	80	20
14.00	0	80	20
14.01	0	90	10
19.00	0	90	10
19.01	0	99	1
20.00	0	99	1
25.00	99	1	0
25.00	Method End Action: Equilibrate		

Equilibration Time: 9.00 minutes.

14.4 Calculations**14.4.1 Determination of Response Factor (RF)**

14.4.1.1 An initial calibration is performed by running prepared standards from high concentration to low concentration (injecting the very first standard a minimum of two times until the response and retention time are constant).

14.4.1.2 A calibration curve is prepared by plotting the concentration of NH₄⁺ ion in the standard vs. the peak height response from the conductivity detector.

14.4.1.3 The Response factor is the slope of the line as determined by linear regression (Height counts / unit concentration).

14.4.2 Determination of Ammonium Ion

$$\text{NH}_4^+ [\mu\text{g/cigarette}] = \frac{\text{peak height} \times \text{volume extractant (mL)} \times \text{final volume (mL)}}{\text{RF} \times \# \text{ cigarettes smoked} \times \text{aliquot volume (mL)}}$$

where the aliquot volume (mL) is the volume quantitatively transferred to the autosampler vial (if necessary).

14.4.3 Determination of Ammonia

$$\text{NH}_3 [\mu\text{g/cigarette}] = \text{NH}_4^+ [\mu\text{g/cigarette}] \times 17/18.$$

where 17/18 corrects for molecular weight.

14.4.4 Determination of Total Particulate Matter (TPM)

$$\text{TPM}(\text{mg/cigarette}) = \frac{[\text{Filter pad and holder after smoking (g)} - \text{Filter pad and holder before smoking (g)}] \times 1000 \text{ mg/g}}{\# \text{ of cigarettes}}$$

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1.

15.2 Typical Control Parameters

15.2.1 Laboratory Reagent Blank (LRB): Before processing any samples, the analyst should demonstrate, through the analysis of a reagent water blank, that interferences from the analytical system, glassware, and reagents are not present.

15.2.2 For each analytical batch, a LRB and laboratory fortified blank (LFB) must be analysed. The blank and spiked samples must be carried through all stages of the sample preparation and measurement steps.

15.2.3 Laboratory fortified matrix (LFM): Analyse a LFM to assess matrix interference. Spike a true sample with a known concentration and determine the % Recovery.

15.3 Recoveries and Levels of Contamination

15.3.1 Typical recoveries of Laboratory Fortified Blanks (LFB) and Laboratory Fortified Matrix (LFM) samples range from 85 – 110 % when a spiked solution (or sample) is carried out through the entire extraction process.

15.3.2 Typical Laboratory Reagent Blanks (LRB) have a value of 0 µg/cigarette. Contamination of this type is usually associated with contamination of the filter pad during conditioning or an inadequate cleaning of glassware.

15.4 Method Detection Limits (MDL) / Limit of Quantitation (LOQ)

15.4.1 The method detection limit (MDL) is determined by analysing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

15.4.2 The MDL (on a ng/cigarette basis) can be varied by modifying the number of cigarettes smoked and the volumes used for extraction and clean up in the procedure.

15.4.3 The practical limit of quantitation (LOQ) is determined by analysing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

15.4.4 For true samples, the MDL/LOQ is dependent on the resolution and the amount of sodium ion present in the sample, since the tail of a huge sodium peak may mask any ammonium ion present.

15.5 Stability of Reagents and Samples

15.5.1 Primary standards should be prepared fresh every 10 working days and stored at 4 °C.

15.5.2 Run standards should be prepared fresh from the stock solution weekly and stored at 4 °C.

15.5.3 Diluted Samples must be run within 48 hours of preparation.

16 MODIFICATIONS FOR INTENSE SMOKING

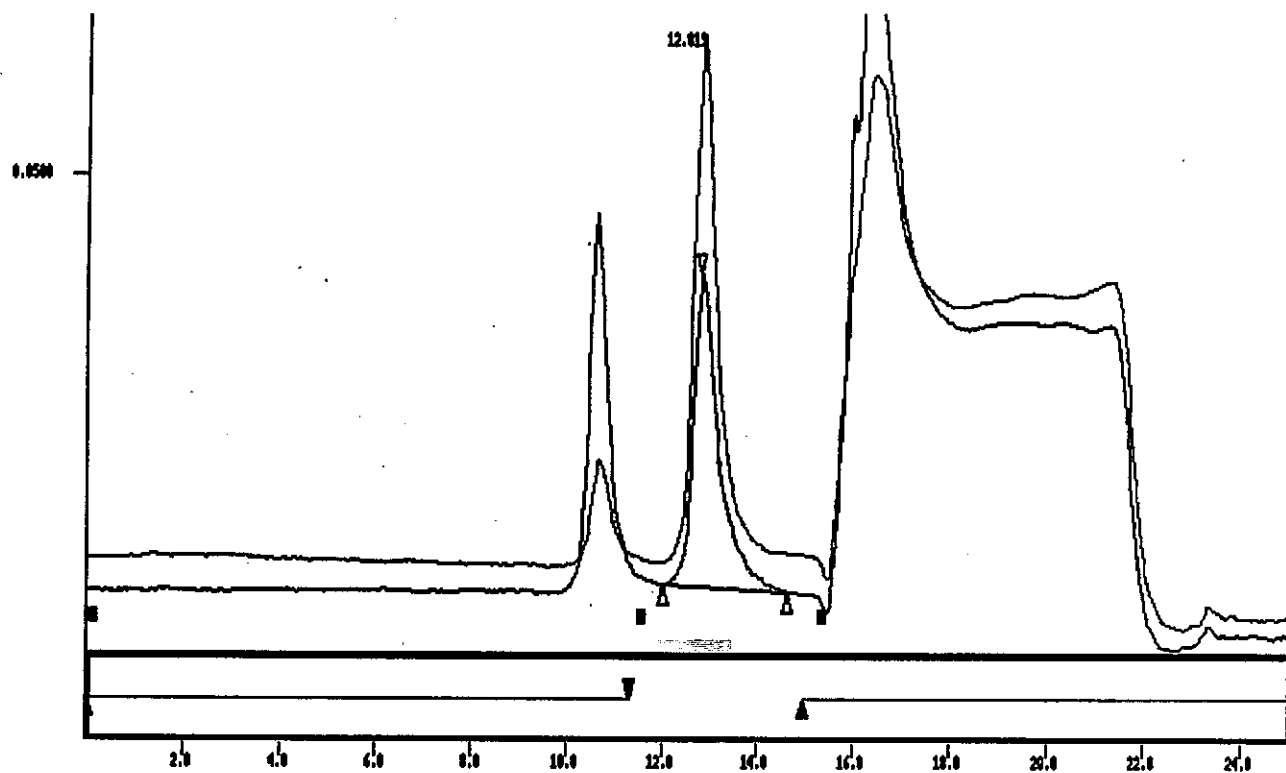
16.1 Under intense smoking conditions, five cigarettes are smoked instead of 20.

17 REFERENCES

17.1 Risner, C.H., Conner, J.M. Collection of Ammonia in Indoor Air by Means of a Weak Cation Exchange Cartridge, *Environmental Toxicology and Chemistry*, Vol. 10, 1991, p. 1417-1423.

17.2 Nanni, E.J., Lovette, M.e., Hicks, R.D., Fowler, K.W. and Borgerding, M.F. Separation and Quantitation of Monovalent and Cationic Species in mainstream Cigarette Smoke Aerosols by High-Performance Ion Chromatography, *Journal of Chromatographic Science*, Vol. 28, August 1990.

17.3 IonPac CS12A Analytical Column, Installation Instructions and Troubleshooting Guide, Document No. 031132, Revision 01, Dionex Corporation, 1995.

APPENDIX**Appendix 1: Typical Chromatogram**

An overlay of a standard and a Reference cigarette with a 5 % offset.

No: T - 102
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of the aromatic amine content (1- and 2-aminonaphthalene and 3- and 4-aminobiphenyl) of mainstream tobacco smoke by gas chromatograph/mass spectrometer (GC/MS).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine, and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.2 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specifications for Reagent Water, Version 1977.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 SUMMARY OF METHOD

- 4.1 Aromatic amines of mainstream tobacco smoke are collected by passing the smoke from 10 cigarettes* through a glass fibre filter disc (pad). The pad is quartered and placed in an Erlenmeyer flask with 100 mL of 5 % hydrochloric acid solution. The flask is shaken for 30 minutes on a wrist-action shaker and the contents filtered into a 500 mL separatory funnel. The internal standard (D₉-4-aminobiphenyl) is spiked into the solution. The filtrate is washed with dichloromethane, made basic with sodium hydroxide solution and extracted with hexane. The hexane extracts are dried with sodium sulphate, derivatized with pentafluoropropionic acid anhydride and trimethylamine, passed through a florisil column, and quantitated by GC/MS.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking analyses as specified in T-115.
- 5.2 Equipment needed for conditioning as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 Volumetric Flasks - Class A – 10 mL, 25 mL, 100 mL.

- 5.5 Pipettes - Class A - 20 µL, 50 µL, 100 µL, 250 µL, 500 µL, 1 mL or equivalent gas-tight syringe covering the range required.
- 5.6 Graduated Cylinder - 50 mL, 100 mL.
- 5.7 125 mL Polymethylpentene (PMP) Erlenmeyer flask with screw-cap (or equivalent).
- 5.8 Separatory Funnels - 500 mL with glass stoppers.
- 5.9 Filter funnels - 10 cm internal diameter (id) with glass wool plugs.
- 5.10 Round-bottom flasks - 500 mL.
- 5.11 Pasteur pipettes - nine inch (disposable) with rubber bulbs.
- 5.12 Conical tubes - 15 mL.
- 5.13 Autosampler vials - 1.5 mL with Teflon lined septa (disposable).
- 5.14 Analytical Balance capable of reading to 0.1 mg.
- 5.15 Rotary Evaporator with water bath set at 40 °C.
- 5.16 Turbo Evaporator with water bath set at 40 °C.
- 5.17 Supelco SPE-system or equivalent.
- 5.18 Wrist action shaker.
- 5.19 GC/MS System - Autosampler, SPI Injector with high-performance insert, GC, Ion Trap Detector (ITD) or equivalent.
- 5.20 GC Column - FS-capillary DB-5MS, 30 m X 0.25 mm ID X 0.25 µm or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 D₆-4-aminobiphenyl - 98 % purity or better.
- 6.2 1-Aminonaphthalene - 95 % purity or better.
- 6.3 2-Aminonaphthalene - 95 % purity or better.
- 6.4 4-Aminobiphenyl - 98 % purity or better.

Note: 3-aminobiphenyl is not available.

- 6.5 Hydrochloric Acid (HCl) - 32 %.
- 6.6 Hexane - Distilled in Glass (DIG).
- 6.7 Dichloromethane - Distilled in Glass (DIG).
- 6.8 Diethylether - Distilled in Glass (DIG).
- 6.9 Benzene - Distilled in Glass (DIG).
- 6.10 Acetone - Distilled in Glass (DIG).
- 6.11 Water - Type I (meets ASTM D 1193 specifications).
- 6.12 Sodium Hydroxide Solution - 50 %.
- 6.13 Sodium Sulphate - Granular.
- 6.14 Florisil SPE Columns - 1 g packing.
- 6.15 Florisil - deactivated 60-100 mesh.
- 6.16 Pentafluoropropionic Acid Anhydride (PFPA) - Reagent.
- 6.17 Trimethylamine - 40 % wt solution in water.
- 6.18 pH Paper - high range.

7 GLASSWARE PREPARATION

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 5 % Hydrochloric Acid - Add 312 mL of 32 % HCl solution to 1 litre of Type I water, dilute to two litre with Type I water. Mix very well.
- 8.2 TMA Solution - Add 2 mL of 40 % Trimethylamine Solution to a conical tube containing 2 mL of hexane. Vortex for 1 minute, let settle and transfer hexane to a 1.5 mL autosampler vial. Cap and store at 4 °C when not in use.
- 8.3 Florisil Elution Solution – 500 mL hexane, 400 mL benzene and 100 mL acetone. Mix well.

9 PREPARATION OF STANDARDS

9.1 Stock Solutions

- 9.1.1 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 4-aminobiphenyl (4-amb) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.2 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 1-aminonaphthalene (1-amn) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.3 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 2-aminonaphthalene (2-amn) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.4 A mixed secondary stock solution is prepared by diluting 100 µL of primary 4-amb solution, 500 µL of the primary 1-amn solution and 500 µL of primary 2-amn solution to 10 mL with hexane.
- 9.1.5 A tertiary stock solution is prepared by diluting 500 µL of the secondary stock solution to 25 mL with hexane. This solution is approximately 200ng/mL in 4-amb and 1000ng/mL in the aminonaphthalenes.
- 9.1.6 Internal Standard Solution (D₉-4-aminobiphenyl)
 - 9.1.6.1 A primary stock solution (100µg/mL) is prepared by accurately weighing 10 mg of pure D₉-4-aminobiphenyl (D₉-4amb) into a 100 mL volumetric flask and diluting to volume with hexane.

9.2 Internal Standard Spiking Solution (200 ng/mL D₉-4amb) (ISTD)

- 9.2.1 The internal standard spiking solution is prepared by diluting 100 µL of the primary D₉-4-aminobiphenyl stock solution to 50 mL with hexane.

9.3 Working Standards

- 9.3.1 Standard 1 (40 ng/mL 4-amb & 200 ng/mL 1- and 2-amn) - Add 2 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL

of PFPA, vortex and let sit a minimum of 30 minutes. Proceed as under Solid Phase Extraction.

9.3.2 Standard 2 (20 ng/mL 4-amb & 100 ng/mL 1- and 2-amn) - Add 1 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFPA, vortex and let sit a minimum of 30 minutes. Proceed as under Solid Phase Extraction.

9.3.3 Standard 3 (10 ng/mL 4-amb & 50 ng/mL 1- and 2-amn) - Add 0.5 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFPA, vortex and let sit a minimum of 30 minutes. Proceed as under Solid Phase Extraction.

9.3.4 Standard 4 (5 ng/mL 4-amb & 25 ng/mL 1- and 2-amn) - Add 0.25 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFPA, vortex and let sit a minimum of 30 minutes. Proceed as under Solid Phase Extraction.

9.3.5 Standard 5 (2 ng/mL 4-amb & 10 ng/mL 1- and 2-amn) - Add 0.1 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFPA, vortex and let sit a minimum of 30 minutes. Proceed as under Solid Phase Extraction.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product is to be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115.

13 SAMPLE GENERATION

13.1 Cigarettes shall be smoked and total particulate matter (TPM) collected as specified in T-115 with the following modifications as detailed below:

13.1.1 Smoking is conducted on 10 alternate ports of a rotary 20 port smoking machine. The machine is set with a two seconds puff duration and four seconds interval.

13.1.2 The mainstream smoke is collected on a 92 mm central Cambridge filter pad.

13.1.3 After smoking, cut the pad into quarters and place in an Erlenmeyer flask. Add 100 mL of 5 % HCl, and cap. Store at 4 °C until extracted.

14 ANALYSIS

14.1 Liquid-Liquid Extraction

14.1.1 Mix sample well on a wrist action shaker for 30 minutes.

14.1.2 Filter the contents of the flask through a filter funnel with a glass wool plug into a separatory funnel. Rinse the flask three times with 5 % HCl and transfer the rinses into the separatory funnel.

14.1.3 Add 100 µL of the ISTD solution to the separatory funnel. Add the stopper to the funnel and shake.

14.1.4 Add 50 mL of dichloromethane to the separatory funnel. Shake with careful venting until there is no more pressure and let settle.

14.1.5 Draw off the dichloromethane layer into a beaker and discard this layer as chlorinated waste.

14.1.6 Repeat 14.1.4 and 14.1.5 twice (total of three rinses).

14.1.7 Slowly add 10 to 15 mL of 50 % NaOH solution to the aqueous phase in the separatory funnel. Mix gently with careful venting, until venting no longer releases pressure, and check that the pH is above 12. If it is not, add 5 mL more NaOH. If more than 20 mL of NaOH needs to be added, check that the HCl solution was made up properly.

14.1.8 Add 50 mL of hexane to the separatory funnel and shake VERY, VERY carefully with venting into the fume-hood. Shake until there is no more pressure and let settle.

- 14.1.9 Prepare a filter funnel with a plug of glass wool and approximately 100 g of sodium sulphate. Rinse the sodium sulfate with approximately 50 mL of hexane into a 500 mL round-bottom flask. Discard the rinse.
- 14.1.10 Drain bottom (aqueous) layer of the contents of the separatory funnel into a beaker and retain.
- 14.1.11 Pass the top (hexane) layer through the sodium sulfate into the 500 mL round bottom flask, spreading the hexane over the entire surface of the sodium sulfate.
- 14.1.12 Pour the aqueous layer in the beaker back into the separatory funnel and add 50 mL of hexane. Shake very carefully and let settle.
- 14.1.13 Repeat 14.1.10 to 14.1.12.
- 14.1.14 After the third extraction with hexane, the aqueous layer can be discarded.
- 14.1.15 Repeat 14.1.11.
- 14.1.16 Rinse the sodium sulphate with approximately 50 mL hexane into the round-bottom flasks.
- 14.1.17 Add 50 µL of the TMA solution and 50 µL of the PFPA. Swirl and observe that a fine mist forms in the flask. Let sit for a minimum of 30 minutes (or overnight).

14.2 Solid Phase Extraction (SPE)

- 14.2.1 Evaporate the sample in the round bottom flask on a rotary evaporator to near dryness.
- 14.2.2 Prepare a florisil column by adding 2 g of florisil to a 1g florisil tube. Add a small amount of sodium sulfate to the top of the florisil.
- 14.2.3 Pre-wash with 5 mL of hexane:benzene:acetone (removing all air bubbles from the column).
- 14.2.4 Add approximately 1 mL of the hexane:benzene:acetone solution to the round-bottom flask (rinsing the solution down the sides of the flask).
- 14.2.5 Transfer the solution to the top of the florisil column and let drain into a 15 mL conical tube.
- 14.2.6 Repeat 12.2.4 and 12.2.5 two more times.
- 14.2.7 Rinse the florisil tube with hexane:benzene:acetone until approximately 15 mL have been collected in the conical tube.

14.2.8 Reduce the volume of the eluate in the tube in the Turbovap Evaporator under a gentle stream of nitrogen (3 to 5 psi) until approximately 1 mL remains.

14.2.9 Make the volume in the tube up to approximately 1 mL if necessary with hexane and vortex briefly (approximately 10 seconds).

14.2.10 Transfer the contents to an autosampler vial, cap and store at 4 °C until injected on the GC/MS.

14.3 GC/MS Operating Conditions

- | | | |
|---------------|-----------------------------------|---|
| 14.3.1 | Injector Temperature | 60 °C for 0.5 minute
200 °C per minute to 280 °C, hold to end of run. |
| 14.3.2 | Column Temperature | 80 °C for two minutes
10 °C per minute to 220 °C
20 °C per minute to 280 °C
Hold at 280 °C for three minutes. |
| 14.3.3 | Transfer Line Temperature: | 250 °C. |
| 14.3.4 | Manifold Temperature: | 240 °C. |
| 14.3.5 | Column Head Pressure: | 12 psi. |
| 14.3.6 | Injection Volume: | 1-2 µL. |
| 14.3.7 | Scan Range: | 100 to 330 amu. |
| 14.3.8 | Ion Peaks Used: | m/z 315 for analyte (3- and 4-aminobiphenyl)
m/z 324 for internal standard (D ₅ -4-aminobiphenyl)
m/z 289 for analyte (1- and 2-aminonaphthalene). |

14.4 A calibration curve (ratio of each analyte's response to the ISTD response versus the amount of the analyte in ng/mL) is generated at the beginning of analysis from the five working standards. Quantitation is performed using the internal standard method available with the GC/MS software. A calibration curve for 3-aminobiphenyl is generated from the calibration for 4-aminobiphenyl. The spectra and retention times for these two analytes are established with the analysis of a control cigarette.

14.5 A Check Standard is run every 20 injections and is analyzed as a sample to confirm that the calibration is still valid. If the result differs by more than 10 % of the expected value for that standard, the calibration process must be repeated and a new calibration curve generated.

14.6 The amount of each analyte is reported in ng/cigarette and is calculated as follows:

$$\text{Analyte (ng/cigarette)} = \frac{\text{Amount of Analyte from curve (ng/mL)} \times \text{Final Volume (1 mL)}}{\text{\# of Cigarettes}}$$

Note: There is no volume dilution factor because all of the sample is concentrated to the final stage. The samples and standards are quantitated in the same manner.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1.

15.2 Recoveries and Levels of Contamination

15.2.1 To determine the presence of aromatic amines in glassware or reagents a laboratory reagent blank (LRB) can be analysed. The LRB is the pad + extraction solution (100 mL of 5 % HCl) spiked with ISTD solution and taken through the sample preparation. LRB results are consistently ND (not detected) for all analytes.

15.2.2 With every set of approximately 20 samples, include a laboratory fortified blank (LFB). A known amount of the tertiary stock solution is added to solution in a separatory funnel along with the ISTD and the solution is taken through the entire procedure, to determine if any aromatic amine is lost during the different stages. Recoveries of 2-aminonaphthalene and 4-aminobiphenyl are typically 90 ± 10 %. Recoveries of 1-aminonaphthalene are somewhat lower (70 to 80 %) due to the more volatile nature of the derivatized 1-aminonaphthalene.

15.2.3 To assess potential matrix interferences, a laboratory fortified matrix (LFM) can be analyzed. A sample of the control cigarette can be split before extraction and each half treated as a separate sample. One of the split halves should be spiked with a known amount of the tertiary stock solution at approximately the level expected in the sample. Recoveries should be very close to 100 %. Typical recoveries are:

4-aminobiphenyl - 98.4 %.

2-aminonaphthalene - 96.8 %.

15.3 Method Detection Limit (MDL) and Limit of Quantitation(LOQ)

For GC/MS analysis, the detection limit can be defined as a peak whose signal to noise ratio (S/N) is three to one. The limit of quantitation can be defined as a S/N of 10 to one. The lowest standard typically run for 4-aminobiphenyl is 1 ng. This peak gives a S/N of approximately 20 to one and a corresponding MDL of approximately 0.01 ng/cigarette and an LOQ of approximately 0.03 ng/cigarette.

15.4 Stability of Reagents and Samples

15.4.1 Store ISTD spiking solution in a 25 mL amber vial with open cap and Teflon-lined septum. Use a 100 μ L gas-tight syringe to transfer the ISTD from the vial to the separatory funnel. Do not touch the sides of the separatory funnel with the tip of the syringe (to avoid contaminating the

ISTD). Wash the syringe with hexane between samples and change the septum daily. Store the ISTD at 4 °C when not in use.

15.4.2 There is no apparent problem with the stability of the stock or underivatized working standards. Derivatize fresh 1 mL aliquots of the working standards as required and replace the red Teflon-lined septa after each injection to minimize contamination from the septa.

15.4.3 Samples should be extracted within one week of being produced.

15.4.4 The area responses of the analytes (including the internal standard) in the calibration standards and the LFBs are occasionally lower than in the samples and LFM. Investigation has suggested that analyte loss occurs if the standards or LFBs are taken to complete dryness during rotary evaporation or turbo-evaporation. The samples do not appear to be affected in the same way, possibly due to the matrix acting as a "keeper" during the solvent removal stages. As a cautionary measure, no samples or standards are taken to complete dryness at any stage of the process.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

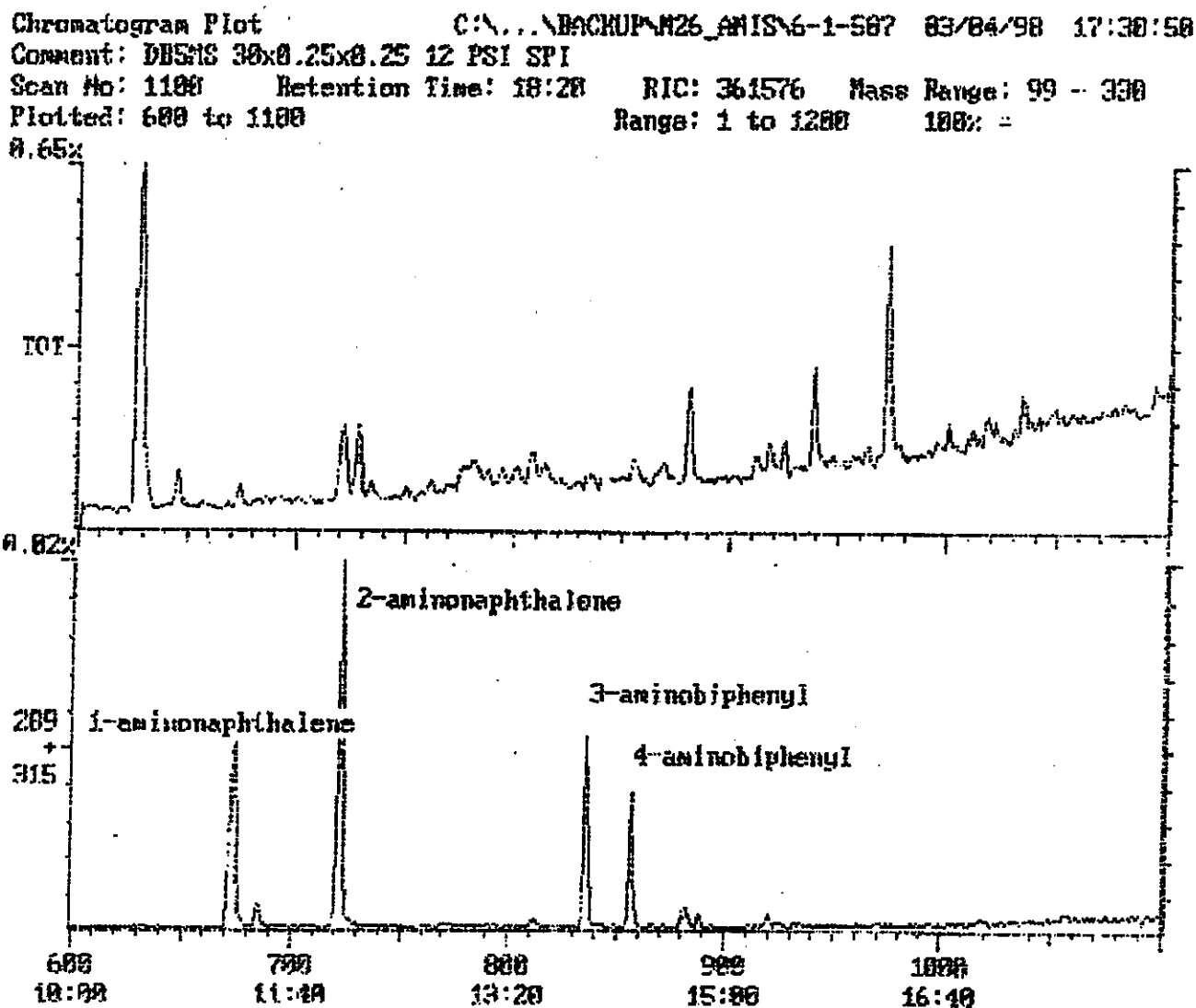
16.1 For very high tar brands or under intense smoking conditions, no more than five cigarettes should be smoked.

17 REFERENCE

- 17.1 Pieraccini, G., F. Luceri, and G. Moneti. New Gas-Chromatographic/Mass-Spectrometric Method for the Quantitative Analysis of Primary Amines in Main- and Sidestream Cigarette Smoke. I, *Rapid Communications in Mass Spectrometry*, 6, 1992, p. 406-409.

APPENDIX

Appendix 1: Typical Chromatogram



No.: T - 103
Date: December 31, 1999
Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the quantitation of benzo[a]pyrene (B[a]P) content in the total particulate matter (TPM) of mainstream (MS) tobacco smoke by reversed phase high performance liquid chromatography (HPLC) via fluorescence detection.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of the Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.3 Methods of Sampling and Testing Tobacco: Determination of Benzo[a]pyrene in Total Particulate Matter of Tobacco Smoke. *National Standard of Canada*, Canadian General Standards Board CAN/CGSB-176.2 No. 1-96, March 1996.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Tobacco product is smoked as specified in T-115.
- 4.2 Total particulate matter, collected on a glass fibre filter disc (pad), is extracted with a sufficient amount of cyclohexane to create an extract with a concentration of approximately 1 mg of wet particulate matter/mL extract.
- 4.3 A portion of this solution is filtered through a 0.45 µm PTFE filter to a 7 mL glass vial for storage. A 2 mL aliquot of the extract is passed through a 1 g (6 mL) silica cartridge and 360 mg NH₂ plus cartridge in series.
- 4.4 The B[a]P is eluted with hexane, evaporated under a constant stream of nitrogen to dryness, and reconstituted to a 1 mL volume with acetonitrile.
- 4.5 The sample is subjected to reversed phase high performance liquid chromatography (HPLC) and quantitated via fluorescence detection.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking analyses as specified in T-115.
- 5.2 Equipment needed to perform conditioning of tobacco product as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 Supelco Visi-Prep Solid Phase Extraction (SPE) unit (24 cartridge unit) or equivalent.
- 5.5 2 mL glass pipettes.
- 5.6 Brinkman Dispensette (10-50 mL) or equivalent.
- 5.7 Micro-pipettes (10, 50, 500, 1000 μ L).
- 5.8 125 mL and/or 250 mL Erlenmeyer (or round bottom) flasks with ground glass joints.
- 5.9 Zymark TurboVap II Concentrator or equivalent.
- 5.10 2 L volumetric flask.
- 5.11 Wrist-action shaker.
- 5.12 Glass transfer pipettes.
- 5.13 Laboratory mixer.
- 5.14 Merck 250 X 4 mm, RP-18e, 5 μ m packing HPLC column or equivalent.
- 5.15 Lichrocart 4-4 Lichrosphere 100 RP-18 endcapped 5 μ m guard column or equivalent.
- 5.16 Analytical balance, capable of measuring to four decimal places.
- 5.17 High Performance Liquid Chromatograph consisting of:
 - 5.17.1 Autosampler.
 - 5.17.2 Tertiary pump.
 - 5.17.3 Fluorescence Detector.
 - 5.17.4 Data collection system.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

- 6.1 Benzo[a]pyrene (B[a]P).
- 6.2 Cyclohexane.
- 6.3 Hexane.
- 6.4 Acetonitrile.
- 6.5 Methanol.
- 6.6 Isopropanol (IPA).
- 6.7 Acetone.
- 6.8 Anhydrous Sodium Sulphate.
- 6.9 Tetrahydrofuran (THF).
- 6.10 16 X 125 mm culture tubes (20 mL).
- 6.11 Glass fibre filter disc (pad) and holders (45 mm).
- 6.12 Disposable 5 cc syringe.

- 6.13 Autosampler vials, caps and teflon lined septa.
- 6.14 Pasteur Pipettes.
- 6.15 1 g silica Sep-Pak cartridges (6 mL capacity).
- 6.16 360 mg NH₂ Plus Sep-Pak cartridge.
- 6.17 7 mL screw top vials with aluminum lined cap.
- 6.18 13 mm 0.45 µm PTFE disposable filters.
- 6.19 Type I water as per ASTM D1193.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Prepare solutions required for analysis, as specified in T-115, in accordance with good laboratory practice.

9 PREPARATION OF STANDARDS

9.1 Preparation of Spiking Solution for Lab Fortified Samples

9.1.1 Primary (1°) B[a]P Stock: Dissolve 10 mg B[a]P into 50 mL Cyclohexane.

9.1.2 Secondary (2°) Stock: Pipette 50 µL of 1° Stock into 50 mL Cyclohexane.

9.1.3 A 10 µL volume of spiking solution is added to a second 2 mL aliquot of a control brand cigarette extract solution prior to solvent substitution (14.2.4 to 14.2.8) and clean-up through the SPE cartridges to make the Laboratory Fortified Matrix (LFM*). Another 10 µL volume of spiking solution is added to a second 2 mL aliquot of the Laboratory Reagent Blank (LRB*) prior to solvent substitution and clean-up through the SPE cartridges to make the Laboratory Fortified Blank (LFB*). The spiking analytical concentration is approximately 2 ng/mL (dependent on stock concentration).

*See section on Quality Control for explanations of these initialisms.

9.2 Preparation of Working Standards

9.2.1 Primary (1°) B[a]P Stock: Dissolve 10 mg B[a]P into 50 mL Acetonitrile.

9.2.2 Secondary (2°) Stock: Pipette 100 µL of 1° Stock into 50 mL Acetonitrile.

Working Standards:

Standard #	Volume of Secondary (2°) Stock (µL)	Final Volume (mL)	Concentration [ng/mL]
1	40	25	0.6400
2	175	25	2.800
3	350	25	5.600
4	600	25	9.600
5	900	25	14.4
6	2 mL of Std 1	10	0.1280
7	4 mL of Std 1	10	0.2560

All weights, volumes, and purity must be recorded and used to accurately calculate the standard concentrations. These concentrations are only representations of standards used in a calibration curve.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115.

13 SAMPLE GENERATION

- 13.1** Cigarettes shall be smoked and TPM collected as specified in T-115.

Note: Five cigarettes per observation are to be smoked under standard conditions. For other tobacco products, select a number such that breakthrough does not occur.

Note: Samples may be stored at -20°C for up to 10 days prior to extraction with cyclohexane.

14 SAMPLE ANALYSIS**14.1 Extraction of Filter Pads**

- 14.1.1** Remove the mainstream pad from its holder, folding it into quarters and wiping the inside of the holder with the clean side of the pad.

- 14.1.2** Transfer the pad into a round bottom flask.

- 14.1.3** Pipette a volume of cyclohexane (minimum amount is 30 mL) into the round bottom flask that is numerically equivalent to the total TPM in mg (rounded to the nearest 10 mL) yielding a concentration of approximately 1 mg TPM/mL of cyclohexane. Record the volume of the cyclohexane used to extract the pad.

- 14.1.3.1** Example: 1. If TPM = 70 mg (total), then cyclohexane volume = 70 mL.

- 14.1.3.2** Example: 2. If TPM = 83 mg (total), then cyclohexane volume = 80 mL.

- 14.1.3.3** Example: 3. If TPM = 57 mg (total), then cyclohexane volume = 60 mL.

14.1.4 Shake the round bottom flask containing the mainstream pad and cyclohexane vigorously on a wrist action shaker for 30 minutes, until there appears to be a homogeneous solution and there is no longer any evidence of localized colour on the pad.

14.1.5 Place the flasks in the dark until they are ready for further processing.

14.2 Sample Clean-up

14.2.1 Filter approximately an 8 mL portion of the cyclohexane extract through a 0.45 µm PTFE disposable organic filter into a 7 mL vial with foil lined cap. Samples may be stored at 4 °C at this point if adequate headspace is left.

14.2.2 Pre-condition both (the silica and the NH₂ plus) cartridges using hexane as recommended by the manufacturer.

Note: All air has to be removed from the packing thus exposing sorbent material to the hexane.

14.2.3 Place the pre-conditioned cartridges on the Visi-prep unit. Add about 1 g anhydrous sodium sulfate to the silica cartridge and wash the cartridges with 10 mL hexane allowing the hexane to flow through the cartridge by gravity.

14.2.4 Once the extract has reached room temperature, pipette a 2 mL aliquot of the extract onto the packing of the Silica cartridge.

14.2.5 Allow the cyclohexane to pass through the SPE cartridges (by gravity) at a rate of approximately one drop/second. Discard the eluant.

14.2.6 Pipette 4 mL of hexane to the cartridge allowing the eluant to gravimetrically pass through the cartridge. Discard the eluant.

14.2.7 Place 20 mL disposable glass culture tubes beneath each of the cartridges.

14.2.8 Gravimetrically elute the B[a]P from the cartridges with 4 X 4 mL additions of hexane.

14.2.9 Add 1 mL of THF to each tube.

14.2.10 Place the tubes containing the 17 mL of collected eluant into the Zymark Turbo-vap.

Note: Conditions are to be set at 40 °C with a Nitrogen pressure of 7.5 psi.

14.2.11 Evaporate the samples to complete dryness.

Note: This will require an initial 20 minute period in which the nitrogen pressure may be slowly increased to a maximum of 10 psi in a manner such as to prevent any loss of sample from splattering.

14.2.12 Remove samples that are completely dry. If some samples are not completely dry, process the samples in additional five minutes intervals.

14.2.13 Pipette 1000 µL of Acetonitrile into each of the dried tubes to dissolve the analyte and any residue that may be present.

14.2.14 Vortex the sample at high speed for approximately 15 seconds.

14.2.15 Using a glass transfer pipette, wash down the sides of the tube five times with the sample, and transfer the sample to an autosampler vial with a screw cap and Teflon faced septa.

14.2.16 The samples are ready for HPLC analysis and may be stored at 4 °C until they are analyzed.

14.3 Instrument Analysis - Reversed Phase High Performance Liquid Chromatography (HPLC) Analysis

14.3.1 Jasco Fluorescence Detector Conditions

Excitation Wavelength:	365 nm.
Emission Wavelength:	425 nm.
Gain:	X 1000.
Attenuation:	32.

Note: A different manufacturer's fluorescence detector may need to be programmed differently to maintain the full calibration range. A slight change in excitation and emission wavelength may be required dependent on manufacturer (i.e. 366 and 424 for the wavelengths).

14.3.2 Autosampler : Injection Volume

Analyze using a 50 µL sample loop and set the parameter for injection volume at 75 µL to ensure a thorough flushing of the sample loop with the sample.

14.3.3 Mobile Phase / Gradient Conditions (Tertiary Gradient System)

Solvent A:	55:45 Acetonitrile: 1 % isopropyl alcohol in Type I water (degassed and filtered using a 0.45 µm nylon filter).
Solvent B:	Methanol.
Solvent C:	Acetonitrile.
Flow:	1.5 mL/minute.
Gradient:	Adjustments to the gradient may be required depending on column conditions and resolution of analyte.

Time (minutes)	Composition		
	% A	% B	% C
0.00	55	0	45
20.00	75	0	25
25.00	100	0	0
28.00	100	0	0
30.00	0	100	0
32.00	0	100	0
34.00	100	0	0
35.00	100	0	0
35.00	Method End Action:		Equilibrate

Equilibration Time: 8.00 minutes.

14.4 Calculations

14.4.1 Determination of Response Factor

14.4.1.1 An initial calibration is performed by running prepared standards from high concentration to low concentration (injecting the very first standard a minimum of two times until the response and retention time are constant).

14.4.1.2 A calibration curve is prepared by plotting the concentration of B[a]P in the standard vs. the peak height response from the fluorescence detector.

14.4.1.3 The Response factor is the slope of the line as determined by linear regression (Height counts/unit concentration).

14.4.2 Determination of B[a]P Delivery [ng/cig]

$$\text{B[a]P [ng/cig]} = \frac{\text{Peak Height} \times \text{Volume Extractant (mL)} \times \text{Final Volume (mL)}}{\text{Response Factor} \times \# \text{ Cigarettes Smoked} \times \text{Aliquot Volume (mL)}}$$

where the aliquot volume (mL) is the volume transferred to the Sep-pak cartridges correcting for any potential previous dilutions in the solvent substitution step. The Response Factor is determined from the calibration curve.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1a and 1b.

15.2 Typical Control Parameters

15.2.1 Each set analysis should contain at least one of each of the following per smoking run (20 port smoking):

- 15.2.1.1** Laboratory Reagent Blank (LRB): to determine background contamination from solutions, glassware, or materials used in the analysis process.
- 15.2.1.2** Laboratory Fortified Blank (LFB): to determine whether there is any loss of analyte as a result of the analysis process.
- 15.2.1.3** Laboratory Fortified Matrix (LFM): by spiking one of the control brand cigarettes: to determine whether there is any loss of analyte as a result of the analysis process and to determine potential matrix effects.
- 15.2.1.4** Reference Sample: to determine the inter-experimental reproducibility of the entire method of analysis
- 15.2.1.5** Duplicate Sample: to determine the reproducibility of the procedure within the same experiment or batch on analysis.

15.3 Recoveries and Levels of Contamination

- 15.3.1** Typical recoveries of Laboratory Fortified Blanks (LFB) and Laboratory Fortified Matrix (LFM) samples range from 85 - 110 % when a spiked solution (or sample) is carried out through the entire extraction process.
- 15.3.2** Recoveries lower than 85 % indicate either an insufficient elution of B[a]P from the solid phase extraction cartridges or a change in response factor (RF) of the fluorescence detector. A change in RF must first be investigated before re-processing of samples is initiated.
- 15.3.3** Typical Laboratory Reagent Blanks (LRB) range from a calculated value of 0 - 0.3 ng/cigarette. Contamination of this type is usually associated with contamination of the filter pad during conditioning or an inadequate cleaning of glassware.

15.4 Method Detection Limits (MDL) / Limit of Quantitation (LOQ)

- 15.4.1** The method detection limit (MDL) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

Note: The MDL (on a ng/cig basis) can be manipulated by modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure.

- 15.4.2** The practical limit of quantitation (LOQ) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

15.5 Stability of Reagents and Samples

15.5.1 Analytical stocks and standard should be stored at -20°C .

15.5.2 Stock standards and stock spike solutions remain stable for up to six months. Although there is no loss of analyte, evaporation (loss) of solvent may be an issue.

15.5.3 Analytical run standards should be freshly prepared every two months.

15.5.4 Samples are stable at 4°C for three weeks after extraction.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 Under intense smoking conditions, the number of cigarettes per observation is reduced to two.

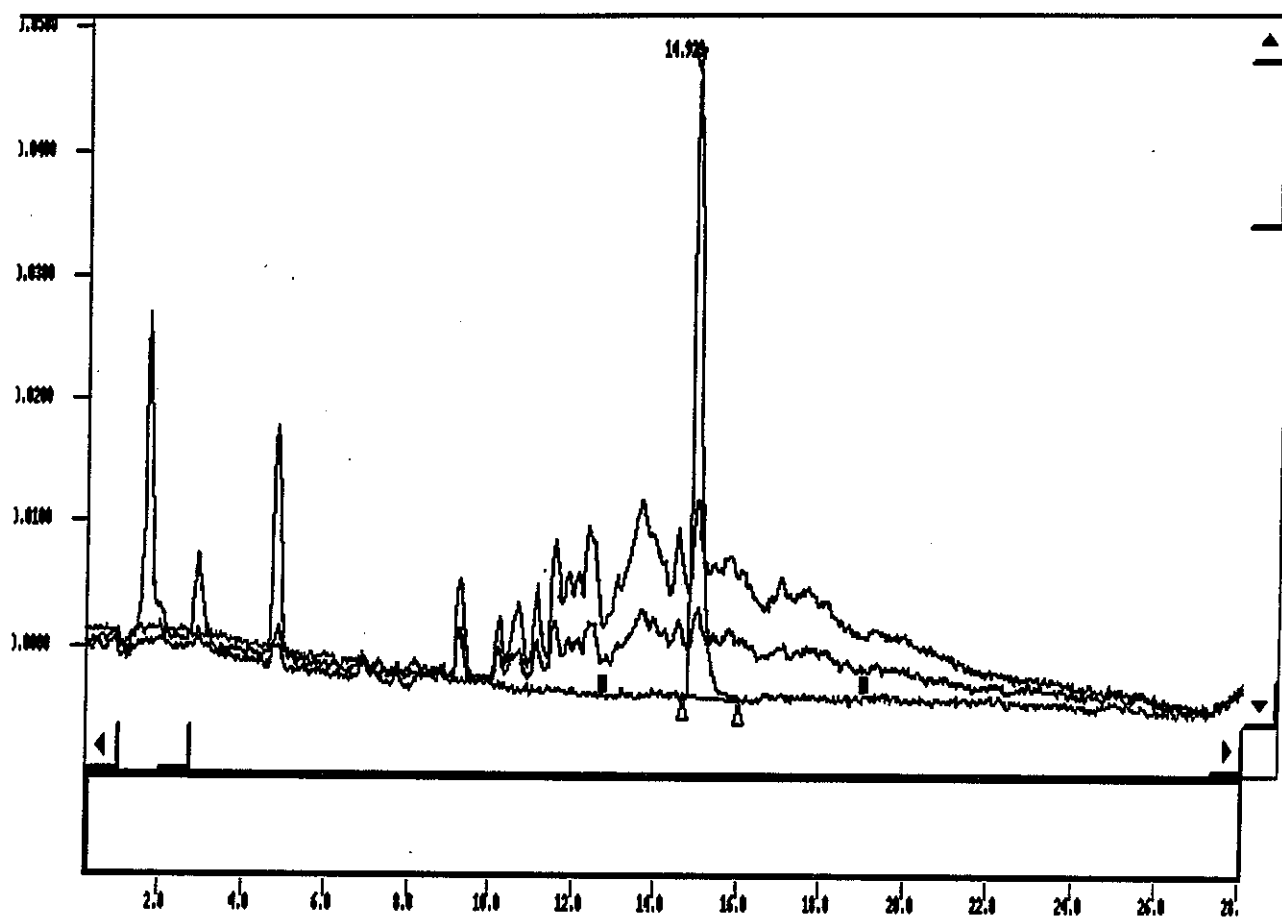
17 REFERENCES

17.1 Dumont, J., Larocque-Lazure, F., and Iorio, C. An Alternative Isolation Procedure for the Subsequent Determination of Benzo[a]pyrene in Total Particulate Matter of Cigarette Smoke, *Journal of Chromatographic Science*, Vol. 31, September 1993, p. 371-374.

17.2 Tomkins, B.A.; Jenkins, R.A.; Griest, W.H.; Reagen, R.R. Liquid Chromatographic Determination of Benzo[a]pyrene in Total Particulate Matter of Cigarette Smoke, *J. Assoc. Off. Anal. Chem.*, Vol. 68, 5, 1985, p. 935-940.

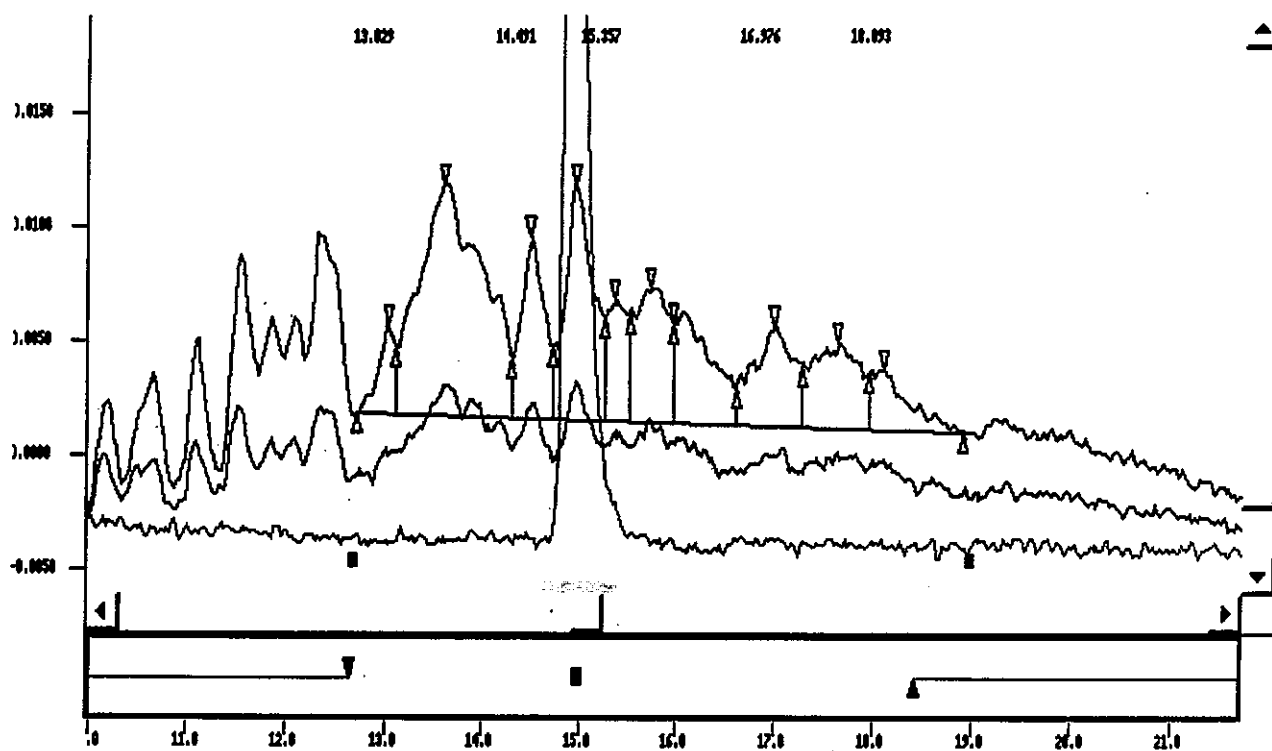
APPENDICES

Appendix 1a: Typical Chromatogram



An overlay of a standard, a high tar reference cigarette and a low tar reference cigarette.

Appendix 1b: Typical Chromatogram



An expanded view of Appendix 1a to show the integrating baseline of a true sample.

No.: T - 104
Date: December 31, 1999
Page: 1 of 13

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the trapping and quantitation of volatile carbonyl compounds (as their 2,4-dinitrophenylhydrazones [DNPH]) in the vapour phase of mainstream tobacco smoke. This is applicable to the carbonyl compounds extracted from the mainstream vapour phase and quantitated from the DNPH trapping solution only. The method is applicable to tobacco smoke generated by both cigarettes and cigars.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 –Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Tobacco product is smoked on alternate ports of a standard 20 port linear smoking machine that has been fitted with Drechsel-type bottles or traps with fritted impingers.
- 4.2 The unfiltered mainstream tobacco smoke is scrubbed of volatile carbonyls by passing each puff through an impinger into a trap containing 80 mL of an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile.
- 4.3 An aliquot of the reacted DNPH-smoke extract is then syringe-filtered, diluted with 1 % trizma base in aqueous acetonitrile.
- 4.4 The samples are subjected to reverse phase high performance liquid chromatography (HPLC) and quantitated via ultra violet detection

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

The sample preparation and analysis should be completed in one day and the solvent waste generated by the HPLC must be stored for disposal by a registered chemical-recycling agency.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Wrist action shaker.
- 5.5 150 mL Erlenmeyer flasks with ground glass stoppers.
- 5.6 12 glass cigarette holders (6.5 cm in length and 8.0 mm internal diameter).
- 5.7 12 glass Drechsel Type traps (capacity 250 mL) with fritted extra coarse impingers.
- 5.8 Nalgene Tubing 1/4" ID X 3/8" OD.
- 5.9 Analytical balance, capable of measuring to four decimal places.
- 5.10 Volumetric flasks 10 mL, 25 mL, 1 L, and 2 L.
- 5.11 Glass micropipettes - assorted volumes (100, 150, 300, 400, 500, 800, 1000, and 2000 µL).
- 5.12 Glass transfer pipettes - 1, 2, 5, 6, 7, 8, and 20 mL.
- 5.13 Glass graduated measuring cylinders 25 mL and 50 mL.
- 5.14 Hot Plate/Stirrer.
- 5.15 PC controlled High Performance Liquid Chromatography System consisting of:
 - 5.15.1 Tertiary gradient pump.
 - 5.15.2 Autosampler with 50 µL sampling loop.
 - 5.15.3 UV Detector.
 - 5.15.4 Data Collection System.
 - 5.15.5 Column: Merck Lichrosphere 250 X 4 mm, 100, RP 18e (5 µm) or equivalent.
 - 5.15.6 Disposable Guard Column: Lichrocart 4 X 4 mm, Lichrosphere RP 18e (5 µm) or equivalent.
- 5.16 Vacuum filter.
- 5.17 Amber bottles 1 L and 4 L.
- 5.18 Dessicator.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Methanol – Distilled in glass (DIG).
- 6.2 Acetonitrile (MeCN) – DIG.
- 6.3 Isopropanol (IPA) – DIG.
- 6.4 Ethyl Acetate – DIG.
- 6.5 Tetrahydrofuran (THF) – DIG.
- 6.6 Reagent Alcohol – HPLC Grade.
- 6.7 Perchloric Acid (60 %).
- 6.8 Hydrochloric Acid (35 %).
- 6.9 Sulphuric Acid, Concentrated (H₂SO₄).
- 6.10 Type I water as per ASTM D1193.
- 6.11 Formaldehyde Solution - 37-41 % (w/v).
- 6.12 Acetaldehyde > 99 % purity.
- 6.13 Acetone – DIG.
- 6.14 Acrolein > 99 % purity.

- 6.15 Propionaldehyde > 97 % purity.
- 6.16 Crotonaldehyde > 99+ % purity.
- 6.17 Methyl Ethyl Ketone > 99+ % purity.
- 6.18 Isobutyraldehyde > 99 % purity.
- 6.19 Butyraldehyde > 99+ % purity.
- 6.20 Trizma Base.
- 6.21 2,4-dinitrophenylhydrazine.
- 6.22 Syringe filter - 0.45 μ m PVDF or equivalent.
- 6.23 Disposable syringes – 5 mL.
- 6.24 Disposable glass Pasteur pipettes.
- 6.25 Rubber bulbs.
- 6.26 Autosampler Vials (amber), caps and Teflon faced septa.
- 6.27 Masking Tape.
- 6.28 Parafilm® (or equivalent).
- 6.29 Helium (UHP).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.
 - 7.1.1 It is extremely important that all possible sources of contamination are removed from the work area e.g. acetone solvent wash bottles.

8 PREPARATION OF SOLUTIONS

8.1 Preparation of DNPH Solution

- 8.1.1 Weigh 6.792 g (24.0 mmol) of commercially available 2,4 dinitrophenylhydrazine. Add to 1 L of fresh acetonitrile in a 2 L volumetric flask. Dissolve DNPH by alternating: gently swirling and warming the flask. Make sure there are no crystals remaining before proceeding. (Warning! Do not sonicate.)
- 8.1.2 After the DNPH is dissolved, add 5.6 mL 60 % perchloric acid with gentle mixing. The solution will turn yellow at this point.
- 8.1.3 Dilute to volume with Type I water. The solution will turn to a bright orange upon addition of the water.
- 8.1.4 Store the solution in a 4 L amber bottle at room temperature in the dark to reduce the chances of DNPH precipitation. This solution, if properly sealed, will remain stable for one week under these conditions.

8.2 Preparation of Trizma Base Dilution Solution (80:20, MeCN:1 % aqueous Trizma)

- 8.2.1 Dissolve 2.00 g of Trizma Base in 200 mL of distilled deionized water (Type I water) in a 1 L volumetric flask. Dilute to volume with acetonitrile.

- 8.2.2** Store in a 1 L amber bottle with Teflon-lined cap at room temperature. This solution should remain stable for several weeks under these conditions.

9 PREPARATION OF STANDARDS

9.1 Preparation of Dinitrophenylhydrazone derivatised carbonyls

- 9.1.1 Dissolve 600 mg commercially available DNPH in 2 mL concentrated H_2SO_4 in a 50 mL Erlenmeyer flask.
- 9.1.2 Stir with a glass rod while adding 3 mL of Type I water (clear solution). Then add 10 mL of reagent alcohol.
- 9.1.3 Add the DNPH solution to a solution of the appropriate aldehyde or ketone containing (each as an individual preparation):
- 120 mg formaldehyde
 - 50 mg acetaldehyde
 - 40 mg acetone
 - 40 mg acrolein
 - 40 mg propionaldehyde
 - 35 mg crotonaldehyde
 - 33 mg methyl ethyl ketone
 - 33 mg butyraldehyde.

Crystallisation generally occurs rapidly.

- 9.1.4 Filter crystals (hydrazones) using a vacuum filter and rinse the crystals with cold (4 °C) reagent alcohol.
- 9.1.5 Recrystallization of hydrazones: Add about 10 mL reagent alcohol to the crystals in a small Erlenmeyer flask, heat and then add 3 mL ethyl acetate dropwise to dissolve crystals. Cool to room temperature.
- 9.1.6 Filter crystals under vacuum, rinse with cold (4 °C) reagent alcohol, air dry and then store in vials in desiccator at -20 °C.

9.2 HPLC Calibration Standards and Working Solutions

9.2.1 Primary (1°) Carbonyl Standards

- 9.2.1.1 Weigh purified hydrazones in the amounts described in **Appendix 1(a)**. Put into individual 25 mL volumetric flasks and dissolve in acetonitrile. Concentration is of the free aldehyde.
- 9.2.1.2 Seal each volumetric flask with parafilm and refrigerate at 4 °C. When properly stored, solutions are stable for up to one year.

9.2.2 Secondary (2°) Carbonyl Standards

- 9.2.2.1 Pipette predetermined volumes of each primary hydrazone standard into a single 25 mL volumetric flask and dilute up to the mark with acetonitrile.

- 9.2.2.2** Seal volumetric flask with parafilm and refrigerate at 4 °C. Prepare new working standards every 20 days. See **Appendix 1(a)**.

9.2.3 Carbonyl Working Standards

- 9.2.3.1** Take appropriate volumes (0.050 to 10 mL) of the 2° carbonyl standard and dilute to 10 mL with acetonitrile to give calibration standards with approximate carbonyl concentrations in the ranges noted in **Appendix 1(b)**.

- 9.2.3.2** Transfer to autosampler vials.

- 9.2.3.3** New carbonyl calibration standards should be prepared every 20 days.

9.2.4 Carbonyl Spiking Solution (see Appendix 1c)

- 9.2.4.1** Pipette predetermined volumes of each primary hydrazone standard into a single 25 mL volumetric flask and dilute up to the mark with acetonitrile.

- 9.2.4.2** Prepare new spiking solution every 20 days.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

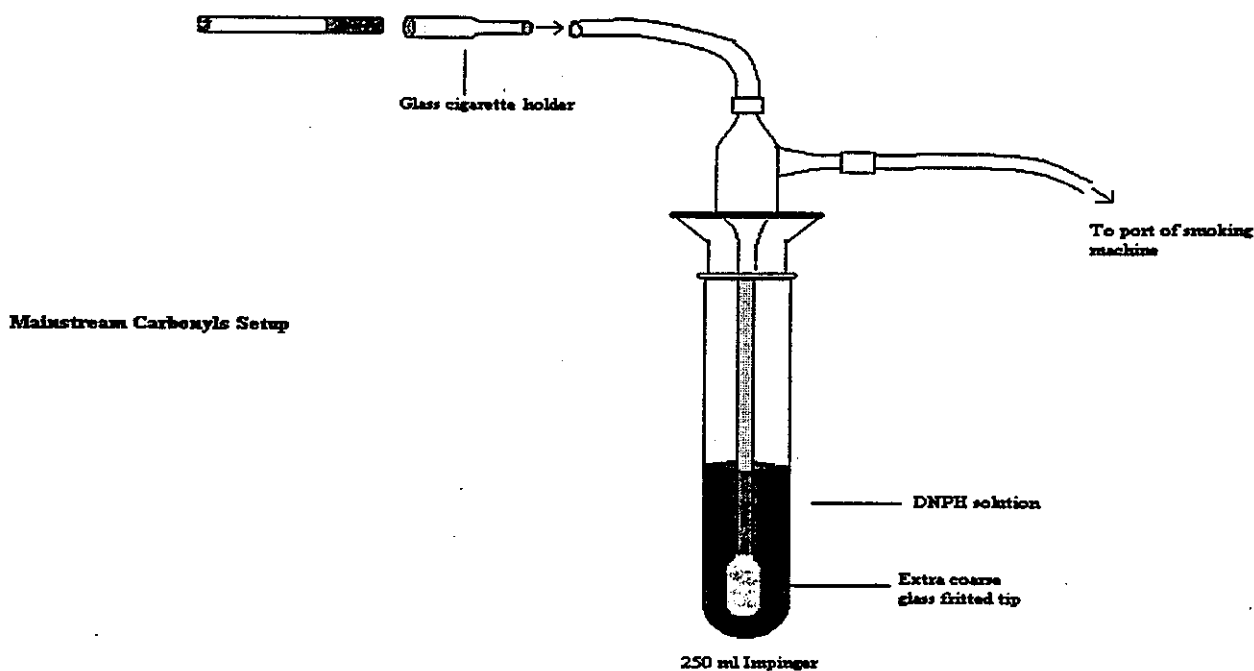
12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115 (with the following modifications as detailed below:)

- 12.2.1.1** It is important to ensure that the mainstream tobacco smoke is characteristic of the test sample before proceeding with the

analysis. Because the mainstream total particulate matter (TPM) is not filtered, no filter pad is present; puff count information must then be used to characterise the smoke extract samples and monitor the smoking process.

- 12.2.1.2** Assemble the carbonyl mainstream apparatus on 12 alternating ports of the linear smoking machine without using the filter pads and holders.
- 12.2.1.3** Connect the special glass cigarette holder (6.5cm in length and 8.0 mm internal diameter) at the back by Nalgene tubing to the 250 mL impinger and trap. See diagram.
- 12.2.1.4** Check and adjust the puff volume drawn by the smoking machine in all of the 12 ports as per T-115. Volumes are checked at the cigarette end of the port (with fritted impinger and DNPH in line) via the brass restrictor.
- 12.2.1.5** The same impinger can be used to adjust each port before smoking begins. Discard the DNPH solution after puff volumes have been measured and adjusted.
- 12.2.1.6** Add 80 mL of fresh 2,4-DNPH solution to each impinger.



13 SAMPLE GENERATION

- 13.1** Cigarettes (two cigarettes*/observation) shall be smoked per observation as specified in T-115.

*For other tobacco products, select a number such that breakthrough does not occur.

14 SAMPLE ANALYSIS**14.1 Mainstream Smoke Extract Solution**

- 14.1.1** One run consists of 12 DNPH smoke extract samples. Process 12 samples at a time but not more than two runs or 24 samples per day. Do not smoke more than can be analysed in a 24 hour period.
- 14.1.2** Rinse the cigarette holder and the Nalgene tubing with the impinger solution by forcing the impinger solution back up the impinger as far as the glass cigarette holder using positive air pressure and then with negative air pressure until air is forced back through the solution.
- 14.1.3** Repeat this rinsing procedure at least three times for each impinger to dissolve any smoke condensate in the gas transfer lines.
- 14.1.4** Allow the DNPH smoke extract solution to sit for least five minutes before continuing with sample preparation.
- 14.1.5** Pipette 6 mL of 1 % Trizma base solution into a 10 mL volumetric flask.
- 14.1.6** Add 4 mL of syringe-filtered DNPH smoke extract to the volumetric flask.
- 14.1.7** Mix the volumetric flask well. Transfer a portion of this solution by Pasteur pipette to autosampler vials in duplicate (a and b). (Rinse each vial first with a few drops, fill to minimise head space).
- 14.1.8** Cap the vials with Teflon faced septa and stored at 4 °C until analysed.
- 14.1.9** Repeat 14.1.5 to 14.1.8 for each smoke extract sample.

14.2 Preparation of Controls and Blanks

- 14.2.1** Prepare at least one LRB, LFB, and one LFM per day of activity as follows to demonstrate that interference from the analytical system, glassware, and reagents are not present.

14.3 Laboratory Reagent Blank (LRB)

- 14.3.1** Pipette 6 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.
- 14.3.2** Add 4 mL of fresh filtered DNPH solution to the volumetric flask. Cap the flask and mix well.

14.3.3 Transfer to two autosampler vials (a and b), cap and store at 4 °C until ready to analyse.

14.4 Laboratory Fortified Blank (LFB)

14.4.1 Add 1 mL of the Carbonyl Spiking Solution and 79 mL of DNPH solution to the 250 mL impinger. Mix well.

14.4.2 Pipette 6 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.

14.4.3 Add 4 mL of the filtered DNPH/Spiking solution (14.4.1) to the volumetric flask. Cap the flask and mix well.

14.4.4 Transfer to two autosampler vials (a and b), cap and store at 4 °C until ready to analyse.

14.5 Laboratory Fortified Matrix (LFM)

14.5.1 Pipette 5 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.

14.5.2 Add 1 mL of the Carbonyl Spiking Solution to the 10 mL volumetric flask.

14.5.3 Add 4 mL of filtered DNPH/smoke extract solution from a control brand to the 10 mL volumetric flask. Cap the flask and mix well.

14.5.4 Transfer to two autosampler vials (a and b), cap and store at 4 °C until ready to analyse.

14.6 REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

14.6.1 Chromatographic Conditions

14.6.1.1 Column Temperature: 30 °C.

14.6.1.2 Injection volume: 20 µL.

14.6.1.3 UV detection at 365 nm.

14.6.1.4 Mobile Phase: Reagents

Solvent A: Prepare 2 L of 30 % Acetonitrile, 10 % THF, 1 % IPA in Type I water, filter and degas. (UHP Helium sparged).

Solvent B: Prepare 2 L of 65 % Acetonitrile, 1 % THF, 1 % IPA in Type I water, filter and degas. (UHP Helium sparged).

Solvent C: Acetonitrile (UHP Helium sparged).

14.6.1.5 Sample Wash: Solvent A.

14.6.1.6 Mobile Phase: Gradient.

Flowrate Time (minutes)	1.5 mL/minute Composition		
0.0	100 % A	0 % B	0 % C
8.0	70 % A	30 % B	0 % C
20.0	47 % A	53 % B	0 % C
27.0	0 % A	100 % B	0 % C
30.0	0 % A	0 % B	100 % C
32.0	0 % A	0 % B	100 % C
34.0	95 % A	5 % B	0 % C
Method End			
Action	100 % A	0 % B	0 % C
(Equilibrate 10 minutes).			

14.6.2 Sample vials are loaded onto the autosampler such that every 8th vial is a standard solution and in such quantities that the total analysis time (14.1 – 14.6) does not exceed 24 hours.

14.6.3 Inject 20 µL of vial (a) of each sample onto the HPLC column and analyze as per the chromatographic conditions listed in 14.6.1. Vial b is the backup sample in the event of a problem.

14.6.4 Elution pattern should be similar to **Figure 1**.

14.7 Calculations**14.7.1 Construct a Calibration Curve:**

14.7.1.1 Twenty µL of each calibration standard is injected onto the HPLC column and analysed as per the chromatographic conditions listed in 12.7. Do in duplicate. Elution pattern should be similar to **Figure 2**.

14.7.2 Determination of Response Factor

14.7.2.1 A calibration curve for each individual carbonyl is prepared by plotting the concentration of the standards versus their respective peak areas.

14.7.2.2 Response factors are calculated for each individual carbonyl compound from the calibration curves.

14.7.3 Sample Quantification

14.7.3.1 The amount of the various carbonyl compounds in smoke samples is quantified by the external standard method. The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.

14.7.3.2 Carbonyl concentrations are reported in ($\mu\text{g/mL}$) by the software.

14.7.3.3 Determination of Mainstream Carbonyl Deliveries in [$\mu\text{g/cigarette}$]

$$\text{e.g. Carbonyl } [\mu\text{g/cigarette}] = \frac{\text{Peak Area}}{\text{Resp. Factor}} \times \frac{\text{DF}}{\text{No. of Cigarettes}}$$

where DF is the dilution factor. The response factor shall be determined from the calibration curve.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Figures 1, 2.

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should include:

A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, glass fibre filter discs (pads), and analyzer effects.

A Laboratory Fortified Matrix (LFM) to evaluate the extent of potential analyte loss.

A standard run as a sample to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) and Limit of Quantitation

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analysing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analysing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as ten times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary Carbonyl standards are prepared as required.

15.4.2 All work standards are to be prepared every 20 days.

15.4.3 All samples are to be analysed as soon as they are prepared and within four hours of the cigarettes being smoked.

16 MODIFICATIONS FOR INTENSE SMOKING

16.1 No modifications for intense smoking are necessary.

17 REFERENCES

17.1 Related Publications

17.1.1 Houlgate, P. R., Dhingra, K. S., Nash, J. S., and Evans, W. H., 1989: Determination of Formaldehyde and Acetaldehyde in Mainstream Cigarette Smoke by high-performance Liquid Chromatography; *Analyst* 114, p. 355-360.

17.1.2 Manning, D.L., Maskerinec, M.P., Jenkins, R.A., and Marshall, A.H. "High Performance Liquid Chromatographic Determinations of Selected Gas Phase Carbonyls in Tobacco Smoke" *Journal of Assoc of Anal Chem*., 66, p. 8-12.

APPENDICES

Appendix 1 – Typical Calibration Standards

(a): Stock Standards

Carbonyl Hydrazone	Primary Standard						Secondary Standard		
	Formula Wt Hydrazone	Formula Wt Carbonyl	Weight (mg)	Purity %	Volume (mL)	Stock [µg/mL]	Vol. (mL) Primary Stock	Dilute to Vol. (mL)	Stock [µg/mL]
Formaldehyde	211.2	30.03	39.72	100	25	225.879	0.5	25	4.51758
Acetaldehyde	225.14	44.05	53.9	100	25	421.834	1.0	25	16.87338
Acetone	239.17	58.08	31.2	100	25	303.064	0.75	25	9.09192
Acrolein	237.15	56.06	32.27	100	25	305.133	0.5	25	6.10266
Propionaldehyde	239.17	58.08	31.18	100	25	302.87	0.5	25	6.0574
Crotonaldehyde	251.18	70.09	27.37	100	25	305.496	0.5	25	6.10992
MEK	253.2	72.11	27.43	100	25	312.477	0.5	25	6.24953
Butyraldehyde	253.2	72.11	23.28	100	25	265.201	0.5	25	5.30402

*In a single 25 mL volumetric flask and made up to volume with acetonitrile.

(b): Carbonyl Working Standards **

	5	20	40	80	200	400	700	1000
Vol. (mL) W/S	0.050	0.200	0.400	0.800	2.000	4.000	7.000	10.000
Carbonyl [µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]
Formaldehyde	0.0226	0.0904	0.1807	0.3614	0.9035	1.8070	3.1623	4.5176
Acetaldehyde	0.0844	0.3375	0.6749	1.3499	3.3747	6.7494	11.8114	16.8734
Acetone	0.0455	0.1818	0.3637	0.7274	1.8184	3.6368	6.3643	9.0919
Acrolein	0.0305	0.1221	0.2441	0.4882	1.2205	2.4411	4.2719	6.1027
Propionaldehyde	0.0303	0.1211	0.2423	0.4846	1.2115	2.4230	4.2402	6.0574
Crotonaldehyde	0.0305	0.1222	0.2444	0.4888	1.2220	2.4440	4.2769	6.1099
MEK	0.0312	0.1250	0.2500	0.5000	1.2499	2.4998	4.3747	6.2495
Butyraldehyde	0.0265	0.1061	0.2122	0.4243	1.0608	2.1216	3.7128	5.3040

**Prepared in 10mL volumetric flasks and made up to volume with acetonitrile.

(c): Spiking Solutions

Carbonyl	LFB Spiking Solution ***					
		Stock [µg/mL]	Volume (mL)	Dilute to Vol. (mL)	Spike [µg/mL]	as Analyzed [µg/mL]
Formaldehyde	Primary	225.879	1.4		31.62307	0.11294
Acetone	Primary	303.064	1.0	10.0	30.30641	0.10824
Butyraldehyde	Primary	265.201	1.0		26.52008	0.09471
Total Butyraldehyde					47.68601	0.1703

***In a single 10mL volumetric flask and made up to volume with acetonitrile.

Figure 1: Analytical Chromatogram of Volatile Carbonyls in DNPH Extract of Mainstream Tobacco Smoke

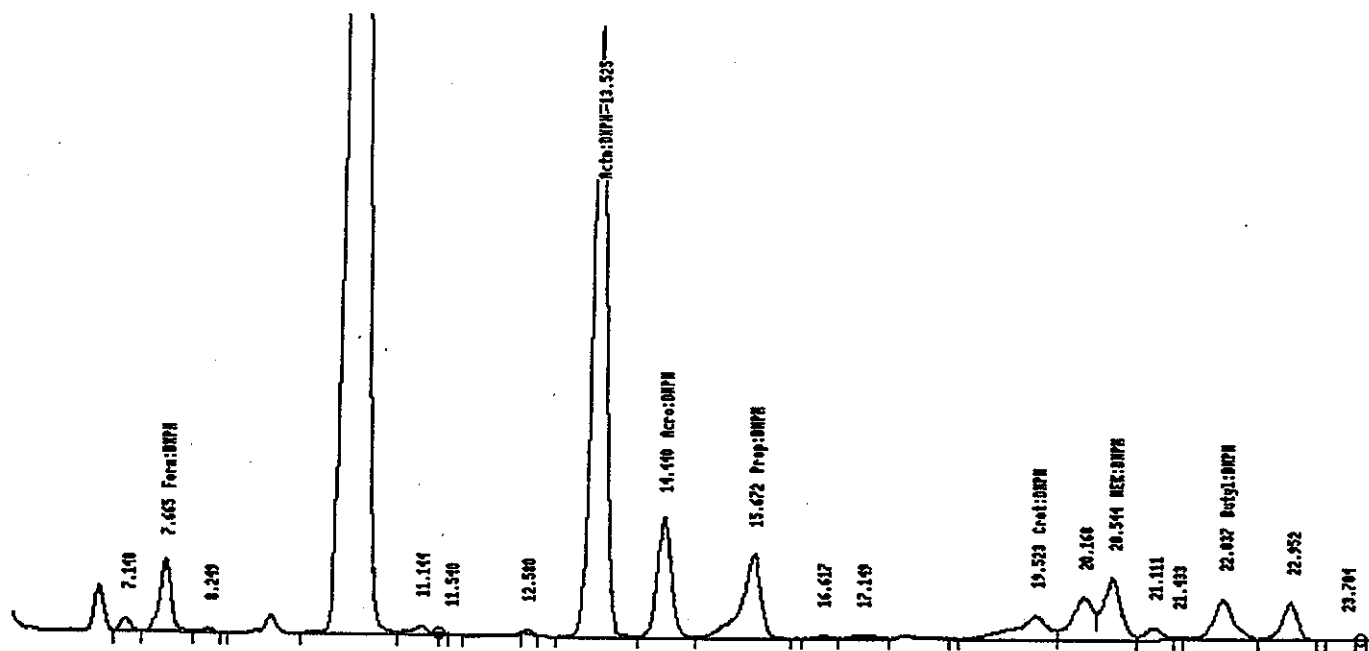
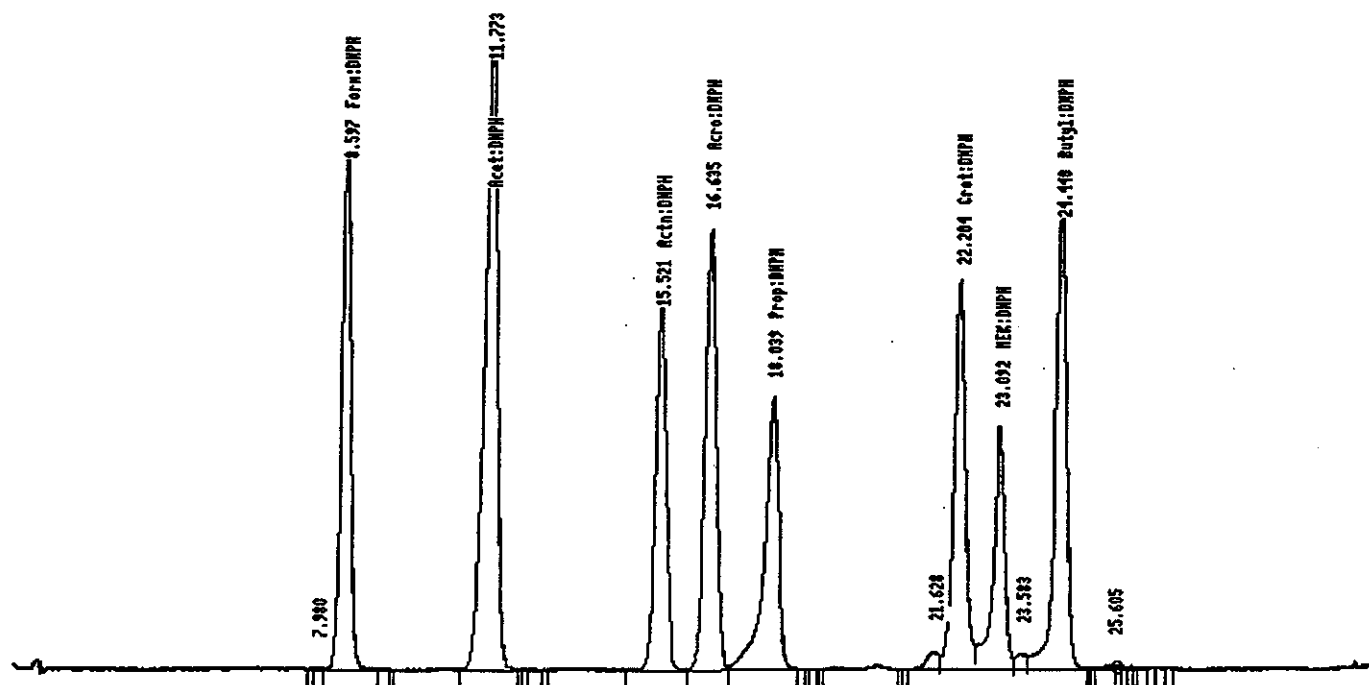


Figure 2: Chromatogram of a typical combined Carbonyl Calibration Standard



No: T - 106
Date: December 31, 1999
Page: 1 of 6

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the determination of the relative retention of nicotine in the filters of filtered cigarettes by gas chromatography (GC).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Five conditioned cigarettes are smoked per port, using an automated 20-port constant volume smoking machine, onto a conditioned, pre-weighed glass fiber filter disc (pad). The pad is then re-weighed and the difference is the Total Particulate Matter (TPM). The pad and filters are extracted separately with Isopropanol (IPA) containing the internal standard, and the extracts analyzed for nicotine by packed column GC with FID. The amount of nicotine in the filters relative to the total amount of nicotine in the pad and filters is the filter efficiency.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking and TPM analysis as described in T-115.
5.2 Equipment needed to perform nicotine analysis as described in T-115.
5.3 Equipment needed for conditioning of tobacco products as described in T-115.
5.4 Anti-static wipes.
5.5 Analytical balance measuring to at least four decimal places.
5.6 50 mL amber serum bottles with Teflon-lined stoppers.
5.7 Constant rate platform shaker.
5.8 Glassware drying ovens.
5.9 Volumetric Flasks - 10 and 25 mL.
5.10 Volumetric pipettes or gas-tight syringe for range 60 to 1000 µL.
5.11 Hewlett-Packard 5890 GC with FID and 6890 autosampler and necessary computer capable of supporting Excel and HP GC software or equivalent.
5.12 Nicotine Column - 6' X 1/8" o.d. (2 metres X 3.2 mm o.d.) stainless steel – 16 % Apiezon L, 2 % KOH, 2 % Carbowax 20M on Chromosorb W: 80-100 mesh or equivalent.

- 5.13 Pipettor (Eppendorf or equivalent) with pipette tips.
- 5.14 20 L volumetric flask.
- 5.15 Dessicant tube.
- 5.16 Magnetic Stirrer and stir bar.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Supplies needed to perform nicotine analyses as described in T-115.
- 6.2 Trans-Anethole (at least 99 % purity) or equivalent as internal standard (ISTD).
- 6.3 Amber autosampler vials with rubber septa lined cap.
- 6.4 Parafilm® or equivalent.
- 6.5 Argon gas.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

8.1 Preparation of Extraction Solution

- 8.1.1 Prepare the extraction solution to contain a concentration of ISTD of 200µL/mL of isopropanol (IPA). For example: pipette 4 mL of anethole into a 20 L volumetric flask. Dilute to volume with isopropanol.
- 8.1.2 Store extraction solution in the dark at room temperature with a desiccant tube on the top and with slow, constant stirring.
- 8.1.3 A blank of the extraction solution is prepared for injection onto the GC for determination of any interfering peaks.

9 PREPARATION OF STANDARDS

9.1 Preparation of GC Calibration Standards

9.1.1 Nicotine Stock

Note: Use gloves and work in a fume hood due to the extreme toxicity of nicotine.

- 9.1.1.1 Upon opening a new bottle of nicotine, purge the bottle with argon gas and store at 4 °C. Discard one month after opening.

- 9.1.1.2 Weigh, using a pipettor, approximately 100 mg pure nicotine into a dry 25 mL volumetric flask and dilute to volume with extraction solution.

9.1.2 Calibration Standards

- 9.1.2.1 Rinse eight dry 10 mL volumetric flasks with extraction solution.

9.1.2.2 Pipette 60, 100, 160, 300, 500, 800, 1200 and 2400 μL of the Nicotine Stock Solution into the corresponding 10 mL volumetric flasks.

9.1.2.3 Dilute to the line with extraction solution and mix.

9.1.2.4 Use a small amount of each standard solution to rinse the 2 mL GC vials designated to each standard.

9.1.2.5 Transfer aliquots of these standards into the pre-rinsed 2 mL GC vials.

9.1.2.6 A vial containing only extraction solution should be run with the standards to represent the "intercept" of the standard curve.

9.1.2.7 Store calibration standards in the dark, covered with Parafilm.

9.1.2.8 Make fresh stocks and calibration solutions weekly or whenever fresh extraction solution is prepared.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Preparation of Cigarettes for smoking

11.1.1 Cigarettes are to be conditioned as specified in T-115.

11.1.2 Cigarettes are to be marked for butt length as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115.

13 SAMPLE GENERATION

13.1 Cigarettes shall be smoked and TPM collected as specified in T-115 with the following modification as detailed below:

13.1.1 Smoke the cigarettes but keep the butts from each port in a separate container until the smoking of the test cigarettes is complete.

14 SAMPLE ANALYSIS

14.1 Extraction (Pad)

- 14.1.1 At the end of the run, remove the pad holders from the smoking machine and weigh them to determine TPM.
- 14.1.2 Open the pad assembly and, with gloves on and using clean tweezers, fold the pad into quarters, TPM inside.
- 14.1.3 Wipe the internal surface of the holder with the clean surface of the pad and transfer the pad into a dry, labeled 50 mL amber serum bottle, TPM side up.
- 14.1.4 Three blanks must be prepared with each smoking run. Place one conditioned pad into each of three dry 50 mL serum bottles and treat as samples.
- 14.1.5 Add 20 mL of the extraction solution to the bottle and seal with a Teflon-lined stopper.
- 14.1.6 Shake the bottles for 30 minutes on a platform shaker.
- 14.1.7 Rinse two autosampler vials with the contents of the bottle and discard the rinsate. Fill each vial, cap and label with run #, port #, and A or B and place A samples on GC autosampler tray for analysis.
- 14.1.8 Store B samples in the dark to be used if necessary.

14.2 Extraction (Butt Filters)

- 14.2.1 Remove and discard all excess tobacco remaining on the butts saved from the smoking of the test cigarettes (13.1.1).
- 14.2.2 Carefully remove the filter paper, which surrounds the filter.
- 14.2.3 Place the butt filter into a 50 mL amber serum bottle.
- 14.2.4 Repeat the procedure for the remaining four butts.
- 14.2.5 When all five filters are in the bottle, add 20 mL of extraction solution and extract as per 14.1.6 to 14.1.8.

Note: If the cigarette filter contains more than one piece or section (e.g. contains charcoal) it is usually required to determine the retention of these sections separately. In this case, the different sections would each be placed into separate bottles and analyzed separately.

14.3 Instrument Analysis: Typical GC Conditions

Oven Temperature:	190 °C.
Injector Temperature:	230 °C.
Detector Temperature:	230 °C.
Carrier Gas:	Purified Helium @ pressure 60 psi.

Flow Rates

FID:	Column flow:	20 mL/minute.
	Column + Hydrogen:	60 mL/minute.
	Column + Air + Hydrogen:	350 mL/minute.

- 14.4** Two µL of each standard and sample are injected onto the GC.
- 14.5** Chromatographic data is collected on the GC Computer with the HP Chemstation software or equivalent.

14.6 Calculations**14.6.1 Calibration Curve**

14.6.1.1 With each new batch of extraction solution prepared, the GC must be recalibrated to determine new slopes and intercepts for nicotine calculations as well as to monitor any changes in GC performance. Each recalibration involves preparing new stock and standard solutions.

14.6.1.2 A calibration curve is generated from the working standards. Quantitation is performed using the internal standard calculation.

14.6.1.3 The ratios are obtained from the chromatograms. Nicotine ratios are calculated as nicotine peak area/ISTD peak area. Ratio versus expected mg/cigarette are plotted for the determination of the nicotine slope and intercept.

14.7 Sample Calculations**14.7.1 TPM**

$$\text{TPM}(\text{mg/cigarette}) = [\text{Pad Weight after}(\text{g}) - \text{Pad Weight before}(\text{g})] \times 1000(\text{mg/g}) / \text{number of cigarettes.}$$

14.7.2 Nicotine in Extracts

Nicotine results are calculated from the calibration curve and are reported in mg/cigarette.

14.7.3 Filter Efficiency

The Filter Efficiency value is determined for each sample by the following calculation:

$$R = F / (M + F) \times 100.$$

R = % nicotine retained by the filter.

F = nicotine content of filters (mg/cigarette).

M = nicotine content from pad (mg/cigarette).

15 QUALITY CONTROL**15.1 Recoveries and Levels of Contamination**

15.1.1 Laboratory Reagent Blanks (LRB) are used to monitor the level of nicotine contamination in the reagents (including glassware and pads). LRB results for nicotine are typically ND (not detected).

15.1.2 Laboratory Fortified Blanks (LFB) are used to evaluate the extent of potential analyte loss during the extraction process. An LFB is prepared by spiking a conditioned CFP with a known amount of nicotine standard. LFBs should be run whenever there is a question about the validity of results, but do not need to be run routinely due to the simplicity of the extraction process and the use of internal standards. Typical average nicotine recovery from a series of 6 LFBs (spiked pad) are:

100.3 ± 0.3 %.

15.2 Method detection limit (MDL)/Limit of Quantitation (LOQ)

15.2.1 This involves the analysis of either a test material with a low level of the analyte or the lowest standard. The standard deviation of 10 observations is determined and the MDL is determined to be three times the standard deviation. The LOQ is determined to be 10 times the standard deviation.

15.3 Stability of Reagents and Samples

15.3.1 Standards should be wrapped with Parafilm® and kept in the dark. They are stable for approximately one week.

15.3.2 Extraction solution is stable but can become contaminated with water over time. For this reason, and to ensure nicotine calibration remains constant, fresh extraction solution should be made weekly.

15.3.3 Each bottle of nicotine, once opened, should only be used for one month. Opened bottles should be purged with argon gas and stored at 4 °C.

16 MODIFICATIONS FOR INTENSIVE SMOKING

16.1 Three conditioned cigarettes are to be smoked per port under intensive conditions.

17 REFERENCE

17.1 Agriculture Canada Research Station, Delhi, Ontario, *Methods of Analysis*, Part V. Filter Efficiency Test, July 1974.

No.: T - 107
Date: December 31, 1999
Page: 1 of 7

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the quantitation of the hydrogen cyanide (HCN) content of mainstream tobacco smoke by an automated continuous flow analyzer, as trapped on the glass fibre filter disc (pad) and in the gas phase.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specifications for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Five conditioned cigarettes* are smoked per port, on alternate ports of an automated linear constant volume smoking machine, onto a conditioned pad, with a trap containing 0.1N NaOH located directly behind the pad.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2 The pad is extracted with 40 mL of 0.1N NaOH on a wrist action shaker for 30 minutes.

- 4.3 Both the pad extract and impinger trapping solutions are analyzed by an automated continuous flow colorimetric analyzer where each sample undergoes on-line dilution. Hydrogen cyanide in the sample is converted to cyanogen chloride by an aqueous solution of chloramine-T. The cyanogen chloride then reacts with pyridine to give glutaconic aldehyde, which, upon reaction with a pyrazolone reagent, forms a coloured complex. A single channel monitors the complex, which is quantified by comparison to an external standard calibration.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

The analysis should be completed in one day, and the waste potassium cyanide solutions generated must be stored for disposal by registered chemical recycling agencies. All pipetting must be done with mechanical devices.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115
- 5.4 Analytical Balance capable of reading to four decimal places.
- 5.5 125 mL Polymethylpentene (PMP) Erlenmeyer flasks with screw caps.
- 5.6 Wrist-action shaker.
- 5.7 5 cc Disposable Syringe.
- 5.8 Syringe Filter – Nalgene SFCA (25 mm) (or equivalent).
- 5.9 Computer-controlled Continuous Flow AutoAnalyzer (or equivalent) consisting of :
 - 5.9.1 Technicon IV Autosampler.
 - 5.9.2 Technicon II Peristaltic Pump.
 - 5.9.3 HCN Manifold.
 - 5.9.4 Single Channel Colorimeter equipped with 15 mm flow cell and 540 nm filter.
- 5.10 70 mL impingers with coarse frits.
- 5.11 50 mL volumetric flasks with ground glass joints.
- 5.12 50 mL graduated cylinders.
- 5.13 Glass filter funnel.
- 5.14 Magnetic Stirrer and stir bars.
- 5.15 1000 μ L variable adjusting volume pipettor.
- 5.16 Sample cups for autoanalyzer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Potassium cyanide.
- 6.2 Chloramine T.
- 6.3 Pyridine.
- 6.4 Sodium Hydroxide (NaOH).
- 6.5 3-methyl-1-phenyl-2-pyrazolin-5-one.
- 6.6 Bispyrazolone.
- 6.7 Potassium dihydrogen phosphate.
- 6.8 Disodium hydrogen phosphate.
- 6.9 Brij-35 solution (30 %).
- 6.10 Type I water, as specified in ASTM D1193.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Chloramine-T Solution

8.1.1 Add 2 g of chloramine-T to 500 mL of Type I water. Mix well. Prepare fresh weekly.

8.2 Saturated Pyrazolone Solution

- 8.2.1** Stir 5 g of 3-methyl-1-phenyl-2-pyrazolin-5-one with 2 L of water for five hours, using a magnetic stirrer and stir bar.

8.3 Pyridine-Pyrazolone Solution

- 8.3.1** Dissolve 0.080 g of bispyrazolone in 80 mL of pyridine in an amber bottle and mix on magnetic stirrer for 30 minutes. After complete solution is obtained, add 400 mL of filtered saturated pyrazolone solution and mix.

8.4 Buffer solution

- 8.4.1** Dissolve 13.6 g of potassium dihydrogen phosphate and 0.28 g of disodium hydrogen phosphate in Type I water and dilute to 1 L. Add 0.5 mL of Brij-35 and mix.

8.5 Sodium Hydroxide (0.1N)

- 8.5.1** Add 8g of NaOH pellets to 2 L of Type I water. Stir until completely dissolved.

9 PREPARATION OF STANDARDS**9.1 Preparation of Cyanide Standards**

- 9.1.1** Prepare a primary stock solution equivalent to 500ppm HCN (60.2 mg of KCN made up to 50 mL with 0.1N NaOH).
- 9.1.2** Then dilute 0.1, 0.3, 0.5, 0.7, 0.9, 1.2 and 1.5 mL aliquots of this solution each to 50 mL with 0.1N NaOH. These standards are equivalent to 1, 3, 5, 7, 9, 12 and 15ppm ($\mu\text{g/mL}$) HCN. These standards should be prepared fresh weekly.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product is to be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

- 12.1** Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications:

12.2.1.1 Load impingers for the vapour phase HCN analysis with 30 mL of 0.1N NaOH. Place the impingers onto the rear section of the smoking machine. Tubing from the top of the impinger (internal stem connection) attaches to the pad holder and tubing from the side of the impinger attaches to the vacuum source of system (syringe).

13 SAMPLE GENERATION

13.1 Cigarettes shall be smoked and TPM collected as specified in T-115.

14 SAMPLE ANALYSIS

14.1 Extraction of Pads

14.1.1 Remove the mainstream pad, fold in half and in half again with the "clean" side facing out. Grasp with a pair of clean tweezers, and wipe the holder. Place the pad into a clean labeled 125 mL Erlenmeyer flask.

14.1.2 Add 40 mL of 0.1N NaOH to each of the Erlenmeyer flasks containing a pad from the analytical run and cap.

14.1.3 Clamp the flasks onto the armature of a wrist action shaker and agitate for 30 minutes. The pad should be disintegrated at the end of the time period.

14.1.4 Filter the pad extract directly into appropriately labeled vials or sample cups using a syringe filter attached to a 5 cc disposable syringe.

14.1.5 Analyse the extract immediately for HCN (within 24 hours).

14.2 Impinger Trapping Solution

14.2.1 The 0.1N NaOH impinger solution that traps the vapour phase HCN is used to rinse the attached tubes and walls of the impinger. The impinger solution is then poured into autosampler cups and analysed for HCN while the pad extracts are shaking.

14.3 Continuous Flow Analysis

14.3.1 The Autosampler is operated at a sampling rate of 20 per hour with a 2:1 sample to wash ratio. Sufficient time should be allowed for the system to become stable with the reagents being pumped.

14.3.2 The samples are only rerun if found to be out of range or if there is a problem with the analysis.

14.3.3 The samples undergo on-line dilution.

14.3.4 Sampling cups containing only 0.1N NaOH are placed at regular intervals to allow for baseline correction.

14.4 Calculations

14.4.1 Construct a calibration curve relating ppm of HCN to peak height with the data obtained from the standards.

14.4.2 Obtain ppm or µg/mL of HCN for each extract and calculate micrograms (µg) per cigarette of HCN in mainstream smoke for both pad and impinger extracts:

Pad HCN (µg/cigarette) = [amount (µg/mL) X 40 (mL)] / No. of Cigarettes smoked.

Impinger HCN (µg/cigarette) = [amount (µg/mL) X 30 (mL)] / No. of Cigarettes smoked.

Total HCN (µg/cigarette) = PAD HCN (µg/cigarette) + IMPINGER HCN (µg/cigarette).

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

15.2.1.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interferences due to glassware, trapping reagents, pads, and analyzer effects.

LRB: Add one conditioned filter pad to a clean 125 mL Erlenmeyer flask, add 40 mL of 0.1N NaOH solution and cap.

15.2.1.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

LFB: Add one conditioned filter pad to a clean 125 mL Erlenmeyer flask, add 39.6 mL of 0.1N NaOH plus 0.4 mL of the 500 ppm HCN stock solution and cap.

15.2.1.3 A Laboratory Fortified Matrix (LFM) to evaluate any potential matrix effects.

LFM: After shaking the flasks, prepare a laboratory fortified matrix (LFM) daily using a control brand:

LFMA – Dilute 5 mL of a control pad extract to 10 mL with 0.1 N NaOH.

LFMB – Dilute 5 mL of a control pad extract with 0.1 mL of the 500 ppm KCN stock solution and make to 10 mL with 0.1N NaOH.

15.2.1.4 Check standards run as samples to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) & Limit of Quantification (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations. Typical values are: 0.036 µg/mL, which gives an impinger MDL of 0.22 µg/cigarette and a pad MDL of 0.29 µg/cigarette.

15.3.2 Limit of Quantification (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations. Typical values are: 0.12 µg/mL, which gives an impinger LOQ of 0.72 µg/cigarette and a pad LOQ of 0.96 µg/cigarette.

15.4 Stability of Reagents and Samples

15.4.1 All primary stock and working KCN standards are prepared fresh weekly.

15.4.2 All autoanalyzer reagents are prepared fresh weekly or as needed.

15.4.3 All samples are analyzed within 24 hours of sample generation.

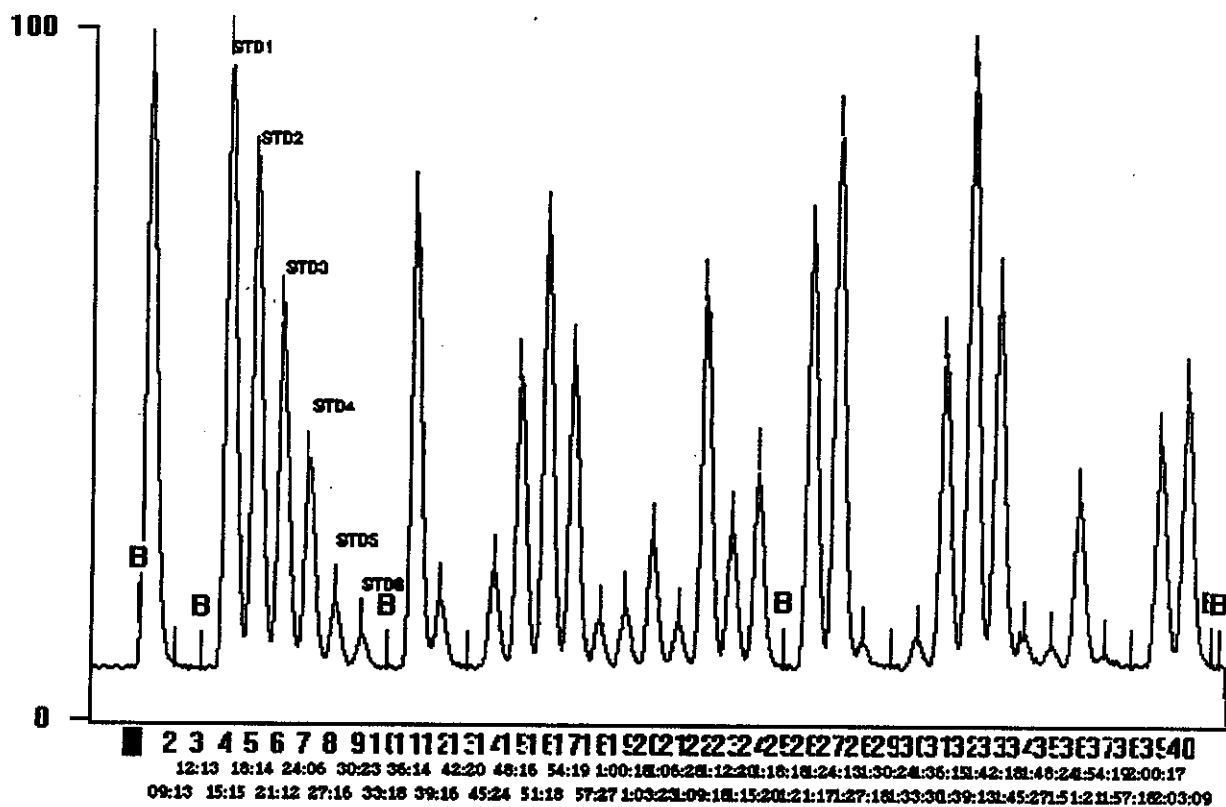
16 MODIFICATIONS FOR INTENSIVE SMOKING

16.1 Under intense smoking conditions the number of cigarettes can be reduced from five to one or two.

17 REFERENCES

- 17.1** Collins, P.F. et al. A Trapping System for the Combined Determination of Total HCN and Total Gas Phase Aldehydes in Cigarette Smoke, *Beitrag zur Tabakforschung*, Vol 7, No.2, 1973.
- 17.2** Rickert, W. S., and P. B. Stockwell. Automated determination of hydrogen cyanide, acrolein, and total aldehydes in the gas phase of tobacco smoke, *J. Autom. Chem.*, 1, 1979, p. 152-154.

Appendix 1: Graph of Colorimeter Output



No: T - 108
Date: December 31, 1999
Page: 1 of 10

1 SCOPE OF APPLICATIONS

- 1.1 This method is to be used to determine the amount of Mercury (Hg) in mainstream tobacco smoke. The method is designed to trap and quantitate Hg in both the particulate phase and gaseous phase components.
- 1.2 Particulate phase mercury cannot be separated from gaseous phase mercury using this type of trapping and analysis system.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Twenty conditioned cigarettes* are smoked as per ISO 3308 on a 20-port rotary Borgwaldt smoking machine. The analyte is collected by passing the mainstream tobacco smoke through two impingers containing an acidified potassium permanganate solution. The impinger solutions are then subjected to microwave digestion.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2 When digestion is complete, the vessels are removed from the digester and allowed to cool. Excess potassium permanganate is reduced with hydroxylamine hydrochloride and the sample is then transferred to a volumetric flask where it is made to volume with Type I water.
- 4.3 The digestate is analysed using cold vapour atomic absorption spectroscopy at 253.7 nm. This method uses a continuous flow vapour generator to reduce the divalent mercury to its atomic state with stannous chloride. A peristaltic pump pushes the reducing agent and sample through a mixing coil to a gas/liquid separator. Nitrogen gas carries the mercury vapour into a flow cell positioned in the burner compartment.

Note: The reaction is very sensitive to fluctuations in temperature so the response must be checked frequently against standards.

Important: The cleaning of glassware and the cleanliness of the environment in which the analysis is performed, has a direct effect on the accuracy and precision of the method. In order to achieve accurate results, all glassware must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 70 mL impinger without frits.
- 5.5 1/4" ester grade Tygon tubing.
- 5.6 1/4" Nalgene connectors.
- 5.7 44 mm Glass fibre filter discs (pads) and cassette.
- 5.8 50 mL, 100 mL, 500 mL, 1000 mL volumetric flasks.
- 5.9 20 mm X 150 mm disposable borosilicate culture tubes.
- 5.10 Pipettor or micro-pipettes for the preparation of working standards.
- 5.11 Pipettor (1-5 mL adjustable volume).
- 5.12 125 mL high density polyethylene (HDPE) storage bottles.
- 5.13 Varian 400P Atomic Absorption Spectrophotometer, or equivalent.
- 5.14 Varian PSC-56 Programmable Sample Changer, or equivalent.
- 5.15 Varian VGA-76 Vapour Generation Assembly, or equivalent.
- 5.16 Varian Mercury Flow Through Cell, or equivalent.
- 5.17 Hollow Cathode Lamp for Hg.
- 5.18 CEM MDS-2100 Microwave Digestion System or equivalent.
- 5.19 CEM ACV-12 Digestion Vessel Assembly (X 2) or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Concentrated HCl.
- 6.2 Concentrated H₂SO₄.
- 6.3 Concentrated HNO₃.
- 6.4 Type I water as per ASTM D1193.
- 6.5 Potassium Permanganate.
- 6.6 H₂O₂ (30-32 %).
- 6.7 Stannous Chloride.
- 6.8 Hydroxylamine Hydrochloride.
- 6.9 Atomic Absorption Reference Standards - Mercury standard solution at 1000µg/mL in 10 % HNO₃.

7 PREPARATION OF GLASSWARE

-
- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.
- 7.2 All glassware and digestion vessels must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

8 PREPARATION OF SOLUTIONS**8.1 Sulphuric Acid / Potassium Permanganate Impinger Solution (20 % H_2SO_4 v/v, 4 % KMnO_4 w/v)**

- 8.1.1 Add approximately 500 mL of Type I water to a 1000 mL volumetric flask.
- 8.1.2 Carefully add 200 mL of conc. H_2SO_4 to the flask and gently swirl and **allow the solution to cool completely to room temperature before proceeding.**
- 8.1.3 Add 40 g of potassium permanganate to the flask and continue to mix until it appears that all the permanganate is dissolved.
- 8.1.4 Make solution to volume with Type I water.

Note: When diluting a concentrated acid solution, it is always important to add the acid to water.

8.2 Hydroxylamine Hydrochloride Solution (10 % w/v)

- 8.2.1 Add 10 g of hydroxylamine hydrochloride to a 100 mL volumetric flask.
- 8.2.2 Add approximately 70 mL of Type I water to the flask to dissolve the solid.
- 8.2.3 Make solution to volume with Type I water.

8.3 Stannous Chloride Solution (25% w/v SnCl_2 in 20 % v/v HCl)

- 8.3.1 Weigh 125 g of Stannous Chloride into an acid-washed 500 mL volumetric flask.
- 8.3.2 Add 100 mL of conc. HCl to completely dissolve the solid material.

Note: Gentle heating may be applied in order to speed up this process.
- 8.3.3 Allow the solution to cool before carefully adding Type I water to make to the 500 mL volume.
- 8.3.4 Mix well and transfer the contents to the 500 mL bottle for the reducing agent channel of the Vapour Generation Assembly.

Note: If any precipitate appears in the bottle or flask, discard the solution and prepare fresh. It is necessary to keep the stannous chloride in solution as well as contaminant free as possible.

8.4 Hydrochloric Acid Solution (20 % v/v HCl)

- 8.4.1 Add approximately 250 mL of type I water into an acid washed 500 mL volumetric flask.
- 8.4.2 Add 100 mL of conc. HCl to the volumetric flask.

8.4.3 Allow the solution to cool before carefully adding type I water to make to the 500 mL volume.

8.4.4 Mix well and transfer the contents to the 500 mL bottle for the acid channel of the Vapour Generation Assembly.

9 PREPARATION OF STANDARDS

9.1 All analytical standards are to be made up in a 12 % (v/v) H_2SO_4 solution immediately prior to analysis, and are to be considered stable for only two days (maximum).

9.2 The purchased standard is in a 10% (v/v) HNO_3 acid solution at a concentration of 1000 $\mu\text{g/mL}$ for stability purposes. The required standards concentrations for Hg are:

Standards:

Standard 1	0.300
Standard 2	0.500
Standard 3	1.500
Standard 4	3.000
Standard 5	5.000
Concentration Units:	ng/mL

9.3 In order to make the proper dilutions, it is necessary to prepare a secondary standard at a concentration of 1 $\mu\text{g/mL}$ also in a 10 % (v/v) HNO_3 acid solution. This secondary solution is considered to be stable for one week (maximum).

9.4 Representative dilutions are as follows:

Primary Stock = 1000 $\mu\text{g/mL}$.

Secondary Stock = 100 μL of Primary Stock to 100 mL = 1 $\mu\text{g/mL}$.

Working Standards:

Standard Concentration = 0.300 ng/mL = 30 μL Secondary Stock to 100 mL.

Standard Concentration = 0.500 ng/mL = 50 μL Secondary Stock to 100 mL.

Standard Concentration = 1.500 ng/mL = 150 μL Secondary Stock to 100 mL.

Standard Concentration = 3.000 ng/mL = 300 μL Secondary Stock to 100 mL.

Standard Concentration = 5.000 ng/mL = 500 μL Secondary Stock to 100 mL.

Note: All aqueous standard dilutions can be made using a pipettor or micropipette and volumetric flasks. The accuracy of the pipettor must be checked if one is to be used.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1 Product is to be conditioned as specified in T-115.
- 11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.
- 11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1 The machine conditions shall be as those specified in T-115, with the following modifications as detailed below:
 - 12.2.1.1 Using ester grade Tygon tubing, directly connect 2 X 70 mL impingers in series, each containing 30 mL of impinger solution (20 % H_2SO_4 v/v, 4% KMnO_4 w/v), between the rear connector position of the smoking machine and the back-up filter cassette on the pneumatics panel using Tygon tubing.
 - 12.2.1.2 Check the set-up for leaks before proceeding further.
 - 12.2.1.3 The pneumatic panel for smoking machine, is adjusted for a 35 mL (± 0.2 mL) puff volume (with impingers in place) and 1.85 second sweep-time, using the supplied timer to measure the adjusted sweep-time.

13 SAMPLE GENERATION

- 13.1 Cigarettes shall be smoked as specified in T-115.
- 13.2 A total of three clearing puffs are taken after smoking is completed to ensure that all the smoke in the dead volume of the system has passed through the impingers.
- 13.3 After smoking is complete, the impinger solutions are transferred to digestion vessels to digest the samples for analysis.

14 SAMPLE ANALYSIS

- 14.1 Using positive pressure, backwash the tubing for each of the impingers with the impinger solution.
- 14.2 The impinger solutions are transferred to a single microwave digestion vessel. First transfer the contents of impinger #1 to the digestion vessel, then the contents of impinger #2 into impinger #1. Then wash the residual acid from impinger #2 with one 5 mL wash of hydrogen peroxide followed by one 5 mL

wash of Type I water. Repeat the wash procedure for impinger #1 using the washes of impinger #2 after transferring the contents of impinger #1 into the digestion vessel.

- 14.3 Carefully add the impinger wash to the same digestion vessel (Caution: high effervescence).
- 14.4 Install the rupture membrane and cap the digestion vessel.
- 14.5 Place the digestion vessel into the turntable and lock into position.
- 14.6 Choose the sample that appears to be the most reactive sample as the reference vessel for monitoring pressure and temperature to control the digestion.
- 14.7 Load the turntable of samples into the microwave digester, and start the digestion program. See Appendix: Microwave Digestion Parameters.
- 14.8 When the digestion is complete, remove the turntable from the microwave and allow the samples to cool to room temperature before opening.
- 14.9 Add 5 mL of the hydroxylamine hydrochloride solution dropwise to each vessel to react with the excess permanganate in the samples.

Note: If the digestion appears to be incomplete, by evidence of tar in the digestate, carefully add 1 to 2 more mL of fresh impinger solution and repeat the original digestion procedure.

- 14.10 When the digestion is complete, remove the turntable from the microwave and allow the samples to cool to room temperature before opening.
- 14.11 Transfer the digestate to a 100 mL volumetric flask and make up to volume using the washings of the digestion vessel with Type I water.

Note: Samples must be analyzed within 48 hours (24 hours is desired) of completing the digestion and taking to volume for stability purposes. Manual dilutions (if necessary) of the digestate should only take place at the time of analysis.

14.12 Sample Dilutions required for Elemental Analysis

14.12.1 No further dilutions of the sample are required, and it may be analysed as is.

14.12.2 A portion of the sample is transferred to a 20 X 150 mm disposable borosilicate culture tube for analysis. The remainder of the solution is kept in the 100 mL volumetric flask until the analysis is complete in order to prevent possible contamination.

Note: Sample volumes are based on "average" literature values. These dilutions may need to be modified depending on: 1. the sample's country of origin; 2. the year in which the sample was grown (environmental factors); 3. the soil type and conditions in which the sample was grown;

4. the type of tobacco used for the sample; 5. the stalk position of the tobacco used for analysis (if not a blended, finished product).

14.13 Analysis of Hg by Cold Vapour Atomic Absorption

14.13.1 Samples are analysed using the parameters established for the instrument at a wavelength of 253.7 nm and a slit width of 0.5 nm.

14.13.2 It is important to analyse the samples for Hg within 48 hours of completing the digestion.

14.13.3 If samples are not analysed within this time frame, the digestate should be returned to the digestion vessel and the secondary digestion procedure performed.

Note: Parameters may differ slightly between instruments.

14.14 Calculations

14.14.1 Results reported from the software, based on a calibration of concentration vs. instrument response, are expressed as [ng/mL] in solution. This result multiplied by the dilution of the sample and divided by the number of cigarettes smoked will calculate the result in a [ng/cigarette] basis.

14.14.1.1 The [ng/cigarette] results can be converted to [µg/cigarette] by dividing this result by 1000.

Note: Representative calculations are as follows:

14.14.1.1.1 Analytical Result (on a per cigarette basis):
$$\text{Analyte [ng/cigarette]} = (\text{Analytical result [ng/mL]} \times 100\text{mL} \times \text{Additional Dilution factor}) / \text{No. of Cigarettes (20)}.$$

15 QUALITY CONTROL

15.1 Each set analysis should contain one of each of the following per day of smoking or batch of up to 24 analysis (20-22 true samples):

15.1.1 Laboratory Reagent Blank (LRB): to determine background contamination from solutions or glassware used in the analysis process.

15.1.2 Laboratory Fortified Blank (LFB): to determine whether there is any loss of analyte as a result of the analysis process.

15.1.3 Control Sample: to determine the inter-experimental reproducibility of the entire method of analysis

15.1.4 Duplicate Sample: to determine the reproducibility of the procedure within the same experiment or batch on analysis.

Note: As an initial evaluation of the method and materials used, it is recommended that a minimum of five blanks be analysed using the method in order to establish control parameters for expected levels of background contamination before any samples are analysed. An LRB outside these control limits is an indicator of possible contamination problems or the use of materials and reagents of different lot numbers.

15.2 Recoveries and Levels of Contamination

15.2.1 Recoveries for a Laboratory Fortified Blank (LFB) for Hg are normally between 85 and 115 %. Variability in this range is associated to differences in the blanks.

15.2.2 Contamination must be monitored with each individual set of samples that are digested and is dependent on the laboratory environment. This ultimately effects the precision of the analysis.

15.3 Method Detection Limit (MDL) / Limit of Quantitation (LOQ)

Note: Individual instruments will have different MDL's and LOQ's depending on the optimization of the instrument.

The MDL is either:

1. The concentration of analyte that yields an absorbance of 0.004 units (the characteristic mass).

Or:

2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

Or:

3. Same as in item number two, using a blank solution.

The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The MDL (on a ng/cigarette basis) can be enhanced by modifying the amount of cigarettes smoked, however this may affect the amount of background contamination observed.

The LOQ is either:

1. The lowest standard used in the preparation of the calibration curve (excluding a blank).

Or:

-
2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

Or:

3. Same as in item number two, using a blank solution.

The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The affect of modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

15.4 Stability of Reagents and Samples

15.4.1 As stated earlier, all samples and analytical run standards must be analyzed within 48 hours of the digestion (24 hours desired).

15.4.2 All solutions for the analysis (other than the impinger solution) are stable for only two weeks because of the probability of contamination problems.

15.4.3 Impinger solutions are stable for a maximum of one day because of precipitation of permanganate and the possibility of contamination.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 Modifications for intense smoking conditions generally include, but are not limited to, a reduction in the number of tobacco products smoked.

17 REFERENCES

17.1 Varian Instruments at Work: Rapid Determination of Mercury in Fish Tissue, a Rapid, Automated Technique for Routine Analysis, No. AA-60, May 1986.

17.2 Varian Instruments at Work: Automated Cold Vapor Determination of Mercury: EPA Stannous Chloride Methodology, No. AA-51, September 1985.

17.3 Van Delft, W. & Vos G. Comparison of Digestion Procedures for the Determination of Mercury in Soils by Cold-Vapour Atomic Absorption Spectrometry, *Analytica Chimica Acta* 209, 1988, p. 147-156.

17.4 Determination of ultratrace-level mercury in sediment and tissue by microwave digestion and atomic fluorescence detection. CEM reference R105.

17.5 The Determination of Total Mercury (Hg) in Air Sampling Solutions, Regulation respecting Mercury - made under the Occupational Health and Safety Act, O. Reg. 23/87, 1987, p. 47-55.

APPENDIX**Appendix 1: Microwave
Digestion Parameters**

Manufacturer: CEM
Model: MDS
2100
**Digestion Vessel
Type:** ACV - Advanced Composite Vessel

Pressure/Temperature/Time Program for the Digestion of MS Smoke Samples

Stage:	1	2	3	4	5
Power %:	70	70	70	0	0
Pressure (psi):	50	125	175	20	150
Run Time (min):	20	15	20	20	20
Time at Parameter:	8	8	15	20	10
Temperature:	95	125	165	20	190
Fan Speed	50	50	50	80	

Note: The temperature and pressure parameters are set as the controlling parameters in this digestion program one of which will define the maximum reached. If either preset is not reached, the microwave oven delivers the designated power for the time programmed in the Run Time function.

Note: These are only suggested parameters as a starting point. The digestion procedure must be optimized for the specific application and instrument used.

No: T - 109
Date: December 31, 1999
Page: 1 of 15

1 SCOPE OF APPLICATIONS

- 1.1** This method is applicable to the determination of nickel (Ni), lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and selenium (Se) in mainstream tobacco smoke by Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Argon Plasma - Atomic Emission Spectroscopy (ICP-AES). The method is designed to quantitate these toxic trace metals in both the particulate phase and gaseous phase of smoke from cigarettes, cigarette equivalents, kreteks, bidis and cigars smoked on a rotary smoking machine.
- 1.2** Particulate phase metals are determined as those metals that become part of the mainstream smoke total particulate matter (TPM), trapped on a glass fibre filter disc (pad) or in an electrostatic precipitator.
- 1.3** Gaseous phase metals are determined as those metals that may have reacted to form a gaseous species or particulate matter that is not retained in the normal TPM condensate.

2 NORMATIVE REFERENCES

- 2.1** American Society for Testing and Materials (ASTM) D 1193-77 - Standard Specifications for Reagent Water, Version 1977.
- 2.2** Health Canada Test Method T-115 - Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Conditioned tobacco product is smoked on a 20-port rotary smoking machine. An electrostatic precipitation generator is utilised to electrostatically precipitate the particulate matter onto a glass electrostatic precipitate (EP) tube. The total particulate matter (TPM) is extracted into 25 mL methanol. The methanol extract is then evaporated using gentle heating while under a constant stream of filtered ultra high purity (UHP) nitrogen. The sample is then subjected to microwave digestion using a mixture of hydrochloric acid, nitric acid and hydrogen peroxide.
- 4.2** The gaseous phase metals are trapped by placing an impinger of a 10 % v/v nitric acid solution between the EP tube and the puff drawing mechanism. The impinger solution is added to the same digestion vessel as the EP tube product and subjected to microwave digestion.
- 4.3** The digestates are then analysed by flameless atomic absorption spectroscopy (or graphite furnace atomic absorption). This method uses pyrolytic coated

partitioned graphite tubes for increased resistivity toward acid, therefore increasing the lifetime of the tube and sensitivity to the analyte.

Note: The analysis of Cd, Pb, Ni, and Cr, can also be achieved by ICP-AES in conjunction with an ultrasonic nebulizer in order to increase sensitivity.

- 4.4 Quantitation is achieved by interpolating the relevant calibration curves prepared from solutions of aqueous metal standards in the same acid concentration to minimize matrix effects. For some metals the use of a matrix modifier is required to prevent loss of analyte during the analysis.

Note: Arsenic and selenium may also be analyzed by hydride generation using sodium borohydride. Extreme care, and a secondary digestion procedure, must be used to ensure these metals are in the proper oxidation state for the hydride reaction to quantitatively occur. This also requires the digestate to be further diluted resulting in a loss in sensitivity.

Important: The electrode tip of the electrostatic precipitator should be made of tungsten or plated with metals that will not interfere with analysis (i.e. Ag, Au etc). The standard EP electrode tip has a Ni plating that, if scratched, yields high background Ni and Cr results.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.2 Equipment needed for conditioning as specified in T-115.
- 5.3 Equipment needed for marking for butt length as specified in T-115.
- 5.4 70 mL impinger without frit.
- 5.5 1/4" ester grade Tygon tubing or equivalent.
- 5.6 1/4" Nalgene connectors.
- 5.7 UHP Compressed Nitrogen.
- 5.8 Glass manifold with Teflon stopcocks.
- 5.9 Tecator 1015 digester or equivalent.
- 5.10 Heinrich Borgwaldt Central Electrostatic Smoke Trap (EP Unit) with tungsten electrode or equivalent.
- 5.11 Heinrich Borgwaldt High Tension Generator, Model 251 or equivalent.
- 5.12 10 mL, 25 mL, 50 mL, 100 mL, 1000 mL volumetric flasks.
- 5.13 Pipettor or micro-pipettes for the preparation of working standards.
- 5.14 Pipettor (1-5 mL adjustable volume).
- 5.15 125 mL HDPE (high density polyethylene) storage bottles.
- 5.16 Atomic Absorption Spectrophotometer.
- 5.17 Graphite Tube Atomizer.
- 5.18 Varian Partition Tubes (Coated) or equivalent.
- 5.19 Hollow Cathode Lamps for : Ni, Pb, Cd, Cr, As, Se and Hg.
- 5.20 Microwave Digestion System with temperature and pressure controls or equivalent.

-
- 5.21** Digestion Vessel Assembly (X 2) or equivalent.
 - 5.22** Alternatively: Varian Axial Vista Simultaneous ICP or equivalent.
 - 5.23** Cetac U-5000AT⁺ Ultrasonic Nebulizer or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Concentrated hydrochloric acid (HCl) – trace metals analysis grade or equivalent.
- 6.2 Concentrated nitric acid (HNO₃) – trace metals analysis grade or equivalent.
- 6.3 Type I water (meets ASTM D1193 specification).
- 6.4 Methanol – distilled-in-glass or equivalent.
- 6.5 Hydrogen Peroxide (32 %).
- 6.6 Ortho-phosphoric acid – trace metals analysis grade or equivalent.
- 6.7 Atomic Absorption Reference Standards - individual standards solutions at 1000 µg/mL.

Note: Reference standards must:

- 1. come with a certificate of analysis, and
- 2. be NIST traceable.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that 0 contamination from glassware does not occur.

Important: The cleaning of glassware and the cleanliness of the environment in which the analysis is performed, directly effects the accuracy and precision of the method. In order to achieve accurate results, all glassware and digestion vessels must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

8 PREPARATION OF SOLUTIONS

8.1 Nitric Acid Impinger Solution (10 % HNO₃ [v/v])

- 8.1.1 Add approximately 500 mL of Type I water to a 1000 mL volumetric flask.
- 8.1.2 Add 100 mL of conc. HNO₃.
- 8.1.3 Make solution to volume with Type I water.

Note: When diluting a concentrated acid solution, always add acid to water.

9 PREPARATION OF STANDARDS

9.1 Elemental Standards and Required Dilutions

- 9.1.1 All standards for graphite furnace analysis are made to a 10% HNO₃ (v/v) acid solution.

Note: For stability purposes, it is desired to dilute the working standards in the same acid as the primary standards.

- 9.1.2 All purchased standards are in 1000 µg/mL concentrations for stability purposes.
- 9.1.3 Primary Standard = 1000 µg/mL.

9.1.4 Secondary Standard (As and Se) = 1 mL of Primary Standard to 10 mL = 100 µg/mL.

9.1.5 Mixed Standard, containing:

100 µL of each Primary Standard (Pb, Ni, Cd).

25 µL Cr Primary Standard.

100 µL As/Se Secondary Standard.

Make up to 100 mL.

Concentrations: Pb, Ni and Cd = 1 µg/mL, Cr = 0.25 µg/mL and As/Se = 0.10 µg/mL.

9.1.6 Preparation of working standards (ng/mL):

Standard #	Mixed Std (µL)	Final Volume (mL)
0	0	100
1	250	100
2	500	100
3	1500	100
4	3000	100
5	5000	100

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product is to be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

The machine conditions shall be as those specified in T-115 with the following modifications as detailed below.

12.2.1 Connect the electrostatic smoke trap (EP Unit) with tungsten electrode to 20-Port Smoking Machine, as per manufacturer's specification.

- 12.2.2** Set the tension generator to 17.5kV for the electrostatic field within the EP unit, and a 0.2 mA current limit.

Note: ALWAYS TURN OFF THE GENERATOR WHEN MAKING ADJUSTMENTS TO THE EP TUBE AND SMOKE TRAIN.

- 12.2.3** Set the control panel for smoking machine, for a two second puff duration, one second intermission, resulting in a 60 second puff frequency.
- 12.2.4** Connect one 70 mL impinger containing 20 mL of 10 % HNO₃ between the EP tube holder and the back-up pad holder of the pneumatics panel using Tygon tubing.
- 12.2.5** Adjust the pneumatic panel for smoking machine, to a 35 mL (+/- 0.2 mL) puff volume (with the EP tube and impinger in place) and 1.85 second sweep-time.

13 SAMPLE GENERATION

- 13.1** Cigarettes shall be smoked and TPM collected as specified in T-115 with the following changes.
- 13.1.1** The EP tube is inserted into the back of the EP unit and then the front of the EP tube is inserted into the head block of the smoke machine. Both ends are gently tightened.
- 13.1.2** The 70 mL impinger, containing 20 mL of fresh 10 % HNO₃ acid, is connected between the EP tube holder and the back-up Cambridge filter cassette on the pneumatics panel using Tygon tubing.
- 13.1.3** The pad in the back-up filter cassette on the pneumatics panel is replaced.
- 13.1.4** The set-up is checked for leaks.
- 13.1.5** Twenty cigarettes are loaded into the ports of the smoking machine using the cigarette load button and positioner.
- 13.1.6** The EP generator is turned on. When the needle reads 17.5kV, cigarettes are lit with an electric lighter.
- 13.1.7** Three clearing puffs are performed once smoking is completed to ensure all of the gaseous phase in the dead volume of the system has passed through the impinger and back-up pad.
- 13.1.8** After smoking is completed, the milli Amp (mA) current on the power supply is recorded.
- 13.1.9** The EP unit is turned off. Allow 10 seconds pass before proceeding to the next step.

13.1.10 The fittings are unscrewed and the EP tube is removed from unit and end-caps are replaced.

13.1.11 The EP tube is weighed and recorded, and the TPM yield per cigarette is calculated using the formula found in the Calculations section.

14 SAMPLE ANALYSIS**14.1 Sample Preparation and Digestion**

- 14.1.1** Twenty-five mL (1 X 12 mL and 1 X 13 mL) of methanol is added to the EP tube after smoking, and the tube is shaken to extract the residue.
- 14.1.2** The extract is transferred to the Teflon microwave digestion vessel (ACV liner).
- 14.1.3** The methanol is evaporated under a gentle flow of filtered nitrogen and gentle heating in a block digester or on a hot plate. The residue is then ready to be digested using the microwave digester.
- 14.1.4** Six mL of HCl is added to the residue.
- 14.1.5** Two mL of conc. HNO_3 is added to the sample, swirling in the acid, and allowing the sample to sit until the original frothing subsides and there is no longer evidence of orange/brown fumes (NO_x formation).
- 14.1.6** Eight mL of hydrogen peroxide is added carefully such that there is no excessive effervescence.
- 14.1.7** Samples are allowed to sit until the effervescence subsides (approximately 10 minutes).
- 14.1.8** The contents of the impinger are added to the digestion vessel.
- 14.1.9** The impingers are rinsed with an additional 2 X 5 mL of hydrogen peroxide and the rinses are added to the digestion vessel.
- 14.1.10** Samples are allowed to sit until the effervescence subsides (approximately 10 minutes).
- 14.1.11** The rupture membrane is installed and the digestion vessel is capped.
- 14.1.12** The digestion vessel is placed into the turntable and locked into position.
- 14.1.13** The sample with the largest TPM content (the most reactive sample) is chosen as the reference vessel for monitoring the temperature and pressure to control the digestion.
- 14.1.14** The turntable of samples is loaded into the microwave digester, and the digestion program is started. **See Appendix 2: Microwave Digestion Parameters.**
- 14.1.15** When the digestion is complete, the turntable is removed from the microwave and the samples are allowed to cool to room temperature before opening.
- 14.1.16** The digestate is inspected. If the digestion appears to be incomplete, the digestion vessels are returned to the microwave for a secondary digestion.

14.1.17 When digestion is complete, the digestate is transferred to a 100 mL volumetric flask and made up to volume by washing the digestion vessel with Type I water.

14.1.18 The contents of the flask are transferred into a 125 mL HDPE storage bottle.

Note: Samples should be stored in the highest concentration of both analyte and acid for stability purposes. Manual dilutions of the digestate should only take place at the time of analysis.

Note: As and Se should be analyzed as soon as possible (within 72 hours) due to a loss in response over time.

14.2 Sample Dilutions required for Individual Elemental Analysis

14.2.1 Samples may require to be diluted so that their absorbances fall within the desired calibration range with a good signal-to-noise ratio and very little matrix effect. Because of minimal matrix effect, standard additions are not required and a standards calibration will suffice.

14.2.2 The analysis of Cd and/or Pb may require a manual dilution prior to analysis by transferring 1000 µL of the digestate to a 10 mL volumetric flask, and making up to volume with Type I water.

Note: When using an ICP-AES for quantitation, the samples may be analyzed without further dilution for Ni, Cr, Pb and Cd.

Note: For As and Se, a multiple injection technique may be required for an adequate instrument response.

Note: These sample dilutions are based on "average" literature values. These dilutions may need to be modified depending on: 1. the sample's country of origin, 2. the year in which the sample was grown (environmental factors), 3. the soil type and conditions in which the sample was grown, 4. the type of tobacco used for the sample, 5. the stalk position of the tobacco used for analysis (if not a blended, finished product).

14.3 Analysis of Ni, Pb, Cd, Cr, As, and Se by Graphite Furnace Atomic Absorption

14.3.1 Samples are analysed using the suggested parameters in **Appendix 1: Instrument Parameters**

Note: Parameters may differ between instruments and must be optimized for the particular instrument used.

14.4 Analysis of Ni, Pb, Cd and Cr by ICP-AES

14.4.1 Samples are analysed using the suggested parameters in **Appendix 3: ICP Parameters**

Note: Parameters may differ between instruments and must be optimized for the particular instrument used.

14.5 Calculations

- 14.5.1 Results reported by the instrument software are expressed as [ng/mL] in solution. This result, multiplied by the dilution of the sample and divided by the number of cigarettes smoked, will calculate the result in a [ng/cigarette] basis.

Analytical Result (on a "per cigarette" basis):

Analyte [ng/cigarette] = (Analytical result [ng/mL] X 100mL X Additional Dilution factor) / No. of Cigarettes (20).

- 14.5.2 The [ng/cigarette] results can be converted to [µg/cigarette] by dividing this result by 1000.

- 14.5.3 Total Particulate matter [mg/cigarette] is calculated using the difference in weight of the EP tube before and after smoking and dividing by the number of cigarettes smoked.

Determination of Total Particulate Matter (TPM):

TPM [mg/cigarette] = [Wt. of EP tube after smoking (g) - Wt. of EP tube before smoking (g)] X 1000 mg/g / 20.

- 14.5.4 Analytical Result (on a "per mg TPM" basis), if desired:

Analyte [ng/mg TPM] = Analyte [ng/cigarette] / TPM [mg/cigarette].

15 QUALITY CONTROL

15.1 Metals Smoking Control Process

- 15.1.1 Each set analysis should contain one of each of the following per day of smoking or batch of up to 24 analyses (20-22 true samples):

Laboratory Reagent Blank (LRB): to determine background contamination from solutions, glassware, or materials used in the analysis process.

Laboratory Fortified Blank (LFB): to determine whether there is any loss of analyte as a result of the analysis process.

- 15.1.2 Control Sample: to determine the inter-experimental reproducibility of the entire method of analysis
- 15.1.3 Duplicate Sample: to determine the reproducibility of the procedure within the same experiment or batch on analysis.

Note: As an initial evaluation of the method and materials used, it is recommended that a minimum of 10 blanks be analysed using the

method in order to establish control parameters for expected levels of background contamination before any samples are analysed. An LRB outside these control limits is an indicator of possible contamination problems or the use of materials and reagents of different lot numbers.

15.2 Recoveries and Levels of Contamination

15.2.1 Recoveries for a Laboratory Fortified Blank (LFB) for Ni, Pb, Cd, and Cr are normally between 85 and 115 %. Variability in this range is associated to differences in the blanks.

15.2.2 Recoveries for a Laboratory Fortified Blank (LFB) for As and Se range from 60 to 85 %. Lower recoveries result from over-heating of the sample while evaporating the methanol.

15.2.3 Contamination must be monitored with each individual set of samples that are digested and is dependent on the laboratory environment. This ultimately effects the precision of the analysis.

15.3 Method Detection Limit (MDL) / Limit of Quantitation (LOQ)

Note: Individual instruments will have different MDL's and LOQ's depending on the optimization of the instrument.

15.3.1 The MDL is defined as:

1. The concentration of analyte that yields an absorbance of 0.004 units (the characteristic mass).

OR

2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

OR

3. As per 2, analyzing a blank a minimum of 10 times.

The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The MDL (on a ng/cigarette basis) can be enhanced by varying the amount of cigarettes smoked, however this may affect the amount of background contamination observed.

15.3.2 The LOQ is either:

1. The lowest standard used in the preparation of the calibration curve (excluding a blank).

OR

2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

OR

3. As per 2, using a blank solution.

The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The effect of modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

15.4 Stability of Reagents and Samples

15.4.1 Secondary and Mixed Standards are stable for one week.

15.4.2 Working standards must be prepared every other day.

15.4.3 All samples must be analyzed within one week of the digestion or samples will have to be re-digested.

16 MODIFICATIONS FOR INTENSIVE SMOKING

16.1 Modifications for intense smoking conditions generally include, but are not limited to, a reduction in the number of cigarettes (or equivalents) smoked.

17 REFERENCES

- 17.1** Environmental Carcinogens - Selected Methods of Analysis, Vol. 8 - Some Metals: As, Be, Cd, Cr, Ni, Pb, Se, Zn, *IARC Scientific Publication*, No. 71, 1986, p. 129-138.
- 17.2** Perinelli, M.A. & Carugno, N. Determination of Trace Metals in Cigarette Smoke by Flameless Atomic Absorption Spectrometry, *Beitrage zur Tabakforschung International*, Band 9, Heft 4, July 1978, p. 214-217.
- 17.3** Bell, Paul & Mulchi, Charles L. Heavy Metal Concentrations in Cigarette Blends, *Tobacco Science*, Vol. 34, 1990, p. 32-34.
- 17.4** NIOSH Method 7300, Elements (ICP), *NIOSH Manual of Analytical Methods*, Vol. 2, Third Edition, 1984.
- 17.5** Varian Analytical Methods for Graphite Tube Atomizers, Varian Australia Pty Ltd, Publication No. 85-100848-00, 1988.
- 17.6** Gawalco, et al. Comparison of Closed-Vessel and Focused Open-Vessel Microwave Dissolution for Determination of Cadmium, Copper, Lead and Selenium in Wheat, Wheat Products, Corn Bran, and Rice Flour by Transverse-Heated Graphite Furnace Atomic Absorption Spectrometry, *Journal of AOAC International*, Vol. 80, No. 2, 1997, p. 379-387.

APPENDICES

Appendix 1: Typical Instrument Parameters

Graphite Furnace Atomic Absorption Analysis of: **Ni**

Method Parameters:

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	4
Slit Width (nm):	0.2
Slit Height:	Normal
Wavelength:	232.0
Sample Introduction:	Sampler Premixed
Measurement Time :	3.1
Replicates:	1
BGD Correction:	On

Graphite Furnace Atomic Absorption Analysis of: **Pb**

Method Parameters:

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	5
Slit Width (nm):	0.5
Slit Height:	Normal
Wavelength:	283.3
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier: Ortho-phosphoric Acid (1000 µg/mL)

Graphite Furnace Atomic Absorption Analysis of: Cd**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	4
Slit Width (nm):	0.5
Slit Height:	Normal
Wavelength:	228.8
Sample Introduction:	Sampler Premixed
Measurement Time :	3.1
Replicates:	1
BGD Correction:	On

Graphite Furnace Atomic Absorption Analysis of: Cr**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	7
Slit Width (nm):	0.2
Slit Height:	Reduced
Wavelength:	357.9
Sample Introduction:	Sampler Premixed
Measurement Time :	3.2
Replicates:	1
BGD Correction:	Off

Matrix Modifier: Ortho-phosphoric Acid (1000 µg/mL)

Graphite Furnace Atomic Absorption Analysis of: As**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	5
Slit Width (nm):	0.2
Slit Height:	Normal
Wavelength:	193.7
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier : Nickel Nitrate (100 µg/mL)**Graphite Furnace Atomic Absorption Analysis of: Se****Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	10
Slit Width (nm):	1
Slit Height:	Normal
Wavelength:	196.0
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier: Nickel Nitrate (100 µg/mL)

Appendix 2: Microwave Digestion Parameters

Microwave Digestion Parameters

Manufacturer: CEM
 Model: MDS 2100
 Digestion Vessel Type: ACV – Advanced Composite Vessels

Pressure/Temperature/Time Program for the Digestion of MS Smoke Samples

Stage:	1	2	3	4	5
Power %:	70	70	70	0	100
Pressure (psi):	45	125	175	20	150
Run Time (min):	20	10	30	20	20
Time at Parameter:	8	8	25	20	10
Temperature:	95	135	190	25	190
Fan Speed:	50 %	50 %	50 %	80%	

Note: Both pressure and temperature are set as the controlling parameters in this digestion program. If the preset pressure or temperature is not reached, the microwave oven delivers the designated power for the time programmed in the Run Time function.

Pressure/Temperature/Time Program a Secondary Digestion

Stage:	1	2	3	4
Power %:	75	75	75	0
Pressure (psi):	95	125	185	20
Temperature:	105	130	160	25
Run Time (min):	15	20	20	20
Time at Parameter:	10	15	15	20
Fan Speed:	50	50	50	80

Note: These are only suggested parameters as a starting point. The digestion procedure must be optimized for the specific application and instrument used.

Appendix 3: ICP-AES Parameters

Power (kw): 1.20
 Plasma Flow (L/minute): 15.0
 Auxiliary Flow (L/minute): 1.50
 Nebulizer Flow (L/minute): 0.65

	Ni	Pb	Cd	Cr
Emmision Wavelength (nm)	221.648	220.353	214.439	267.716

Sample Introduction Settings

Sample Uptake Delay(s): 40
 Pump rate (rpm): 20
 Instrument Stabilization Delay(s): 15
 Rinse Time(s): 10

General Settings

Replicates: 3
 Replicate Read Time(s): 3.0
 Number of Standards Defined: 5

Ultrasonic Nebulizer Set-up

Heater: 140
 Cooler: 2

No: T - 110{PRIVATE }
Date: December 31, 1999
Page: 1 of 13

1 SCOPE OF APPLICATIONS

- 1.1 This method describes the determination of Nitric Oxide (NO) and Total Oxides of Nitrogen (NO_x) in the gas phase of mainstream tobacco smoke and their quantification by a dual channel chemiluminescence analyzer. This is applicable to the NO and NO_x generated in the gas phase of mainstream tobacco smoke from cigarettes on a puff by puff basis.

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 One cigarette is smoked on a standard single port smoking machine and the unfiltered mainstream vapour phase is exhausted into an evacuated smoke mixing chamber (SMC) puff by puff.
- 4.2 The gas is mixed, and at precise and consistent intervals, an aliquot of each puff is routed by vacuum through a filter to a dual channel chemiluminescence nitrogen oxides analyzer where the gas stream is split immediately into two channels.
- 4.2.1 In channel A, the sample stream is reacted with ozone and the resultant chemiluminescent emission is directly proportional to the NO concentration in the sample.
- 4.2.2 In channel B, the sample stream is chemically reduced first by a catalytic converter and then mixed with ozone in the reaction cell where the resultant chemiluminescent emission is due to NO_x or NO + NO₂.
- 4.2.3 The NO₂ concentration is then derived electronically by subtracting the NO signal from the NO_x signal.
- 4.3 Selective photomultiplier detection monitors the reaction cell gas stream and the NO and NO_x found in the vapour phase of mainstream tobacco smoke are quantified by external standard calibration procedures.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with

the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Dual Channel Chart Recorder.
- 5.5 Dual Channel Chemiluminescence Nitrogen Oxides Analyzer.
- 5.6 V/F Integrator.
- 5.7 Digital Multimeter.
- 5.8 Bubblemeter.
- 5.9 Round Bottomed Three Neck boiling flasks – 1 L (SMC for Burly type tobacco).
- 5.10 Round Bottomed Three Neck boiling flask – 5 L (SMC for Flue cured type tobacco).
- 5.11 Round Bottomed Three Neck boiling flask – 12 L (SMC for cigars).
- 5.12 Round Bottomed boiling flask – 1 L (PEC).
- 5.13 Flow Meter.
- 5.14 Mechanical Stirrer.
- 5.15 Teflon coated magnetic stirrers, football shape.
- 5.16 Gas Regulator CGA 660 - Inlet 0-3000, Delivery 0-100.
- 5.17 Gas Regulator CGA 580 - Inlet 0-4000, Delivery 0-100.
- 5.18 Lighter.
- 5.19 Vacuum Pump (Make-up).
- 5.20 Rubber Stoppers.
- 5.21 Gas Flow meter (FM3).
- 5.22 Gas Flow meter (FM4).
- 5.23 Glass fibre filter holders (X2) 44 mm [threaded, screw cartridge] see diagram 2.
- 5.24 Glass tubing 1/4" OD.
- 5.25 Nalgene Tubing 1/4" ID x 3/8" OD.
- 5.26 Teflon (TFE) tubing 1/4" OD (6.35 mm) x 5.8 mm ID.
- 5.27 Inert Valves (X2) with three port housing and plug with 90 ° right angled flow (STC3 & STC4).
- 5.28 Balance capable of measuring to four decimal places.
- 5.29 Exhaust Canopy.
- 5.30 Vacuum Pumps (Exhaust) (X2).
- 5.31 Stop watch.
- 5.32 Barometer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical grade in quality.

- 6.1 Compressed Primary Standard Gas Mixture – 990 ppm NO balance nitrogen (certified).
- 6.2 Compressed Primary Standard Gas Mixture – 508 ppm NO balance nitrogen (certified).
- 6.3 Compressed Primary Standard Gas Mixture – 260 ppm NO balance nitrogen (certified).
- 6.4 Compressed Primary Standard Gas Mixture – 160 ppm NO balance nitrogen (certified).
- 6.5 Compressed Primary Standard Gas Mixture – 70 ppm NO balance nitrogen (certified).
- 6.6 Compressed Primary Standard Gas Mixture – 35 ppm NO balance nitrogen (certified).

- 6.7 UHP Nitrogen (Zero Gas).
- 6.8 Methanol.
- 6.9 Reagent Alcohol HPLC Grade.
- 6.10 Glass fibre filter discs (pads), 44 mm in diameter, with no more than 5% acrylic type binder.
- 6.11 Chart Paper.

7 PREPARATION OF GLASSWARE

- 7.1 All round bottomed glassware is rinsed three times with ethanol and once with methanol and then dried in an oven at 200 °C.
- 7.2 All Teflon three-way stopcocks are disassembled and rinsed with ethanol to remove the tar and once with methanol and allowed to air dry.
- 7.3 The piston assembly of the single port smoking machine is disassembled and the piston is cleaned and rinsed with methanol and then allowed to air dry. The piston is then reassembled, free of any lubricants.
- 7.4 The solenoid valve assembly is disassembled and cleaned with methanol.

8 SAMPLING

- 8.1 The sampling of tobacco products for the purpose of testing shall be as those specified in T-115.

9 TOBACCO PRODUCT PREPARATION

- 9.1 Product shall be conditioned as specified in T-115.
- 9.2 Cigarettes shall be marked for butt length as specified in T-115.
- 9.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

10 SMOKING MACHINE PREPARATION

10.1 Ambient Conditions

- 10.1.1 The ambient conditions for calibration shall be between 21 and 23 °C.

10.2 Machine Conditions

- 10.2.1 The machine conditions shall be as those specified in T-115 with the following modifications.

- 10.2.1.1 Allow the smoking machine to warm up on the automatic cycling for several minutes and ensure that the piston is oil-free.

- 10.2.1.2 Because the analysis is dynamic and ongoing, it is important to ensure that the mainstream tobacco smoke generated is characteristic of the test sample. Also, because the sample

stream is unfiltered; a pad in the mainstream path is not used. Therefore puff count information is the only criterion that can then be used to characterize the vapour phase samples and monitor the smoking process.

10.2.2 Fit a conditioned pad into each of the exhaust and flow meter filter pad holders with the rough side towards the incoming smoke.

10.2.3 The NO_x Analyzer inlet pad is changed every second observation.

10.3 Set-up of Mainstream Flow Detection Equipment

10.3.1 The NO/NO_x Analyzer is pumped down (left on) overnight, then the ozone generator and molycon catalytic converter are activated.

10.3.2 The mainstream NO/NO_x equipment and tubing are assembled as per Diagram 1 in the Appendix.

10.3.3 The FM1 and filter are connected inline to the Make-up air Pump on the downstream side of the NO/NO_x Analyzer inlet. The vacuum flow is calibrated to 0.9 L/minute (SS ball) and is meant to augment the total flow to the NO/NO_x Analyzer (total flow 1.5 L/minute).

10.3.4 The reaction vessel (SMC) is assembled upstream of the NO/NO_x Analyzer inlet as per the following configuration:

10.3.4.1 A glass tube is inserted into the left-hand neck of the SMC through a rubber stopper. This is the exhaust port through which the SMC is evacuated after every puff. The glass tube is connected to a Teflon stopcock (STC3) which is also connected to an inline filter and then to an unrestricted flow Vacuum pump (which is exhausted to the outside). STC3 rotates, and directs the vacuum stream either to the SMC or the room air (when a puff has occurred).

10.3.4.2 Two glass inlet lines are inserted through a rubber stopper in the centre neck of the SMC. One tube is a pressure equalization device whereas the second tube is connected directly to the outlet port of the single port smoking machine.

10.3.4.3 A glass tube is inserted through a rubber stopper into the right hand neck of the SMC. This is connected to a Teflon stopcock (STC4) which is connected directly to a T at the inlet port of the NO/NO_x Analyzer. The STC4 is rotated with every puff at precise intervals to direct the vacuum from the NO/NO_x Analyzer either to the SMC or the room air.

10.3.5 Insert a magnetic stirrer bar into the SMC and mount the SMC directly over the mechanical stirrer.

10.3.6 Electronic output from the NO/NO_x Analyzer is routed to the V/F Integrator which is connected to the digital multimeter.

10.3.7 An output signal is also directed to the two-pen chart recorder where one pen records the NO output and the other record the NO_x output.

10.3.8 Prior to smoking, record:

10.3.8.1 V/F Integrator Span setting at the beginning and end of the experiment.

10.3.8.2 NO/NO_x span pots settings at the calibration gas settings.

10.3.8.3 The average channel counts from the calibration step.

10.3.8.4 Instrument status checks and flow rates.

11 INSTRUMENT ANALYSIS

11.1 Performance Test of the NO/NO_x Analyzer

11.1.1 The NO/NO_x Analyzer electronic output to the two-pen chart recorder and to the V/F Integrator is synchronised in the range of 10mV (ppm)

11.1.2 Various NO/NO_x Analyzer instrument tests are performed prior to any smoking or calibration.

11.1.3 Test T1, T2, and T3 outputs and measure on the analogue scale. This should remain constant day to day.

11.1.4 Switch the Range PPM to the Optic and Electric test modes. Allow to stabilize at least 15-20 minutes before reading. These outputs should be consistent day to day but some variation is experienced.

11.1.5 Switch the Range PPM to 10 for the rest of the experiment.

11.2 Zero the NO/NO_x Analyzer with Nitrogen

11.2.1 Connect the Nitrogen to FM3 to deliver 1.8 L/minute to the inlet port of the pressure equalisation chamber (PEC).

11.2.2 There are two outlets from the PEC, one to the exhaust and the other is connected to STC4 which is connected to the SMC (Smoke mixing chamber) inlet.

11.2.3 Set the Nitrogen regulator to deliver 10 psi.

11.2.4 STC4 is directed to deliver flow to the NO/NO_x Analyzer.

11.2.5 Allow to equilibrate 30 minutes.

11.2.6 Adjust Zero pots on NO/NO_x Analyzer to give an average of 0.000 over 60 seconds on the digital multimeter.

11.3 Confirm the mainstream NO/NO_x configuration with Nitrogen (zero gas)

-
- 11.3.1** Connect the Nitrogen to FM3 to deliver 1.8 L/minute to the inlet port of the pressure equalisation chamber (PEC).
 - 11.3.2** There are two outlets from the PEC, one to the exhaust and the other is connected to STC4 which is connected to the SMC (Smoke mixing chamber) inlet.
 - 11.3.3** Set the Nitrogen regulator to deliver 10psi.
 - 11.3.4** SMC exhaust is directed to room air.
 - 11.3.5** STC4 is directed to deliver flow to the NO/NO_x Analyzer.
 - 11.3.6** Make certain the mixing bar inside the SMC is rotating.
 - 11.3.7** Allow to equilibrate 30 minutes.
 - 11.3.8** Read zero values over 60 seconds on the digital multimeter. Results should agree with 12.2.6.
- 11.4 Span the NO/NO_x Analyzer**
- 11.4.1** Connect the 9 ppm NO primary standard to FM3 to deliver 1.8 L/minute to the inlet port of the pressure equalization chamber (PEC).
 - 11.4.2** There are two outlets from the PEC, one to the exhaust and the other is connected to STC4 which is connected to the SMC (Smoke mixing chamber) inlet.
 - 11.4.3** Set the gas regulator to deliver 10psi.
 - 11.4.4** STC4 is directed to deliver flow to the NO/NO_x Analyzer.
 - 11.4.5** Allow to equilibrate for 30 minutes.
 - 11.4.6** Adjust Span pots on NO/NO_x Analyzer to give an average of 9.000 over two minutes on the digital multimeter. (Adjust the span pots to give a corresponding voltage to concentration i.e. NO=9.1 NO_x=9.3).
 - 11.4.7** Record Span settings and adjust attenuation on plotter if necessary.
 - 11.4.8** Optional: The above procedure can be repeated with a 5 ppm NO standard gas or another standard NO gas that fall in the linear range.
- 11.5 Confirm the mainstream NO/NO_x configuration (Span)**

-
- 11.5.1** Connect the 9 ppm NO primary standard to FM3 to deliver 1.8 L/minute to the inlet port of the pressure equalization chamber (PEC).
 - 11.5.2** There are two outlets from the PEC, one to the exhaust and the other is connected to STC4 which is connected to the SMC (Smoke mixing chamber) inlet.
 - 11.5.3** Set the gas regulator to deliver 10psi.
 - 11.5.4** SMC exhaust is directed to room air.
 - 11.5.5** STC4 is directed to deliver flow to the NO/NO_x Analyzer.
 - 11.5.6** Make certain the mixing bar inside the SMC is rotating.
 - 11.5.7** Allow to equilibrate for 30 minutes.
 - 11.5.8** Read the actual Span voltage over 60 seconds on the digital multimeter.

11.6 Calibrate the NO_x mainstream set-up

11.6.1 Connect the 990ppm NO primary standard to FM3 to deliver 1.8 L/minute to the inlet port of the PEC.

11.6.2 Set the gas regulator to deliver 10 psi.

11.6.3 The outlet line from the PEC chamber is connected directly to the smoking (inlet) port of the single port smoking machine. The exhaust port of the smoking machine is connected directly the inlet port of the SMC.

11.6.4 Sample the contents of the SMC as per the following schedule:

11.6.4.1 Initiate automated smoking machine operation to take one 35 cc, two-second puff every 60 seconds.

11.6.4.2 As the smoking machine piston is drawing the puff, rotate STC3 (SMC exhaust) to room air. STC4 (SMC sample line) is already directed to room air.

11.6.4.3 The 35 cc puff is mixed in the SMC by the stirring bar for five seconds.

11.6.4.4 At t=7 seconds STC4 is rotated to the sample line to the NO/NO_x Analyzer

11.6.4.5 The NO_x Analyzer samples the SMC for 10 seconds.

11.6.4.6 At t=17 seconds both STC3 and STC4 are rotated simultaneously. STC3 is positioned to allow vacuum purging of the SMC until the start of the next puff. STC4 is positioned to direct room air to the NO/NO_x Analyzer inlet line.

11.6.5 This sampling procedure is repeated as many times as necessary to effect the following outcome:

11.6.5.1 First the NO/NO_x span pots are adjusted to give the expected calibration voltage on the digital multimeter and the chart recorder.

11.6.5.2 Use a 5 L smoke mixing chamber when testing Burly type tobacco and a 1L smoke mixing chamber when smoking Flue-cured tobacco.

$$\frac{990 \times 0.035\text{L}}{5\text{L (SMC)}} = 6.93\text{mV}$$

or

$$\frac{170 \times 0.035\text{L}}{1\text{L (SMC)}} = 5.95\text{mV}$$

11.6.5.3 Read baseline from V/F Integrator.

11.6.5.4 Add baseline to expected voltages to give actual target values.

11.6.5.5 Repeat sampling procedure and adjust the threshold pots on the V/F Integrator to give reproducible counts on each channel. Record and average the counts. This figure is inserted into the calculation equation at a latter stage.

11.6.5.6 The NO/NO_x Analyzer is now calibrated for operation.

11.6.5.7 Read the 500 ppm NO balance nitrogen when using the 5 L flask and read the 70 ppm NO balance nitrogen gas mixture when using the 1 L flask for confirmation.

11.6.6 Turn off gases and disconnect line from PEC to smoking machine. Be sure room and smoking machine is evacuated of calibration gas before starting smoking procedure.

12 SMOKING PROCEDURES

12.1 Turn the **Puff Counter** switch to **ON** and switch to automation.

12.2 Weigh each cigarette and insert into the single port smoking machine.

12.2.1 Gently insert the cigarette into the labyrinth seal or dental dam in the cigarette holder to a depth greater than 9 mm. Withdraw the cigarette until the 9 mm mark is just visible.

12.2.2 Light the cigarette and initiate the puff count according to the following schedule. Be sure to light the cigarette on the first puff.

12.2.2.1 Normal lighting procedure is 15 second warm-up beginning at t-18 seconds followed by a five-second ignition. (three seconds prior to puff plus the two-second puff)

12.2.3 Record the puff by puff NO and NO_x counts.

12.3 Record

12.3.1 The barometric pressure and the room temperature. (Do not proceed if room temperature is less than 21°C).

12.3.2 Volume of the SMC and the calibrated puff volumes of the single port smoking machine.

12.3.3 V/F Integrator Span setting at the beginning and end of the experiment.

12.3.4 NO/NO_x span pots settings at the calibration gas settings.

12.3.5 The average channel counts from the calibration step.

12.3.6 Instrument status checks and flow rates.

- 12.4 Position an exhaust canopy above the cigarette to draw off the sidestream smoke.
- 12.5 The unfiltered smoke from each puff is injected directly into the SMC and mixed.
- 12.6 Initiate the sampling procedure as per step 11.6.4 of the calibration procedure. It is very important that this aspect of the procedure be repeated as precisely and consistently as possible to achieve reproducible results.
- 12.7 Record the counts of each channel for each puff on a run sheet and the number of puffs to the end of smoking.
- 12.8 When the cigarette has been smoked to the predetermined butt length. Remove the butt with a pair of tweezers and extinguish in a jar of water. Then take two clearing puffs at the same intervals and record the counts.
- 12.9 The mainstream smoke pattern should be similar to Figure 1.
- 12.10 At the end of smoking, re-test the 990 ppm standard gas as per 11.6 (read only).

13 CALCULATIONS

- 13.1 The total counts for each channel for each test brand are summarized and the mainstream NO and NO_x deliveries in [µg/cigarette] are quantitated.

$$\text{NO } \mu\text{mole/cigarette} = \frac{\text{TC}_{(\text{NO})}}{\text{AC}_{(\text{NO})}} \times \frac{990}{22.4} \times 0.035 \times \frac{273}{273 + ^\circ\text{C}} \times \frac{\text{BP}}{\text{SBP}}$$

$$\text{NO } \mu\text{g/cigarette} = \frac{\mu\text{mole}}{\text{cigarette}} \times \frac{\mu\text{g}}{\mu\text{mole}}$$

and

$$\text{NO}_x \mu\text{mole/cigarette} = \frac{\text{TC}_{(\text{NO}_x)}}{\text{AC}_{(\text{NO}_x)}} \times \frac{990}{22.4} \times 0.035 \times \frac{273}{273 + ^\circ\text{C}} \times \frac{\text{BP}}{\text{SBP}}$$

$$\text{NO}_x \mu\text{g/cigarette} = \frac{\mu\text{mole}}{\text{cigarette}} \times \frac{\mu\text{g}}{\mu\text{mole}}$$

where:

- TC_(NO) is the total puff by puff counts while smoking (including two clearing puffs at the end) of the NO channel.
- TC_(NO_x) is the total puff by puff counts while smoking (including two clearing puffs at the end) of the NO_x channel.
- 990 is the Calibration gas 990 ppm (µL/L). To be used with the 5 L flask.
- 260 is the Calibration gas 260 ppm (µL/L). To be used with the 1 L flask.
- AC_(NO) is the average counts per puff per NO channel of the calibration gas.
- AC_(NO_x) is the average counts per puff per NO_x channel of the calibration gas.
- °C is the room temperature.
- 22.4 µL/µmole.
- BP is the barometric pressure (inches Hg).

- SBP is the barometric pressure at standard temperature and pressure (29.92 inches Hg).

14 QUALITY CONTROL

14.1 Typical Graph

14.1.1 see Figure No.1

14.2 Recoveries and Levels of Contamination

Each analytical run of test cigarettes should also include:

14.2.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, reagents, and analyzer effects (see section 7.2). In this case it would be the Zero gas.

14.2.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss. The 9ppm NO standard gas is the LFB.

14.2.3 Test a reference sample such as 1R4F to determine the inter-experimental reproducibility of the entire method of analysis.

14.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

14.3.1 Method Detection Limit (MDL)

14.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

14.3.2 Limit of Quantitation (LOQ)

14.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

14.4 Stability of Reagents and Supplies

14.4.1 All primary standard gas mixtures are maintained at room temperature (22 °C).

14.4.2 All samples are analyzed on a puff by puff basis.

15 MODIFICATIONS FOR INTENSE SMOKING

15.1 No modifications are necessary under intense smoking conditions.

16 REFERENCES

-
- 16.1** Rickert, W.S., Robinson, J.C., and Collishaw, N.E. Decay of Cigarette Smoke NO_x in Ambient Air Under Controlled Conditions, *Environmental International*, 13, 1987, p. 399-407.
- 16.2** Norman, V., Ibrig, A.M., Larson, T.M., and Moss, B.L. The Effect of Some Nitrogenous Blend Components on NO/NO_x and HCN Levels in Mainstream and Sidestream Smoke, *Beitrige zur Tabakforschung International*, 12, No. 2, 1983, p. 55-62.
- 16.3** Williams, T.B. The Determination of Nitric Oxide in Gas Phase Cigarette Smoke by Non-Dispersive Infrared Analysis, *Beitrige zur Tabakforschung International*, 10, No. 2, 1980, p. 91-99.

APPENDICES

Figure 1. Graph of Vapour Phase NO and NOx in Mainstream Tobacco Smoke



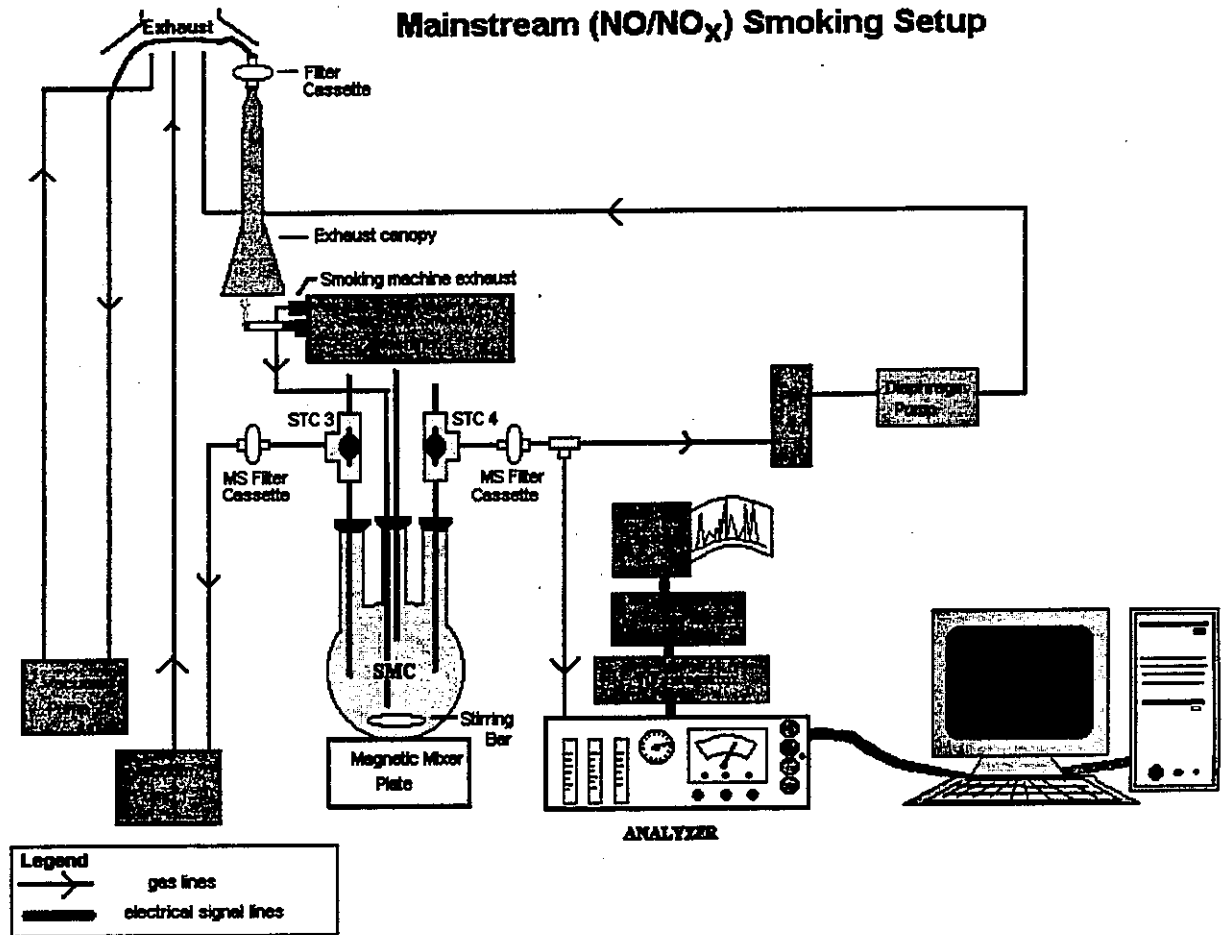
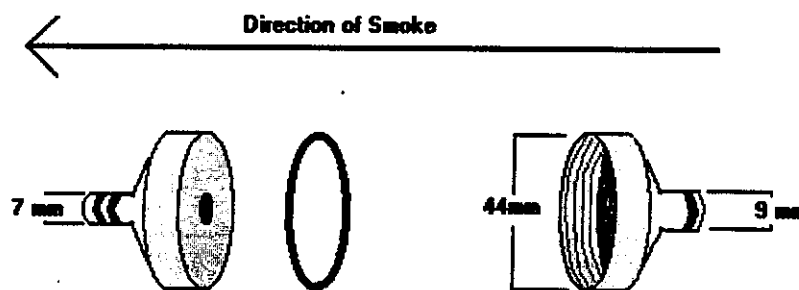
Diagram 1: Set-up of mainstream NO_x analysis

Diagram 2: Glass fibre filter disc holder: Threaded screw cartridge

Pad Holder Setup (Measurements are inner diameters only)

No.: T - 111
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the determination of tobacco specific nitrosamines (TSNA) in mainstream tobacco smoke.
- 1.2 The volatile nitrosamines (VNA) are formed during the processing of tobacco and during the smoking of tobacco products. The tobacco processing methods include air-, sun-, flue- and fire-curing, aging and fermentation.
- 1.3 The generation of mainstream smoke is achieved under standard machine smoking conditions for cigarettes as specified in T-115.
- 1.4 This method is suitable for the quantitative determination of four tobacco specific N-nitrosamines in mainstream (MS) tobacco smoke only: N-nitrosanornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Mainstream smoke of 10 cigarettes* is trapped onto a 92 mm glass fibre filter disc (pad). The TSNA are concentrated by extraction with dichloromethane, followed by column chromatography onto basic Alumina. The fraction containing TSNA is eluted, then quantitatively analysed by combined gas chromatography-thermal energy analysis (GC-TEA). N-nitrosoguvacoline (NG) is used as an internal standard.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

All four TSNA are carcinogenic in several species of laboratory animals. Extreme care should be taken in handling these compounds. The exhaust of the TEA detector should be vented properly in order to reduce exposure to possible excess ozone (O₃).

5 APPARATUS

- 5.1 Equipment needed to perform smoking of tobacco product as specified in T-115.
- 5.2 Equipment needed to perform conditioning as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 250 mL Round-bottom flask with ground glass joint.
- 5.5 Volumetric Flasks - 2, 10, 50, 100 mL.
- 5.6 Amber flask or storage bottle.
- 5.7 Aluminum foil.
- 5.8 Silanized glass wool.
- 5.9 Burrell wrist action shaker or equivalent.
- 5.10 LC Column with Frit and Stopcock, 300 mm X 22 mm ID X 25 mm OD (Supelco 64754) or equivalent.
- 5.11 Short Stem Glass Funnels.
- 5.12 Glass Pasteur Pipettes.
- 5.13 Zymark TurboVap II Concentrator equipped with 200 mL Tubes with Graduated 1 mL stem or equivalent.
- 5.14 Thermal Energy Analyzer (Thermo-Electron Corp.) interfaced to GC or equivalent.
- 5.15 Gas chromatograph (GC), equipped with temperature programmable injector and electronic flow control.
- 5.16 GC column - 30 m X 0.32 mm X 3.0 µm DB-1 fused silica capillary column.
- 5.17 Non-ultra violet (UV) lighting.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Dichloromethane (DCM) - Distilled in Glass (DIG).
- 6.2 Acetone - DIG.
- 6.3 Methanol - DIG.
- 6.4 Basic alumina.
- 6.5 Aluminum foil.
- 6.6 Glass fibre filter pads - 92 mm with holders.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

8.1 1:1 Acetone:Dichloromethane solution

- 8.1.1 Mix Acetone and Dichloromethane together in a 1:1 v/v basis.

9 PREPARATION OF STANDARDS

9.1 N-nitrosoguvacoline (NG) internal standard (ISTD)

9.1.1 Prepare a solution at 5000 ng/mL in dichloromethane.

9.2 TSNA mixed standard solution

9.2.1 Prepare a mixed standard dilution stock solution of NNN, NAT, NAB and NNK in dichloromethane at the following range of concentrations:

9.2.1.1 NNK at 3000 ng/mL.

9.2.1.2 NNN and NAT at 1500 ng/mL.

9.2.1.3 NAB at 500 ng/mL.

9.2.2 Store this solution in an amber flask and protect from light.

Note: Concentrated solutions are stable for at least six months if stored at – 20 °C in such a manner as to prevent loss of solvent from evaporation.

9.2.3 Build a calibration curve ranging from 20 ng/mL (for NAB) to 2000 ng/mL (for NNK) containing NG as an internal standard at 500 ng/mL in each of the standards.

Note: Individual calibration stocks are stable for two months if stored at – 20 °C in such a manner as to prevent loss of solvent from evaporation.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product is to be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.1.2 Non-UV lighting shall be used in the rooms in which sample generation and sample analyses are conducted.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 for a rotary machine.

13 SAMPLE GENERATION

13.1 Samples shall be smoked and TPM collected as specified in T-115 with the following modifications:

13.1.1 Place 10 cigarettes (or little cigars), one in every other position, in the smoking machine and smoke the samples.

13.1.2 After smoking 10 cigarettes (or little cigars), perform three clearing puffs and remove the pad holder from smoking machine.

14 SAMPLE ANALYSIS**14.1 Extraction of Filter Pads**

14.1.1 Remove the pads from the holders and place each into a 250 mL round bottom flask wrapped with Aluminum foil.

14.1.2 Add 200 µL of NG internal standard solution onto each pad (an amount equivalent to the concentration of NG in the standards on a ng/mL basis).

14.1.3 Add 100 mL dichloromethane to each flask.

14.1.4 Extract on a wrist action shaker for 30 minutes.

14.2 Sample Concentration

14.2.1 Filter out the pad material from the extract using a plug of silanized glass wool placed in a short stem funnel directly into a 200 mL Zymark tube.

14.2.2 Rinse the flask with 20 mL DCM. After the initial extract has dripped through the funnel, add the rinse to the funnel.

14.2.3 Repeat Step 14.2.2.

14.2.4 After all the solution has stopped dripping through the funnel, place the sample into the TurboVap II Concentrator set at 38°C and 10psi nitrogen.

14.2.5 Concentrate samples to approximately 5 mL.

14.3 Column Chromatography Clean-up Procedure

14.3.1 Prepare a basic alumina column by adding 50 mL of DCM to an empty, dry, glass LC column.

14.3.2 Add 10 g (+/- 0.2 g) of oven dried (110°C) basic alumina to the liquid in the column. Stir the alumina slurry with a glass rod to remove any possible air pockets.

14.3.3 Drain the liquid from the column to waste, closing the stopcock when the solution is at the level of the alumina.

- 14.3.4 Wash the alumina by adding 50 mL of DCM to the column. Discard the liquid and close the stopcock when the solution is at the level of the alumina.
- 14.3.5 Add the 5 mL sample from the TurboVap tube to the alumina column using a glass Pasteur pipette attempting not to disturb the alumina packing.
- 14.3.6 Discard the liquid from the column, close the stopcock when the solution is at the level of the alumina.
- 14.3.7 Rinse the TurboVap tube with 10 mL DCM, washing the lower portion (25 %) of the tube with repeated flushing using a Pasteur pipette.
- 14.3.8 Add the 10 mL rinse from the TurboVap tube to the alumina column using a glass Pasteur pipette attempting not to disturb the alumina packing.
- 14.3.9 Discard the liquid from the column, close the stopcock when the solution is at the level of the alumina.
- 14.3.10 Rinse the TurboVap tube and alumina column with an additional 40 mL DCM attempting not to disturb the alumina packing, draining the liquid from the column to waste.
- 14.3.11 Place a clean 200 mL TurboVap tube beneath the LC Column to collect the sample (eluate).
- 14.3.12 Elute the TSNA from the Alumina column by adding 50 mL of 1:1 Acetone:DCM to the column attempting not to disturb the Alumina packing.
- 14.3.13 Collect the liquid from the column into the TurboVap tube, closing the stopcock when the solution is at the level of the Alumina.
- 14.3.14 Repeat steps 14.3.12 and 14.3.13, four more times, collecting the eluate in the same TurboVap tube (a total of 250 mL collected).

Note: This will require evaporating a portion of the eluate on the TurboVap before the final 50 mL can be collected into the tube.

14.4 Sample Re-Concentration

- 14.4.1 Place the samples into the TurboVap II Concentrator set at 38 °C and 9 psi nitrogen.
- 14.4.2 After the samples have been concentrated to approximately 150 mL, increase the pressure to 10 psi.
- 14.4.3 Concentrate samples to 0.8 mL or until the sensor turns the concentration off (approximately 45 minutes).

14.4.4 Transfer the concentrate and rinse to a 2 mL volumetric flask and make to volume with 1:1 Acetone:DCM, using the solvent to perform a rinse of the TurboVap tube.

14.4.5 Transfer the contents of the flask to an amber autosampler vial with Teflon-lined septa for GC analysis.

14.5 GC-TEA Operating Conditions

14.5.1 Carrier flow rate (He): 2.8 mL/minute using electronic flow control (velocity = 60 cm/second).

14.5.2 Injector temperature: programmable 35 to 220 °C.

14.5.3 Oven temperature: programmed 50 to 170 to 212 °C.

14.5.4 TEA interface temperature: 240 °C.

14.5.5 TEA furnace temperature: 500-525 °C (dependent on analyzer sensitivity).

14.5.6 Analysis Run Time: 35 minutes.

14.6 Blank test

14.6.1 Blank tests using purified nitrosamine-free air should be performed periodically in order to ensure the absence of nitrosamine traces in the analytical environment, or their formation during analysis.

14.7 GC-TEA calibration

14.7.1 Inject 1.5 µL of the TSNA mixed standard solution and determine peak areas for the four components.

14.8 TSNA determination

14.8.1 Inject 1.5 µL of the sample concentrate (12.4.5) and determine areas of the peaks having retention times corresponding to NNN, NAT, NAB and NNK.

Note: See Appendix 1 for representative chromatograms.

14.9 Calculations

14.9.1 The content, m_{cg} (ng/cigarette), of a given TSNA is obtained from:

$$m_{cg} = CV_s/N.$$

where

C = analytical Concentration determined by ISTD calibration of given TSNA.

V_s = final volume of concentrate.

N = number of cigarettes/cigarette equivalents/little cigars/cigars/kreteks/bidis smoked.

15 QUALITY CONTROL

15.1 Typical Chromatograms

15.1.1 See Appendix 1.

15.2 Method Detection Limits (MDL) / Limit Of Quantitation (LOQ)

15.2.1 The method detection limit (MDL) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

15.2.2 The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume of the extract and dividing this by the number of cigarettes smoked.

15.2.3 The MDL (on a ng/cigarette basis) can be enhanced by modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure.

15.2.4 The practical limit of quantitation (LOQ) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

15.2.5 The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume of the extract and dividing this by the number of cigarettes smoked.

15.2.6 The effect of modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

16 MODIFICATIONS FOR INTENSIVE SMOKING

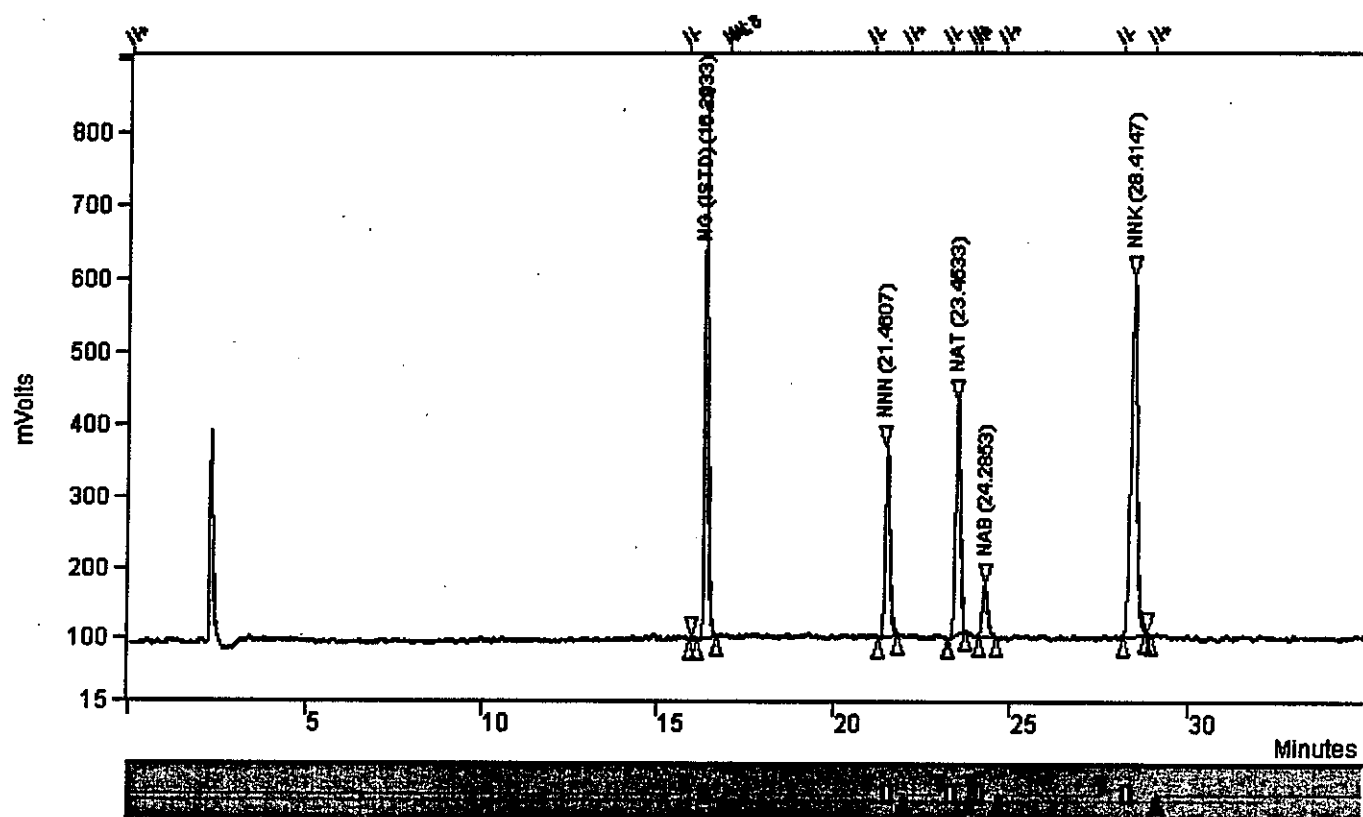
16.1 Under intense smoking conditions, the number of cigarettes and/or the final solution volumes may be modified to maintain the same calibration range as with standard smoking conditions.

17 REFERENCES

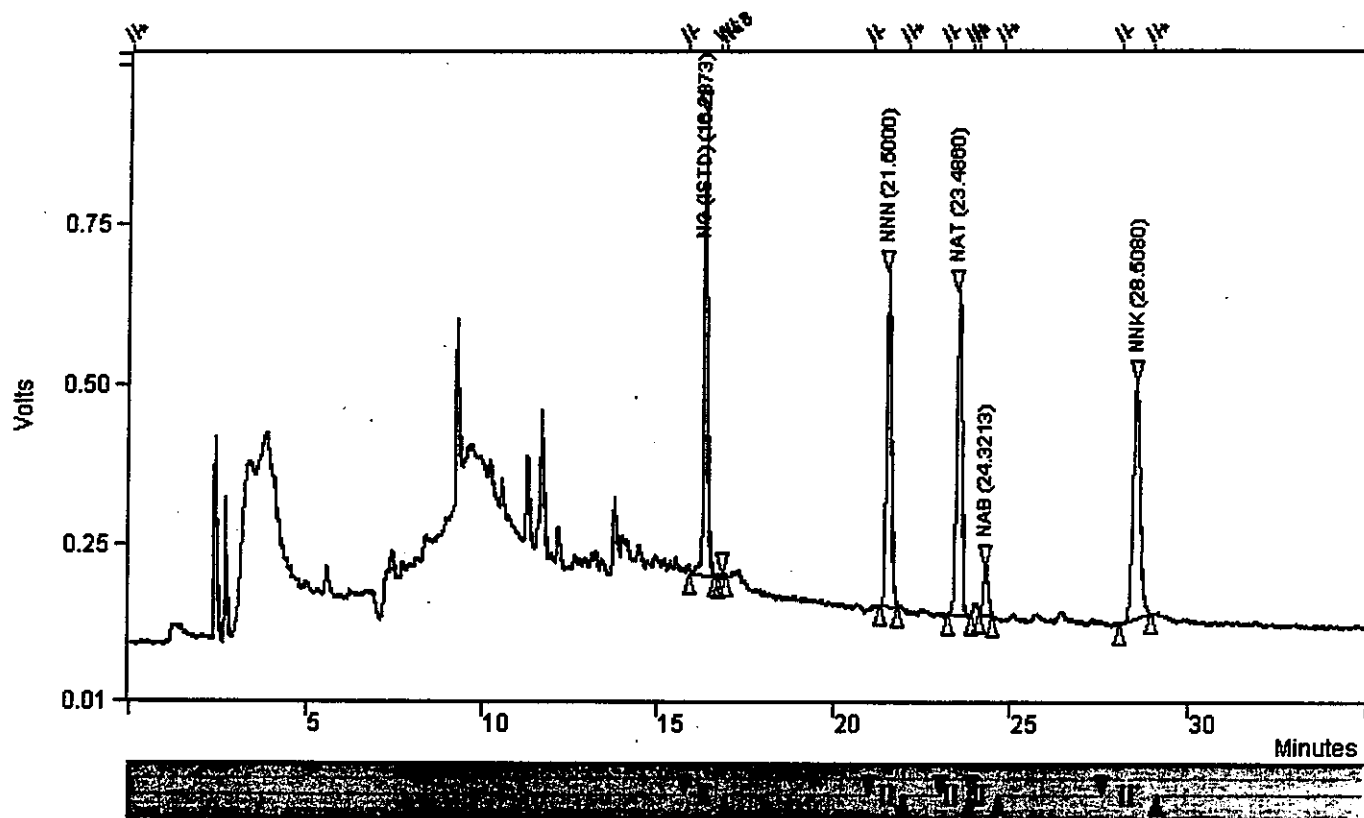
- 17.1 Fisher, S., and Spiegelhalter, B. Improved Method for the Determination of Tobacco Specific Nitrosamines (TSNA) in Tobacco Smoke, *Beitrag zur Tabakforschung International*, 14, 1989, p. 145-153.
- 17.2 Fisher, S., Castonguay, A., Kaiserman, M., Spiegelhaider, B., and Preussmann, R. Tobacco-specific nitrosamines in Canadian cigarettes, *J. Cancer Res. Clin Oncol.*, 116, 1990, p. 563-568.
- 17.3 Adams, J.D., Brunnemann, K.D. & Hoffmann, D. Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific N-nitrosamines by gas-liquid chromatography with a thermal energy analyser, *J. Chromatogr.*, 256, 1983, p. 347-351.

APPENDICES

Appendix 1: Typical Chromatograms



A representative chromatogram of Std 4.



A representative chromatogram of 1R4F mainstream smoke.

No.: T - 112

Date: December 31, 1999

Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of the pyridine, quinoline and styrene content of mainstream tobacco smoke by gas chromatograph/mass spectrometer (GC/MS).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Method Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Pyridine, styrene and quinoline are collected by passing the mainstream smoke of 20 cigarettes* through a 92 mm glass fiber filter disc (pad) and into cryogenic traps containing methanol. The pad is placed into an Erlenmeyer flask and the internal standard is added. The pad is extracted with the impinger solutions and the extracts are injected onto a GC/MS for quantitation.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking analyses as specified in T-115.
- 5.2 Equipment needed to perform conditioning of tobacco product as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 Analytical balance capable of measuring to at least four decimal places.
- 5.5 70 mL glass impingers with extra-coarse frits.
- 5.6 Tygon tubing with connectors.
- 5.7 Dewar flasks.
- 5.8 Thermometer (-100 to 40 °C).
- 5.9 125 mL polymethylpentene (PMP) Erlenmeyer flasks with screw-caps or equivalent.
- 5.10 20 mL graduated cylinder.
- 5.11 Wrist-action shaker.

- 5.12 10, 25, 50 and 100 mL volumetric flasks.
- 5.13 Volumetric pipettes or gas-tight syringes for range 100 to 1000 μ L.
- 5.14 Autosampler vials with caps and Teflon-lined septa.
- 5.15 Varian Saturn I GC/MS system consisting of an 8100 autosampler, a 3400 GC with a 1077 split/splitless injector and an ion trap detector (ITD) (or equivalent).
- 5.16 Supelcowax 30 m X 0.25 mm X 0.25 μ m column (or equivalent) with 1 m X 0.25 mm deactivated fused silica transfer line.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Dry ice.
- 6.2 Isopropanol (IPA).
- 6.3 Methanol - Distilled-in-Glass.
- 6.4 D₅ - Pyridine - purity equal to or greater than 98 %.
- 6.5 D₇ - Quinoline - purity equal to or greater than 98 %.
- 6.6 Pyridine.
- 6.7 Quinoline.
- 6.8 Styrene.
- 6.9 Disposable 5 cc syringe.
- 6.10 Syringe filters - 0.45 μ m PTFE 25 mm (or equivalent).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Not applicable.

9 PREPARATION OF STANDARDS

- 9.1 A primary stock solution of pyridine is prepared by accurately weighing approximately 100 mg of pyridine into a 10 mL volumetric flask. The flask is filled to the mark with methanol and mixed well. [Concentration: approximately 10 mg/mL].
- 9.2 A primary stock solution of quinoline is prepared by accurately weighing approximately 100 mg of quinoline into a 100 mL volumetric flask. The flask is filled to the mark with methanol and mixed well. [Concentration: approximately 1 mg/mL].
- 9.3 A primary stock solution of styrene is prepared by accurately weighing approximately 100 mg of styrene into a 10 mL volumetric flask. The flask is filled to the mark with methanol and mixed well. [Concentration: approximately 10 mg/mL].
- 9.4 A mixed secondary stock solution is prepared by transferring 100 μ L of each stock solution into a 50 mL volumetric flask, making to the mark with methanol and mixing well. [Concentration: approximately 20, 2 and 20 μ g/mL, respectively].

- 9.5** An internal standard stock solution of D₅-pyridine is prepared by accurately weighing 100 mg of D₅-pyridine into a 10 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 9.6** An internal standard stock solution of D₇-quinoline is prepared by accurately weighing 25 mg of D₇-quinoline into a 25 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 9.7** An internal standard (ISTD) spiking solution is prepared by diluting 2 mL of each of the ISTD stock solutions to 100 mL with methanol and mixing well. Aliquots of this spiking solution are stored in 25 mL vials with Teflon-lined caps and at minus 20 °C. [Concentration: approximately 200 and 20 µg/mL, respectively].
- 9.8** Five calibration standard solutions are prepared by adding 100 µL ISTD spiking solution to each of five 10 mL volumetric flasks. The sides are rinsed with methanol, then appropriate aliquots (e.g. 2, 1, 0.5, 0.25 and 0.1 mL) of the secondary stock solution are added to each flask. The flasks are filled to the mark with methanol and mixed well.
- 9.9** The solutions are transferred to a series of labeled autosampler vials, capped with Teflon-lined septa and stored at minus 20 °C until use.

Note: Each vial is only used once.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Product shall be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

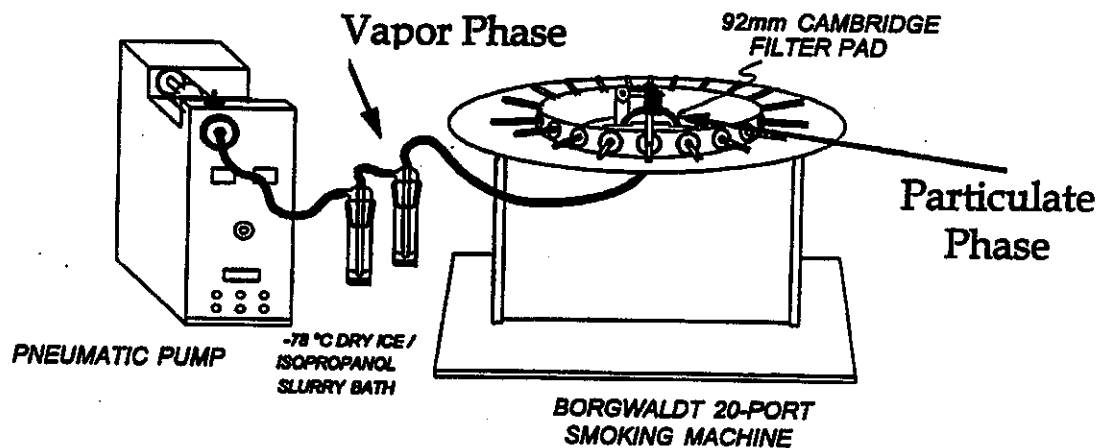
12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications for a rotary machine as pictured in the following diagram:



12.2.2 Prepare the impingers by adding 20 mL of methanol into each impinger.

12.2.3 Immerse the impingers into a dry-ice/IPA bath (temperature at or below -70 °C).

12.2.4 Insert a pad holder with pad into the syringe of the smoking machine, and then hook up in series two impingers to the pad holder. Attach the first impinger to the large 92 mm filter holder.

13 SAMPLE GENERATION

13.1 TPM is collected as described in T-115.

14 SAMPLE ANALYSIS

14.1 Extration of filter pads

14.1.1 Cut the 92 mm pad in quarters and place into a clean 125 mL PMP Erlenmeyer flask. Spike the pad with 400 µL of the ISTD solution.

14.1.2 Transfer the contents of the first impinger into the flask. Rinse the first impinger with the contents of the second impinger and transfer the rinse into the flask.

14.1.3 Close the flask and shake on the wrist-action shaker for 30 minutes.

14.1.4 Pour 4 mL of the solution into a 5 mL syringe fitted with a syringe filter.

14.1.5 Fill two labeled autosampler vials to the base of the neck and cap with an autosampler cap and Teflon-lined septum.

14.1.6 Store samples at -20°C for up to 48 hours prior to analysis.

14.2 Instrument Analysis - GC/MS Conditions

14.2.1 Injector temperature: 250°C .

14.2.2 Column temperature: 70°C for two minutes.

3°C per minute to 150°C .

20°C per minute to 250°C , hold three minutes.

14.2.3 Column pressure: 12 psi or constant flow of 1.0 mL/minute.

14.2.4 Transfer line temperature: 240°C .

14.2.5 Manifold temperature: 240°C .

14.2.6 1 μL of the methanol solution is injected at 5 μL per second onto the GC/MS, which is run in the splitless mode. (Split flow 20 mL/minute).

14.2.7 The GC/MS is operated in full-scan mode (50 to 200 amu). The following ion peak areas are used for quantitation:

D ₅ -pyridine	84
D ₇ -quinoline	136
Pyridine	79
Quinoline	129
Styrene	104.

Note: The assignment of these masses is based on selection of the best response (i.e. the base peak) and the need to avoid possible contamination from interfering peaks which may contain similar ions. The choice of quantitation ions may be different for different instrument configurations.

Note: Quantitation may be based on peak heights if interfering peaks cannot be completely resolved (see Appendices 1b and 1c).

14.3 Calibration Curve

14.3.1 A calibration curve is generated at the beginning of each sample set or "project". Each standard solution is injected once and a calibration file is built using the method for internal standard quantitation available with the Saturn quantitation software.

14.3.2 A "check standard" is analyzed every 20 samples and at least once per run. This standard is treated as a sample and the observed value is compared to the expected value for that standard.

14.3.2.1 If the results are within 10 % of expected, the calibration is still valid.

14.3.2.2 If the results differ by more than 10 % of expected, the calibration is no longer valid and a new calibration curve must be generated.

14.4 Sample Calculation

- 14.4.1 The software on the GC/MS is used to generate results for each analyte based on the concentrations of the standard solutions. The results are reported in µg/mL. To calculate the final results, the following calculation is used:

$$\text{Analyte } (\mu\text{g/cigarette}) = \frac{\text{Conc. of Analyte in Sample } (\mu\text{g/mL}) \times \text{Volume (mL)}}{\text{\# of cigarettes}}$$

15 QUALITY CONTROL

15.1 Typical Chromatograms

- 15.1.1 See Appendices.

15.2 Recoveries and Levels of Contamination

- 15.2.1 This involves the use of laboratory reagent blanks (LRB) to evaluate potential interference of the reagents. One LRB should be analysed every 20 samples. LRB preparation: A 92 mm pad is quartered and placed in a PMP Erlenmeyer with 400 µL of the ISTD solution and 40 mL of methanol. The LRB is then treated as a sample through the rest of the procedure. Typical results are non-detected (ND) for all analytes.

Note: In lieu of an LRB, a smoking blank can be used to monitor contamination of reagents and the air in the smoking room. This involves conducting a smoking run with the same number of puffs as a control cigarette but with no cigarette in place. Typical result for a smoking room blank is between 0.06 and 0.12 µg/mL (equivalent to 0.12 and 0.24 µg/cigarette) for pyridine and styrene and ND for quinoline.

- 15.2.2 A laboratory fortified blank (LFB) may be analyzed to evaluate the extent of potential analyte loss. A 92 mm pad is quartered and placed in a flask with 400 µL of the ISTD solution, an appropriate aliquot of the secondary mixed stock solution and 40 mL of methanol. The LFB is then treated as a sample through the rest of the procedure. The recoveries should be close to 100 %.

- 15.2.3 A laboratory fortified matrix (LFM) may be analysed to assess potential matrix interference. LFM preparation: A sample of a control brand is smoked and the pad transferred to the flask. The pad is spiked with the ISTD solution and an aliquot of the mixed secondary stock solution. The impinger solutions are added to the pad and the sample is taken through the remainder of the procedure. The recoveries should be close to 100 %.

15.3 Method Detection Limit (MDL)/Limit of Quantitation (LOQ)

The MDL can be defined as the level which gives a signal to noise ratio of three to one. The LOQ can be defined as the level which gives a signal to noise ratio of 10 to one. MDLs should be determined for each system and may differ from instrument to instrument.

Note: Because this method involves the analysis of the methanol soluble components of whole tobacco smoke, with no sample clean-up, the chromatography must be very carefully monitored so that the peaks are sharp and the analytes of interest are well resolved from other components.

15.4 Stability of Reagents and Samples

15.4.1 Stock solutions are stable for at least six months if stored at -20°C .

15.4.2 Calibration standards are stable for at least one week if stored at -20°C .

15.4.3 Once punctured, the more volatile pyridine may be lost so vials are typically used once and discarded.

15.4.4 Samples are stable in the freezer for at least one week if the septum has not been punctured. It is essential that at least two vials be prepared for each sample as the vial is discarded once punctured.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 Under intense smoking conditions, 10 cigarettes are smoked instead of 20.

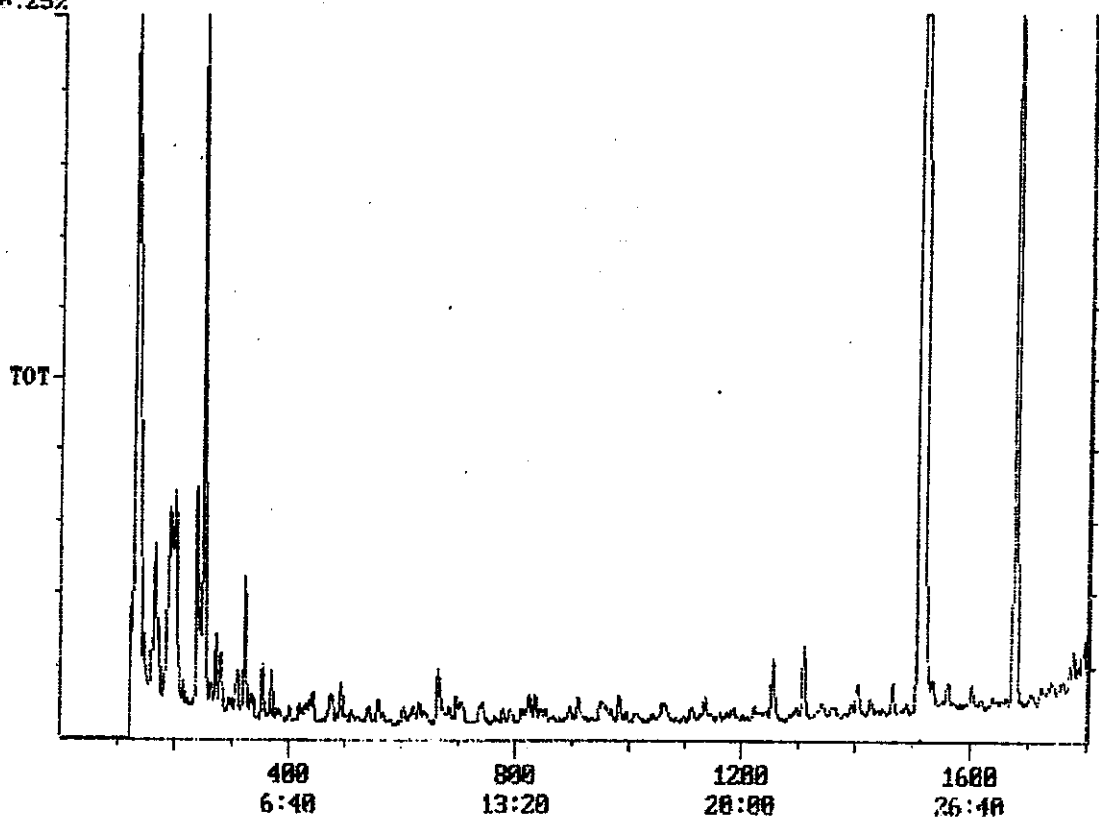
17 REFERENCES

- 17.1** White, E., Uhrig, M., Johnson, T., Gordon, B., Hicks, R., Borgerding, M., Coleman, W., and Elder, J. Quantitative Determination of Selected Compounds in a Kentucky 1R4F Reference Cigarette Smoke by Multidimensional Gas Chromatography and Selected Ion Monitoring - Mass Spectrometry, *Journal of Chromatographic Science*, 26, 1990, p. 393-399.

APPENDICES

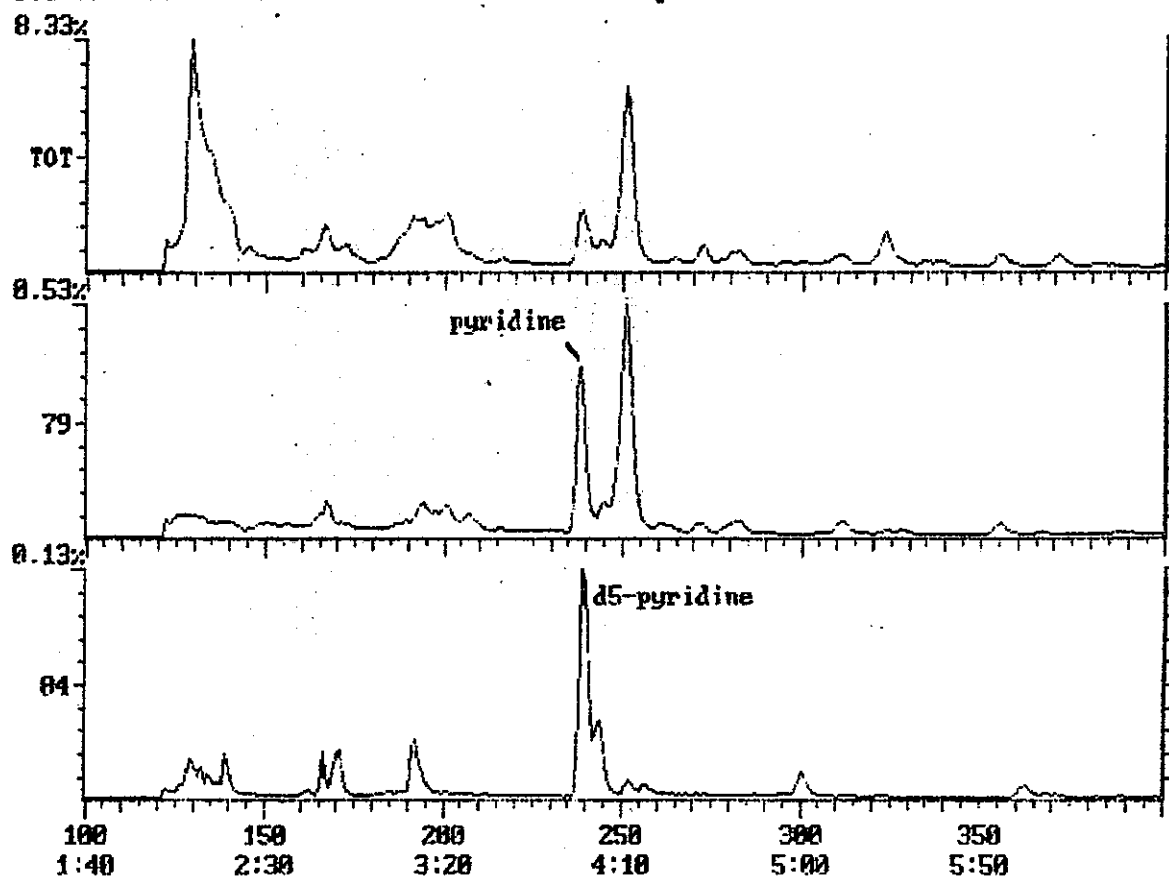
Appendix 1a: Typical Chromatogram

Chromatogram Plot C:\SATURN\DATA\1-18-587 Date: 03/20/98 19:37:43
Comment: SUPELCOWAX 30X0.25X0.25 12PSI 70CSTART INJ250C FASTINJ(5)
Scan No: 1800 Retention Time: 30:00 RIC: 84272 Mass Range: 50 - 200
Plotted: 1 to 1800 Range: 1 to 1800 100% = 13572090
6.25%



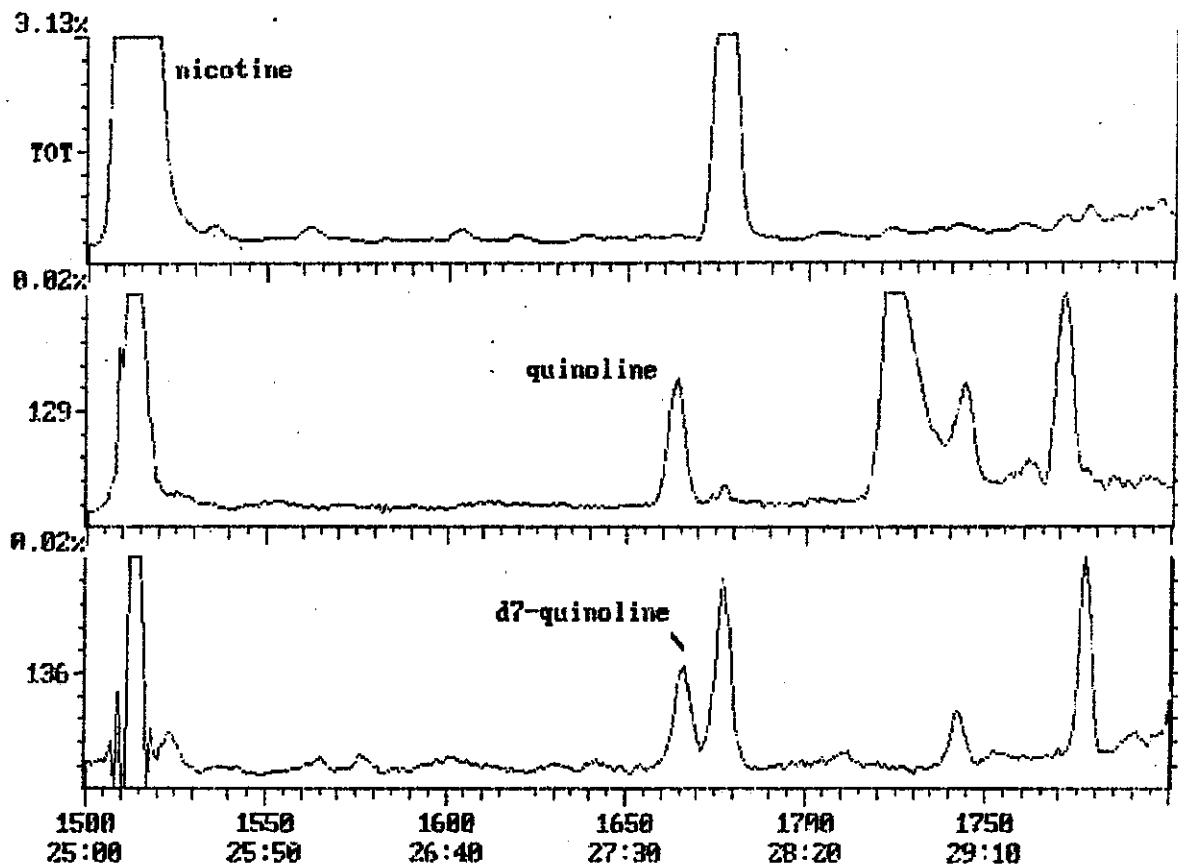
Appendix 1b: Typical Chromatogram - Pyridine

Chromatogram Plot C:\SATURN\DATA\1-10-507 Date: 03/20/98 19:37:43
Comment: SUPELCOWAX 30X0.25X0.25 12PSI 70CSTART INJ250C FASTINJ(5)
Scan No: 400 Retention Time: 6:40 RIC: 30251 Mass Range: 50 - 200
Plotted: 100 to 400 Range: 1 to 1800 100% = 13572090



Appendix 1c: Typical Chromatogram - Quinoline

Chromatogram Plot C:\SATURN\DATA\1-10-507 Date: 03/20/98 19:37:43
Comment: SUPELCOWAX 30X0.25X0.25 12PSI 70CSTART INJ250C FASTINJ(5)
Scan No: 1800 Retention Time: 30:00 RIC: 84272 Mass Range: 50 - 200
Plotted: 1500 to 1800 Range: 1 to 1000 100% = 13572098



See following page for Appendix 1D: Typical Chromatogram - Styrene

Print Date: 15 Nov 1999 12:07:34

Chromatogram Plots

File: c:\saturn\waldata\990925pq\ap1.mss b2 3-10-507.sms

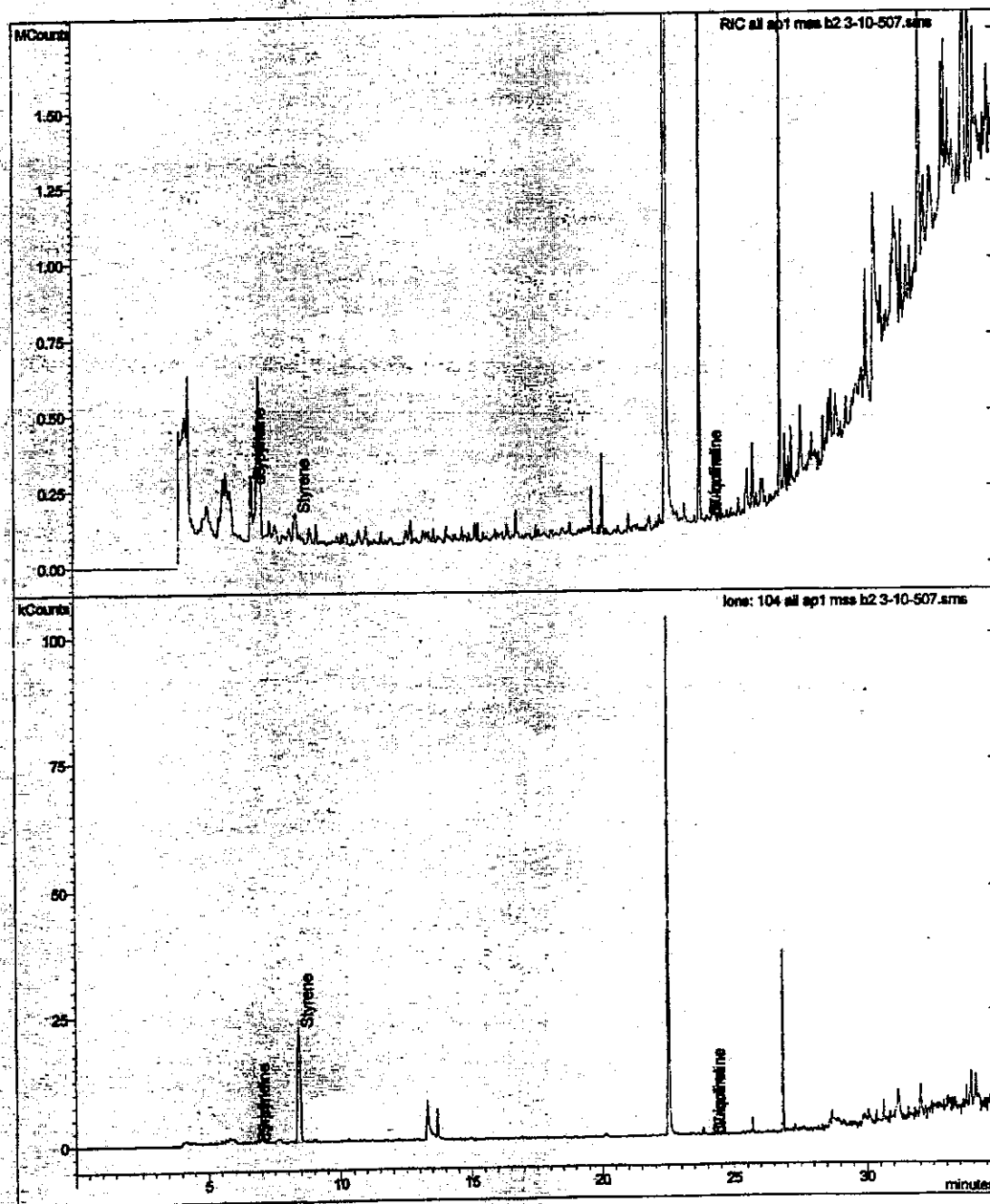
Sample: AP1 MSS B2 3-10-507

9/25/99 10:52 PM

Sample Notes:

Operator:

Scan Range: 1 - 2099 Time Range: 0.00 - 34.97 min.



No.: T - 113
Date: December 31, 1999
Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1** This method is to be used to determine the pH of mainstream tobacco smoke on a puff-by-puff basis in order to observe the acid-base effects of mainstream smoke on an aqueous film during the smoking process. This method is applicable to cigarettes, cigarette equivalents, kreteks, bidis and cigars.

2 NORMATIVE REFERENCES

- 2.1** American Society for Testing and Materials (ASTM) D1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine, and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

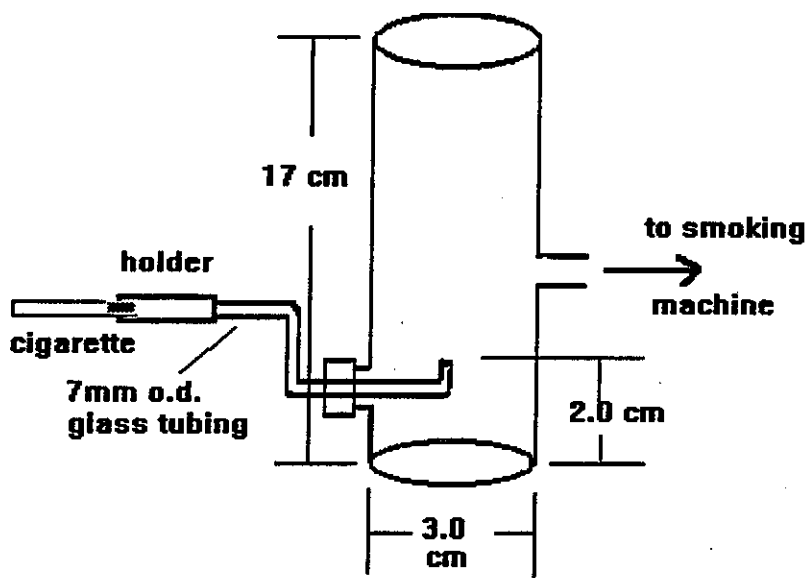
- 4.1** This method uses a modified combination electrode, placed between the tobacco product and filter of the smoking machine, to measure the pH of mainstream tobacco smoke on a puff-by-puff basis. Cigarettes are first conditioned as per T-115 and smoked using a Borgwaldt single port smoking machine. By using this modified electrode, the acid-base effect of both the condensate and gas phase is monitored and information on the puff-by-puff experiences of a smoker may be obtained.
- 4.2** This method utilizes a chart recorder to accurately record the changes in pH during the smoking process.
- 4.3** An alternative to using a chart recorder is to directly transfer pH data to a spreadsheet at a fixed print interval. All data should be plotted on a chart to ensure proper inflections during the smoking process are observed.
- 4.4** An average pH of the smoke is determined by averaging the median pH of the individual puffs and/or averaging the total number of data points transferred, if data can be directly transferred to a spreadsheet program at a defined print interval.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform conditioning as specified in T-115.
- 5.4 Accumet pH Meter 915 (Fisher Scientific) or equivalent, with RS232 and/or analog signal output.
- 5.5 Auto-Temp compensator (Fisher Scientific #13-620-16) or equivalent.
- 5.6 Linear chart recorder or Labtronics software for direct data transfer.
- 5.7 pH Combination Electrode with Ag/AgCl Internal Reference, Ceramic Junction.
- 5.8 Linen thread, size 16/2.
- 5.9 Whatman filter paper.
- 5.10 One inch diameter plastic oil funnel (to remove sidestream smoke).
- 5.11 Vacuum Pump.
- 5.12 Rubber sleeve material (normally used to attach cigarettes to the holders).
- 5.13 Glass Smoke Chamber as described by A.J. Sensabough (see Figure 1).
- 5.14 Anti-static cloth.
- 5.15 Glass cigarette holder.
- 5.16 Rotameter (to adjust sidestream flow rate).

Figure 1: Smoke Chamber for the Determination of Mainstream Smoke pH



6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 pH Electrode Storage Solution.

- 6.2 4M KCl saturated with silver Electrode Filling Solution.
- 6.3 pH=5 Buffer, potassium hydrogen phthalate.
- 6.4 pH=6 Buffer, potassium hydrogen phthalate.
- 6.5 pH=7 Buffer, potassium and sodium phosphate.
- 6.6 Type I water as per ASTM D1193.
- 6.7 Methanol.
- 6.8 Decon 75 (Decon) or similar glassware cleaning agent.

7 PREPARATION OF GLASSWARE

- 7.1 Not applicable.

8 PREPARATION OF SOLUTIONS

- 8.1 No solutions are required to be prepared since all buffers are commercially available.

9 PREPARATION OF STANDARDS

- 9.1 Not applicable.

10 SAMPLING

- 10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1 Product is to be conditioned as specified in T-115.
- 11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.

12 SMOKING MACHINE, ELECTRODE AND pH METER PREPARATION

12.1 Ambient Conditions

- 12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

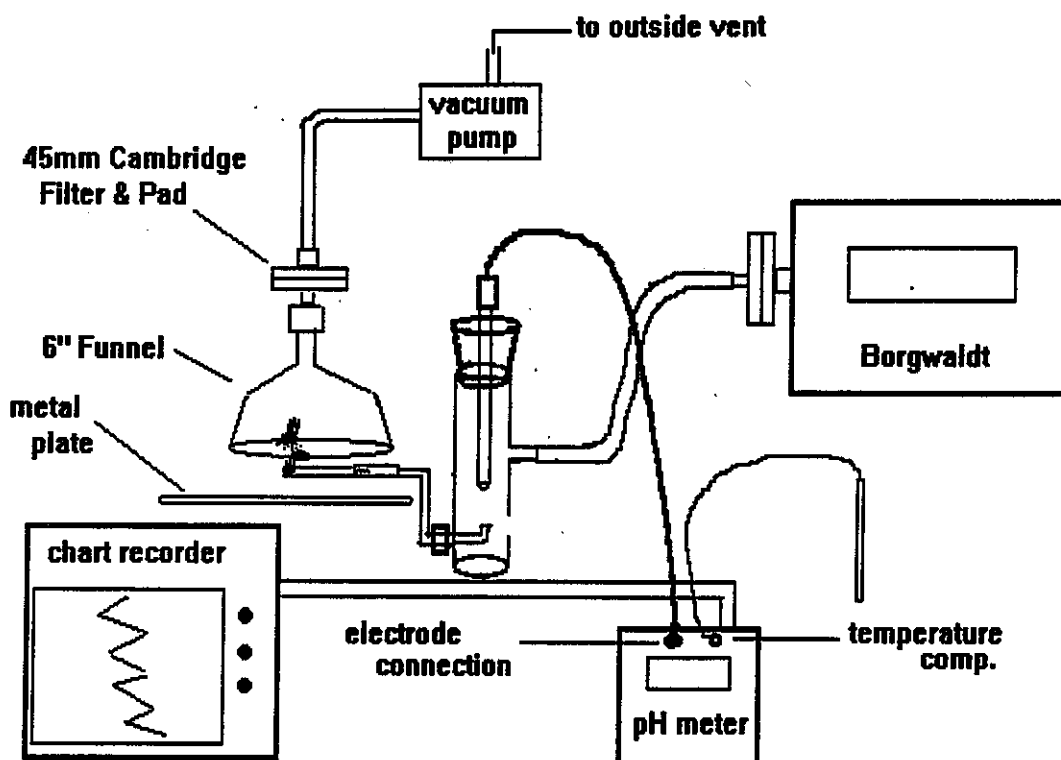
- 12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.2 Creation of Smoke Train (Preparation of Smoking Apparatus)

- 12.2.2.1 Assemble the smoke train as described in the A.J. Sensabough procedure. The resulting sidestream smoke (not analyzed) is drawn away from the analyst by placing a funnel over the cigarette and drawing the smoke away by means of a vacuum pump at a flow rate of 7.5 L per minute (or smoking under some other sort of ventilation). The positioning of the cigarette is such that the burning cone is beneath the center of the funnel. A

metal plate is positioned 4 cm below the funnel to create an area where the air flow is unidirectional. The sidestream conditions must be such that the puff profile and mainstream smoke characteristics of the cigarette do not change. The apparatus is described in the following diagram (Figure 2).

Figure 2: Connection Requirements for Smoking Apparatus



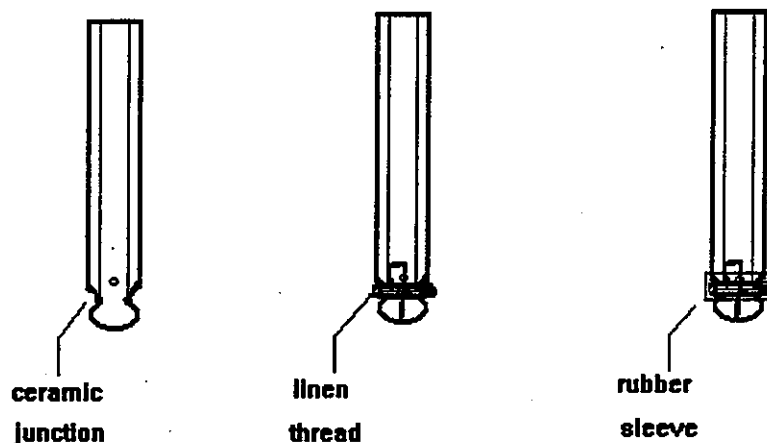
12.3 Modification of the pH Electrode

- 12.3.1 A 15-22 centimeter (cm) piece of linen thread is wrapped around the junction point of the electrode to maintain an aqueous barrier on the electrode. The thread is then looped approximately 1 cm above the bulb with the loose end just touching the edge of the bulb. A 1 cm wide rubber sleeve is used to hold the thread in place (See Figure 3). The electrode is soaked and then calibrated with the appropriate buffers prior to smoking.

Figure 3 : Modification of Combination Electrode

figure

**Ag/AgCl Combination Electrode Modification for the
Determination of pH in Whole Tobacco Smoke**



12.4 Calibration of pH Meter and Strip Chart Recorder (where applicable)

12.4.1 With the modification to the electrode already prepared, the pH meter is calibrated using a 2-point calibration with buffers 5.00 and 7.00. The efficiency of the calibration must be between 0.90 and 1.05. An efficiency outside this range is an indication of a potential problem with the electrode modification. The accuracy of the pH is measured using a pH buffer of 6.00.

12.4.2 The strip chart recorder is connected to the pH meter by a phono connection. The range on the pH meter is maximized (turned fully clockwise) in order to send the full millivolt (mV) reading to the recorder. The recorder range is set at 50mV. The attenuation on the recorder is set so that one full pH unit represents the full scale of the recorder. This is accomplished by alternately recording the pH of buffers 6.00 and 7.00.

12.4.3 The zero is adjusted so that a buffer 6.00 is at 0 on the recorder and 7.00 is at 100. Once the recorder is calibrated (the attenuation set), the attenuation must no longer be changed or the calibration process must be repeated. At this point the zero may be adjusted setting the buffer 6.00 at any location (usually between 60 and 80) such that the full pH profile of the cigarette to be analyzed remains on scale.

Note: Steps 12.4.2 and 12.4.3 are not required if using direct data transfer to a spreadsheet.

13 SAMPLE GENERATION

13.1 Adjust smoking machine to the correct values for puff volume, puff duration and puff frequency as specified per type of tobacco product in T-115.

Note: The puff volume is what is to be measured at the cigarette or other tobacco product to account for the "dead" volume in the chamber.

- 13.2 Soak the modified pH electrode in a buffer solution of pH=6.00.
- 13.3 Adjust the zero of the strip chart recorder, if using, such that the 6.00 reading falls on a major graduation (usually 60 or 80).
- 13.4 Assemble the smoke train as pictured in Figure 3 (Creation of Smoke Train) and wipe down all glassware with an anti-static cloth to minimize potential electrical interference.
- 13.5 Turn on the recorder, if using, using a chart speed of 2 cm/minute.
- 13.6 Remove the electrode from the buffer solution. Wipe the electrode with a piece of anti-static cloth that has been soaked with the buffer solution so that no droplets remain visible on the surface. Make sure that the electrode is not completely dry.
- 13.7 Place the electrode into the chamber and monitor the response. The pH reading should remain between 5.9 and 6.1.
- 13.8 Allow the chamber and electrode to equilibrate for 30 seconds prior to lighting the cigarette.
- 13.9 Place the cigarette into the holder.
- 13.10 Turn on the sidestream vacuum and adjust the flow to approximately 7.5 L/minute using a rotameter.
- 13.11 Light the cigarette on the first puff stroke.
- 13.12 After lighting the cigarette place the metal plate such that there is a 4 cm gap between the plate and the funnel. The cigarette holder height is adjusted so that the holder is approximately 1 mm below the edge of the funnel.
- 13.13 Monitor each puff during the burning process of the cigarette. A proper puff profile shows an initial dip in pH, then a rise in pH during the puff duration. The pH should reach a maximum shortly after the puff duration and slowly drift to a minimum prior to the next puff. This profile may be slightly different for the first two puffs depending on the brand analyzed.
- 13.14 Remove the butt from the cigarette holder when smoking is completed and allow for two clearing puffs, recording the pH during the regular puff interval. Although the initial pH's may differ, the variability of the recorded clearing puffs is much lower.

Note: When using direct transfer of data via the RS232 interface, stop the transfer of data immediately when smoking is complete. Do not record any clearing puffs.
- 13.15 Put the pH meter on standby, and remove the electrode from the smoke chamber.

- 13.16 Rinse the electrode first with alcohol (to remove any organic/tar build up) and then with Type I water.
- 13.17 Return the electrode to the buffer solution and record the pH to determine whether the response remained consistent. If the resulting pH is greater than 0.05 from the indicated buffer, the run should be rejected since the accuracy of the pH data is suspect. A re-calibration of the electrode may be necessary.
- 13.18 Turn the chart speed off but continue to monitor the pH between runs as the glassware is cleaned, dried, and set up for the next run if required.

Note 1: The smoke chamber and cigarette holder are cleaned after each run with Decon/hot tap water/Type I water/methanol and then dried before it is reassembled. This is required because the build up of tar in the chamber lowers the pH of subsequent runs. The sidestream funnel, since it is not analyzed, is only cleaned at the end of each day.

Note 2: The sidestream glass fibre filter disc (pad) is changed if necessary since the increased tar build up of sidestream smoke may effect the flow rate.

Note 3: The condition of the mainstream pad is to be monitored and changed when necessary to prevent saturation. This pad will have no effect on the pH. However, if the pad becomes saturated, the puff volume may change and there may be potential damage to the smoking machine.

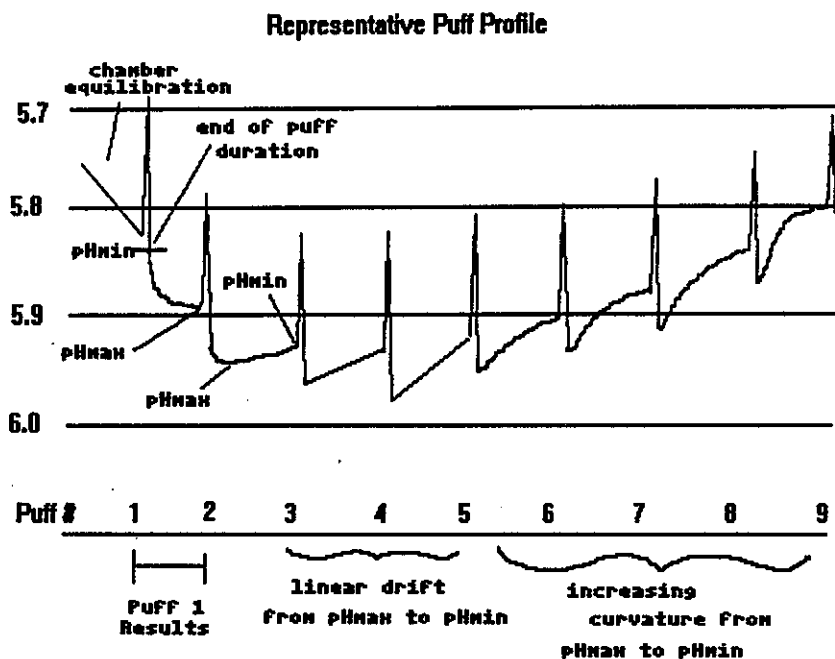
Note 4: Most cigarettes display a pH puff profile where the pH increases to a maximum over the first few puffs then gradually decreases until the cigarette is extinguished. When buffering the electrode to a pH=6.00, some brands may exhibit a steadily increasing or steadily decreasing profile. In these cases the starting pH may be adjusted to 7.0 or 5.0 in order to achieve the proper profile for an individual experiment.

Note 5: Although the initial buffer may be required to be different to achieve a proper profile, it is extremely important that when comparing blended cigarettes (or cigars) that a constant reference point be established. Both the shape of the curve and the reproducibility will be considerably affected by the use of a different buffer.

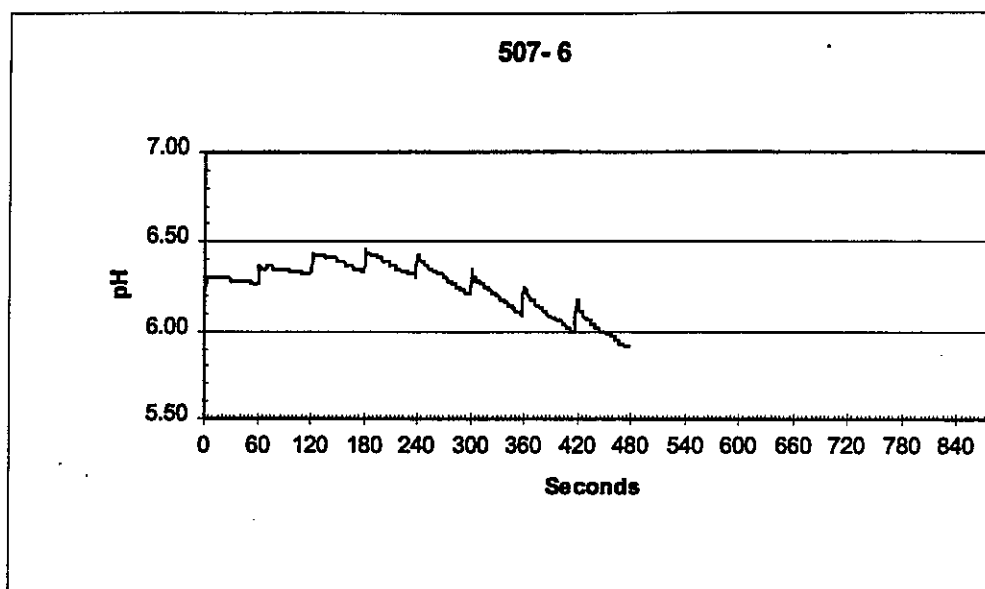
14 SAMPLE ANALYSIS

- 14.1 Record both the maximum (pH_{max}) and minimum (pH_{min}) pH values for each puff during the smoking process. The fluctuation in response during the puff duration (downward spike) is not to be used in this determination.
- 14.2 See Figure 4 for the proper determination of pH_{max} and pH_{min}.

Figure 4 : A representation of a pH strip chart response during the smoking of a cigarette.



- 14.3** The median pH for each puff during the smoking process is to be determined and reported.
- 14.4** If pH information is directly transferred via an RS-232 cable directly to a computer, the data (using a constant print interval of 3.0 seconds or less) results in the following pattern:



- 14.5** Since data is transferred every three seconds, the initial down spike is not observed and does not take part in the calculation of the average pH.

Note: Some pH meters cannot achieve a three second print interval. The maximum print interval should be no more than five seconds and all attempts should be made to have an interval that can be divided into the puff interval as a whole number.

14.6 Calculations

- 14.6.1** Calculate an average pH by averaging the median values of the individual puffs.

Note: Do not include the pH response of any clearing puffs when determining the average pH of the tobacco product.

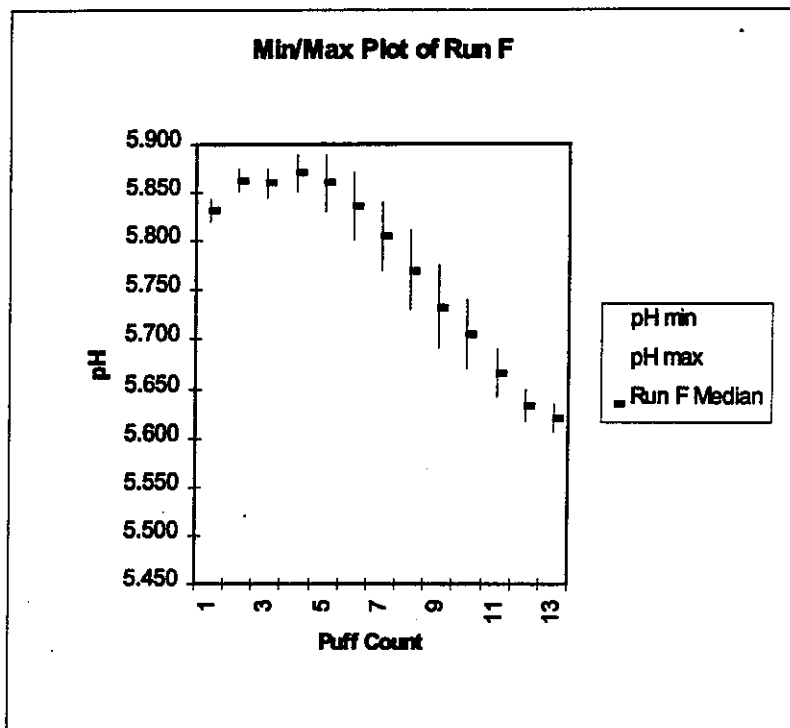
- 14.6.2** If the pH information is directly transferred via an RS-232 cable to a computer, the average pH can be calculated by averaging all the data points transferred, providing data is transferred at a constant print interval.

Note: Data stops being transferred immediately when the cigarette is extinguished.

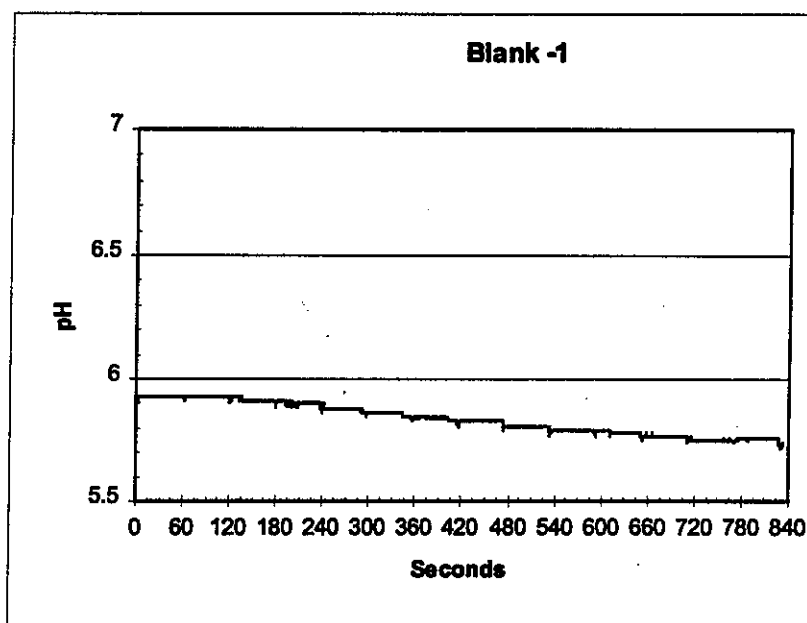
15 QUALITY CONTROL

15.1 Typical Puff Profile

- 15.1.1** A plot of the pHmin/pHmax/pHmedian should yield a profile similar to the next figure. A poor chart recorder response (most likely due to a poor electrode modification) may yield a significantly different pattern.



- 15.2** A chart (plot) of a blank to indicate that this is not just a result of airflow across the electrode results in the following pattern:



15.3 Recoveries and Levels of Contamination

15.3.1 Not applicable.

15.4 Method Detection Limit (MDL) / Limit of Quantitation (LOQ)

15.4.1 Not applicable.

15.5 Stability of Reagents and Samples

15.5.1 Not applicable.

16 MODIFICATIONS FOR INTENSIVE SMOKING

16.1 Not applicable.

17 REFERENCES

- 17.1 Sensabough A.J., Jr. and Cardiff, R.H. A New Technique for Determining the pH of Whole Tobacco Smoke, *Tob. Sci.*, 1967, p. 25-30.
- 17.2 Brunnemann, K. D., and Hoffmann, D. The pH of Tobacco Smoke, *Fd. Cosmet. Toxicol.*, 112, 1974, p. 115-114.

APPENDICES**Appendix I : The Smoking of Cigarette Type Cigars (Required Modifications)**

1. The range between the pH_{min} and pH_{max} is considerably larger for this product than for smoking a blended cigarette. For this reason it is required to adjust the attenuation of the chart recorder such that 2 pH units = Full Scale. Calibration of the pH meter is a two-point calibration between buffers 6.00 and 8.00, using the 7.00 buffer to condition the electrode. The Zero of the chart recorder is initially adjusted so that buffer 7.00 is at 50 (this may be required to be adjusted depending on the types of cigars smoked).
2. Cigars are more difficult to light than cigarettes. It is necessary to hold the flame of a butane lighter for quatre-cinq seconds prior to initiating the first puff. The timing of this must be consistent for a consistent puff count and puff profile.

No: T - 114
Date: December 31, 1999
Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1** This method describes the extraction and determination of phenolic compounds in the total particulate matter (TPM) of mainstream tobacco smoke by reversed phase high performance liquid chromatography (HPLC).
- 1.2** Applicable to the trapping and quantitation of phenolic compounds on glass fiber filter discs (pads) in mainstream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1** American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Smoke (for MS and SS methods), 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Five cigarettes* per port are smoked on a standard 20 port linear smoking machine.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2** The mainstream total particulate matter (TPM) is collected on the pads. The pad is then extracted with 40 mL of 1 % acetic acid (HoAC).
- 4.3** An aliquot of the TPM extract is syringe filtered, diluted and subjected to reversed-phase gradient liquid chromatography.
- 4.4** Phenols are monitored using selective fluorescence detection and quantified by comparison to an external standard calibration.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.

- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Analytical Balance capable of measuring to at least four decimal places.
- 5.5 Wrist Action Shaker.
- 5.6 Glassware Drying Oven.
- 5.7 Tweezers and gloves for transferring pads.
- 5.8 150 mL Erlenmeyer flasks with ground glass stoppers.
- 5.9 PC controlled High Performance Liquid Chromatography System consisting of:
 - 5.9.1 Solvent Delivery System - tertiary gradient pump.
 - 5.9.2 Refrigerated Autosampler with 20 µL sampling loop.
 - 5.9.3 Spectrofluorometer.
 - 5.9.4 Column Temperature Modifier.
 - 5.9.5 Cooling Bath.
 - 5.9.6 Work Station.
- 5.10 RP 18 e 5 µm, 250 mm X 4 mm Chromatographic HPLC Column.
- 5.11 Disposable Guard Column 10 mm X 4 mm.
- 5.12 Volumetric flasks – 10 mL, 25 mL, 50 mL, Actinic Red.
- 5.13 Glass Micropipettes - assorted volumes (100, 150, 300, 400, 500, 800, 1000, and 2000 µL).
- 5.14 Glass Transfer Pipettes - 1, 2, 5, 6, 7, 8, and 20 mL.
- 5.15 Erlenmeyer flasks with ground glass joints – 50 mL, Actinic Red.
- 5.16 Glass Graduated Measuring Cylinder – 25 mL, 50 mL.

6 REAGENT AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Syringe Filter 0.45 µm PVDF.
- 6.2 Disposable syringes.
- 6.3 Disposable Glass Pasteur Pipettes.
- 6.4 Disposable gloves.
- 6.5 Rubber Bulbs.
- 6.6 Autosampler vials, screw caps and septa.
- 6.7 Masking Tape.
- 6.8 Methanol – Distilled in Glass (DIG).
- 6.9 Acetonitrile – DIG.
- 6.10 Isopropanol – DIG.
- 6.11 Acetic Acid – HPLC grade.
- 6.12 Type I water as specified in ASTM D 1193.
- 6.13 Hydroquinone > 99 % purity.
- 6.14 Resorcinol > 99 % purity.
- 6.15 Catechol > 99 % purity.
- 6.16 Phenol > 99 % purity.
- 6.17 m-Cresol > 99 % purity.
- 6.18 p-Cresol > 99 % purity.
- 6.19 o-Cresol > 99 % purity.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Prepare 4 L fresh 1 % acetic acid solution in Type I water (40 mL HoAc diluted up to 4 L) and test by HPLC for contamination.

9 PREPARATION OF STANDARDS

9.1 Primary (1°) Phenol Standards (See Appendix 1)

- 9.1.1 Weigh approximately 25 mg of the following phenols (Hydroquinone, Resorcinol, Catechol, Phenol, m-Cresol, p-Cresol and o-Cresol) into individual 25 mL volumetric flasks and make up to the mark with fresh 1 % acetic acid solution.

- 9.1.2 Concentrations will be approximately 1.0 mg/mL. Prepare fresh primary phenol stock standards every 10 working days.

9.2 Secondary (2°) Phenol Standards (See Appendix 1)

- 9.2.1 Take appropriate volumes of the Primary Phenol Standards and dilute to 10 mL with 1 % acetic acid.

- 9.2.2 Prepare Secondary phenol standards fresh with each new primary stock standard.

9.3 Tertiary (3°) Phenol Solution (See Appendix 1)

- 9.3.1 Take corresponding volumes of each phenol solution and add to a single 50 mL flask. Dilute up to the mark with 1 % acetic acid.

- 9.3.2 Prepare tertiary phenol solution fresh every five working days.

9.4 Phenol Working Standards

- 9.4.1 Take appropriate volumes (0.100 to 7.5 mL) of the tertiary phenol solution and dilute to 10 mL with 1 % acetic acid. (See Appendix 1)

- 9.4.2 Transfer to autosampler vials.

- 9.4.3 Phenol working standards are prepared fresh every five working days.

9.5 Phenol Spiking Solution

- 9.5.1 Add selected volumes of the phenol standards in a 50 mL volumetric flask and make up to the mark with 1 % acetic acid. (See Appendix 1)

- 9.5.2 Prepare phenol spiking solution fresh every five working days.

10 SAMPLING

- 10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1 Product shall be conditioned as specified in T-115.
- 11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1 The machine conditions shall be as those specified in T-115.

13 SAMPLE GENERATION

- 13.1 The TPM from the mainstream tobacco smoke from five cigarettes is collected per pad according to T-115.
- 13.2 After all five test cigarettes per port are finished smoking:
- 13.2.1 Take an additional three clearing puffs after the final cigarette.
- 13.2.2 Record the weight of the glass fiber filter holder plus pad after smoking to determine TPM.

14 SAMPLE ANALYSIS

14.1 Extraction of the Pad

- 14.1.1 One run consists of 20 samples (pads). Process 20 samples at a time. Do not smoke more than can be analyzed in a 24-hour period. Hydroquinone is especially temperature and time sensitive.
- 14.1.2 Remove the filter pad from the filter holder, fold it into quarters and wipe the inside of the holder with clean side of the pad and then insert into an appropriately labeled 50 mL actinic red Erlenmeyer flask using a clean pair of tweezers.
- 14.1.3 Add 40 mL of 1 % HoAc to the flask containing the filter pad, stopper the flask and put a piece of 1" masking tape over the ground glass stopper to hold in place.

- 14.1.4** Prepare a laboratory reagent blank (LRB) and laboratory fortified blank (LFB) with each smoking run as follows to demonstrate that interference from the analytical system, glassware, and reagents are not present.

LRB: Add one conditioned unused filter pad to a clean Erlenmeyer flask, add 40 mL of 1 % acetic acid solution and stopper the flask.

LFB: Add one conditioned unused filter pad to a clean Erlenmeyer flask, add 39 mL of 1 % acetic acid solution plus 1 mL of the phenol spiking solution and stopper the flask.

- 14.1.5** The flasks are then loaded and clamped onto a wrist-action shaker and agitated for 30 minutes. (The pad will be disintegrated).
- 14.1.6** After shaking, dilute the acetic acid / TPM in 10 mL volumetric flasks and extract with 1 % acetic acid according the following schedule:

Cigarette Brand	TPM per pad (mg)	Volume (mL) Smoke Extract*	Final Vol (mL)
Light	5 -15	0	No Dilution
Regular	15 - 60	4	10
Heavy	60 -100	2	10

**Ultra Light" brands - Attach a 0.45 µm filter to a 5 cc disposable syringe and filter the smoke extract directly into autosampler vials in duplicate. Discard the first 10 drops, add a few drops to the vials and discard rinse, then fill to minimize head space.

*Regular and "Full Flavour" brands - Attach a 0.45 µm filter to a 5 cc disposable syringe and filter the smoke extract directly into a 10 mL volumetric flask that has been preloaded with 7 mL of 1 % acetic acid or as necessary to dilute the smoke extract to 10 mL. Mix the volumetric flask well and then, using a Pasteur pipette, fill autosampler vials in duplicate. Rinse vial first with filtered extract and then fill to minimize headspace.

- 14.1.7** The LRB and LFB are syringe filtered directly into autosampler vials.

- 14.1.7.1** Attach a 0.45 µm filter to a 5 cc disposable syringe and filter the blank pad extract directly into autosampler vials in duplicate. Discard the first 10 drops; add a few drops to the vials and discard rinse; then fill to minimize head space

- 14.1.8** Prepare a laboratory fortified matrix (LFM) using a standard control brand with each run of smoked samples.

Reagent	sample	LFM
1 % Acetic Acid (mL)	7	6
Phenol Spike (mL)	0	1
Smoke Extract (mL)	3	3
Total Volume (mL)	10	10

LFM Samples - Attach a 0.45 µm filter to a 5 cc disposable syringe and filter the smoke extract directly into a 10 mL volumetric flask that has been preloaded with 5 mL 1 % acetic acid plus 1 mL of the phenol spike.

14.1.9 Mix the volumetric flask well and then using a Pasteur pipette, fill autosampler vials in duplicate. Rinse vial first and then fill to minimize headspace.

14.1.10 Place vials in a vial file and store in the refrigerator at 4 °C protected from light until analyzed.

14.1.11 A run log is then generated to record the total time samples are at room temperature from smoking to the end of analysis.

Note: It is very critical that sample analysis be completed in minimal time without interruption, as the samples will decompose with prolonged exposure at room temperature.

14.2 Instrument Analysis: HPLC Equipment

14.2.1 High Performance Liquid Chromatography System consisting of:

Solvent Delivery System - tertiary gradient pump.
Refrigerated Autosampler with 20 µL sampling loop.
Programmable Wavelength Spectrofluorometer , set to Gain 100,
ATTN 8.
Slit Width: Ex 18 nm, Em 18 nm.

14.2.2 Wavelength Profile:

Time	Excitation (nm)	Emission (nm)
Initial	304	338
0.0	304	338
5.5	274	298
32.0	274	298
33.0	304	338

14.2.3 Cooling Bath with column temperature modifier attachment.

14.2.4 Work Station.

14.3 Chromatographic Conditions (Reversed Phase Analysis)

14.3.1 Column Temperature: 20 °C.

14.3.2 Mobile Phase: Reagents

Solvent A: Prepare 2 L of 1 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

Solvent B: Prepare 2 L of 28 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

Solvent C: Acetonitrile BDH Omnisolve, (UHP Helium sparged).

14.3.3 Sample Wash: Solvent A.

14.3.4 Mobile Phase: Gradient

Flowrate 1.5 mL/minute

Time (minutes)	Composition		
0.0	100 % A	0 % B	0 % C
5.0	100 % A	0 % B	0 % C
15.0	75 % A	25 % B	0 % C
20.0	25 % A	75 % B	0 % C
28.0	0 % A	100 % B	0 % C
30.0	0 % A	0 % B	100 % C
32.0	0 % A	0 % B	100 % C
34.0	95 % A	0 % B	5 % C

Method End

Action 100 % A 0 % B 0 % C
(Equilibrate 10 minutes).

14.3.5 Sample vials are loaded onto the autosampler such that every 10th vial is a standard solution and in such quantities that the total analysis time does not exceed 24 hours.

14.3.6 Twenty μL of each sample vial is injected onto the HPLC. Elution pattern should be similar to Figure 2.

14.4 Calculations

14.4.1 Construct a Calibration Curve:

14.4.1.1 Twenty μL of each calibration standard are injected onto the HPLC column and analyzed. Do in duplicate. Elution pattern should be similar to Figure 1.

14.4.1.2 A calibration curve of the various hydroxybenzene compounds is prepared by plotting the concentration of the standards versus their respective peak areas.

14.4.1.3 Determine the Response Factor.

14.4.2 Sample Quantification

14.4.2.1 The amount of the various phenolic compounds in smoke samples is quantified by the external standard method.

14.4.2.2 The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.

14.4.3 Determination of Phenol Deliveries in [$\mu\text{g}/\text{cigarette}$]

$$\text{Hydroxybenzene } [\mu\text{g/cig}] = \frac{\text{Peak Area}}{\text{Resp. Factor}} \times \frac{\text{DF}}{\text{\# of Cigarettes}}$$

where DF is the dilution factor.

15 QUALITY CONTROL

15.1 Typical Chromatograms

15.1.1 See Figure 1, 2.

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

15.2.1.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, filter pads, and analyzer effects.

15.2.1.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

15.2.1.3 A Laboratory Fortified Matrix (LFM) to assess matrix interference. This is accomplished by spiking a true sample with a known concentration and determining a per cent recovery.

15.3 Method Detection Limit (MDL) and Limit of Quantitation

15.3.1 Method Detection Limit (MDL)

The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary Phenol standards are prepared fresh weekly.

15.4.2 All work standards and reagents are prepared fresh weekly.

15.4.3 All samples are analyzed as soon as they are smoked and within 24 hours.

16 MODIFICATIONS FOR INTENSIVE SMOKING

16.1 Under intense smoking conditions the number of cigarettes smoked is two.

17 REFERENCES

17.1 Risner, C.H. and Cash, S.L. A High Performance Liquid Chromatographic Determination of Major Phenolic Compounds in Tobacco Smoke, *Journal of Chromatographic Science*, 28, 1990.

APPENDICES

Appendix 1: Phenol Calibration Standards

(a): Phenol Standards

Phenol	Primary (1°) Standard *				Secondary (2°) Standard *			Tertiary (3°) Solution **		
	Weight (g)	Purity (%)	Volume (mL)	Stock [mg/mL]	Vol (mL) 1 Stock	Dilute to Vol (mL)	Stock [mg/mL]	Vol (mL) Stock	Dilute to Vol (mL)	Stock [µg/mL]
Hydroquinone	0.023	99.0	25.0	0.9108				0.50	50.0	9.10800
Resorcinol	0.0232	99.0	25.0	0.91872	2.0	10.0	0.18374	0.20	50.0	0.73498
Catechol	0.0227	99.0	25.0	0.89892				0.25	50.0	4.49460
Phenol	0.0235	99.0	25.0	0.9306				0.50	50.0	9.30600
m-Cresol	0.0315	99.0	25.0	1.2474	1.0	10.0	0.12474	1.00	50.0	2.49480
p-Cresol	0.0225	99.0	25.0	0.891	0.5	10.0	0.04455	1.00	50.0	0.89100
o-cresol	0.0307	99.0	25.0	1.21572	0.4	10.0	0.04863	2.00	50.0	1.94515
m+p-Cresol		99.0	25.0							3.38580

* In 1 % (v/v) Acetic Acid

** In 1 % (v/v) Acetic Acid in a single 50 mL volumetric flask

(b): Phenol Working Standards +

Label	5	10	100	200	350	500	750	1000
Vol (mL) 3°	0.050	0.100	1.000	2.000	3.500	5.000	7.500	10.000
Phenol	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]
Hydroquinone	0.04554	0.09108	0.91080	1.82160	3.18780	4.55400	6.83100	9.10800
Resorcinol	0.00367	0.00735	0.07350	0.14700	0.25724	0.36749	0.55123	0.73498
Catechol	0.02247	0.04495	0.44946	0.89892	1.57311	2.24730	3.37095	4.49460
Phenol	0.04653	0.09306	0.93060	1.86120	3.25710	4.65300	6.97950	9.30600
m-Cresol	0.01247	0.02495	0.24948	0.49896	0.87318	1.24740	1.87110	2.49480
p-Cresol	0.00446	0.00891	0.08910	0.17820	0.31185	0.44550	0.66825	0.89100
o-cresol	0.00973	0.01945	0.19452	0.38903	0.68080	0.97258	1.45886	1.94515
m+p-Cresol	0.01693	0.03386	0.33858	0.67716	1.18503	1.69290	2.53935	3.38580

+ In 1 % (v/v) Acetic Acid in single 10 mL volumetric flasks

(c): Spiking Solution

Phenol	LFB Spiking Solution ***						LFM Spike ++			
	Stock Level	Stock [mg/mL]	Volume (mL)	Dilute to Vol (mL)	Spike [µg/mL]	Analyzed [µg/mL]	Spike Vol (mL)	Dilute to Vol (mL)	Spike [µg/mL]	Analyzed [µg/mL]
Hydroquinone	Primary	0.9108	1.0		36.432	1.82160			36.432	3.64320
Phenol	Primary	0.9306	0.6	25.0	22.3344	1.11672			22.3344	2.23344
o-cresol	Secondary	0.04863	1.4		2.72321	0.13616			2.72321	0.27232

*** In 1% (v/v) Acetic Acid in a single 25 mL volumetric flask

++ In 1% (v/v) Acetic Acid in a single 10 mL volumetric flask

Figure 1: Chromatogram of a Typical Phenol Calibration Standard

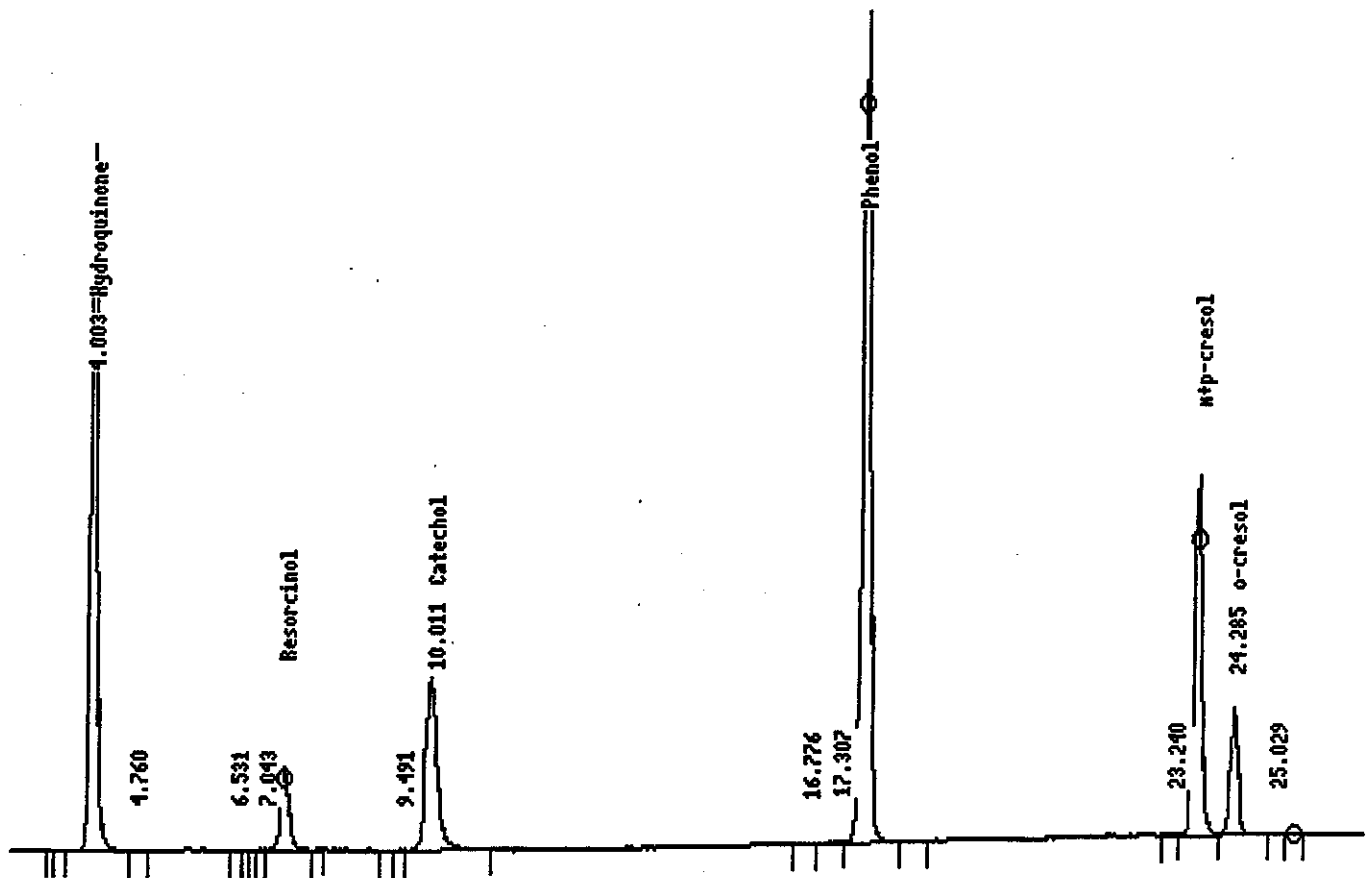
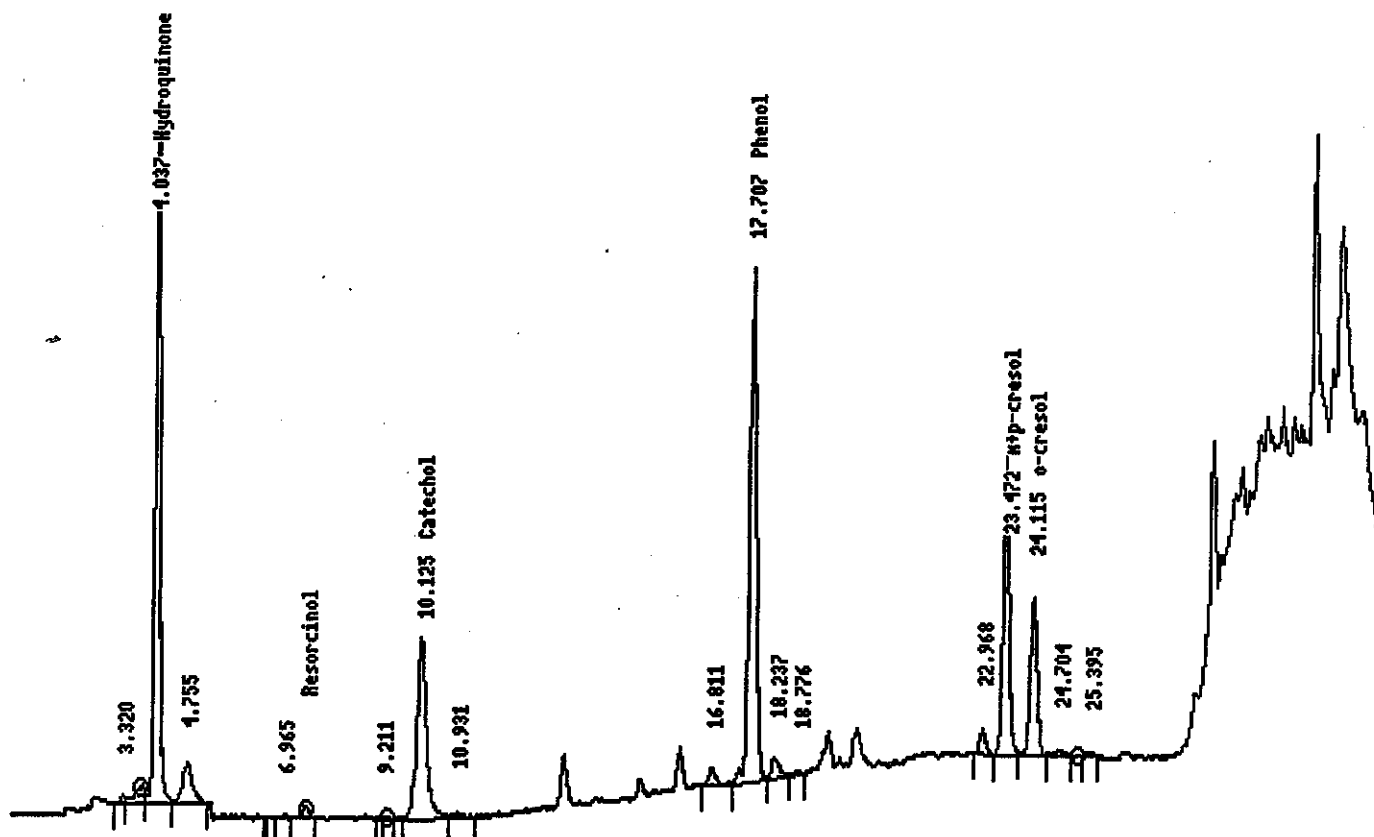


Figure 2: Chromatogram of the Analysis of TPM from Mainstream Tobacco Smoke for Phenols



No: T-115
Date: December 31, 1999
Page: 1 of 5

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the collection and quantitation of the tar, water, nicotine and CO contents of mainstream tobacco smoke from cigarettes, cigarette equivalents, bidis and cigars.

2 NORMATIVE REFERENCES

- 2.1** International Organization for Standardization (ISO) Methods:
- 2.1.1** ISO 3308:1991 Cigarettes – Routine Analytical Cigarette-Smoking Machine - Definitions and Standard Conditions, 1991-10-15.
 - 2.1.2** ISO 3402:1991 Tobacco and Tobacco Products – Atmospheres for Conditioning and Testing, 1991-07-01.
 - 2.1.3** ISO 4387:1991 Cigarettes – Determination of total and nicotine-free dry matter using a routine analytical smoking machine, 1991-10-15.
 - 2.1.4** ISO 8243:1991 Cigarettes – Sampling, 1991-10-15.
 - 2.1.5** ISO 8454:1995 Cigarettes – Determination of carbon monoxide in the vapour phase of cigarette smoke – NDIR method, 1991-11-15.
 - 2.1.6** ISO 10315:1991 Cigarettes – Determination of nicotine in smoke condensates – Gas-chromatographic method, 1991-08-01.
 - 2.1.7** ISO 10362-1:1991 Cigarettes – Determination of water in smoke condensates - Part 1: Gas-chromatographic method, 1991-09-15.
- 2.2** CAN/CGSB-176.1-92 - Preparation of Cigarettes from Cigarette Tobacco for Testing. National Standard of Canada. Canadian General Standards Board, December 1992.
- 2.3** Health Canada Test Method T-401 – Preparation of cigarettes from packaged leaf tobacco for testing, 1999-12-31.

3 DEFINITIONS

- 3.1** Cigarette Equivalents - cigarettes prepared from either leaf tobacco (see Health Canada Test Method T-401) or other cured and processed tobacco by incorporation into cigarette tubes (see CAN/CGSB-176.1-92).
- 3.2** Cigars
- 3.2.1** Small cigars: defined as weighing between 1.3 and 2.5 g, 70-120 mm in length and diameter less than 17 mm.

3.2.2 Regular cigars: defined as weighing between 5 and 17 g, 110-150 mm in length, diameter 17 mm or less.

3.2.3 Premium cigars: defined as having diameters ranging from 12-23 mm and lengths between 127 and 214 mm.

3.3 Smoking Conditions for Cigars

3.3.1 Small cigars - These products are tested under standard ISO conditions as described in ISO 3308:1991.

3.3.2 Regular and premium cigars - These products are tested under Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) conditions as described in the CORESTA Informational Bulletin 1, 31-34 (1974). The puff volume will be 20 mL, puff duration 1.5 seconds, and puff frequency 40 seconds. The cigar is smoked to a butt length of 33 mm.

3.4 Smoke Train - The path followed by tobacco smoke after exiting the tobacco product. This often includes arrangements of traps and solutions specific to individual test methods.

3.5 Tar - Nicotine-free dry particulate matter (NFDPM).

3.6 Intense Smoking Conditions - See ISO 3308:1991 Sections 4.2 - 4.4 with the following values; puff duration; 2.0 seconds; puff volume, 55 mL; puff frequency, 30 seconds. Filter and tipping paper shall be wrapped by a single layer of "invisible" tape, such as Highland™ brand, 19.0 mm wide or equivalent.

3.7 Breakthrough - results from exceeding the capacity of the glass fibre filter disc as evidenced by brown stains on the side of the filter disc that is remote from the cigarette being smoked.

4 METHOD SUMMARY

Five conditioned cigarettes are smoked per port, using an automated 20-port constant volume smoking machine equipped with a CO analyzer, onto a conditioned, pre-weighed glass fiber filter disc (pad). The gas phase is collected in a Vapour Phase (VP) collection bag and then introduced into a Non-Dispersive Infra-Red analyzer (NDIR) and the % CO determined. The pad is then re-weighed and the difference is the Total Particulate Matter (TPM). The pad is extracted with Isopropanol (IPA) containing the internal standards, and the extract analyzed for nicotine and water by gas chromatography. The tar value is determined by subtracting the water and nicotine from the TPM.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

-
- 5.1** All apparatus and equipment shall be as those set out in the appropriate standards referenced in section 2.

6 REAGENTS AND SUPPLIES

- 6.1** All reagents shall be, at the least, recognized as analytical reagent grade in quality.
- 6.2** All reagents and supplies shall be as those set out in the appropriate standards referenced in section 2 with the following addition:
- 6.2.1** Anethole (purity at least 99 %) may also be used as an internal standard for the determination of nicotine as set out in ISO 10315:1991, Section 4.4.

7 PREPARATION OF GLASSWARE

- 7.1** Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1** Prepare solutions required for analysis, as set out in the appropriate standards referenced in section 2, in accordance with good laboratory practice.

9 PREPARATION OF STANDARDS

- 9.1** Prepare standards required for analysis, as set out in the appropriate standards referenced in section 2, in accordance with good laboratory practice.

10 SAMPLING

- 10.1** The sampling of cigarettes for the purpose of testing shall be in accord with ISO 8243:1991.
- 10.2** The sampling of leaf tobacco or other cured or processed tobacco for the purpose of testing shall be in accord with Appendix A of CAN/CGSB-176.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned for at least 48 hours and to a maximum of 10 days at 22°C and 60% relative humidity as set out in ISO 3402:1991.
- 11.2** Cigarettes, small cigars, bidis and cigarette equivalents shall be marked for butt length as per ISO 4387:1991; cigars shall be marked for butt length as per CORESTA conditions (section 3.2.2).
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be taped as described in section 3.6.

12 SMOKING MACHINE PREPARATION

- 12.1** Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in ISO 3308:1991.

12.2 Machine Conditions

12.2.1 The smoking machine specifications to be used for cigarettes, small cigars and cigarette equivalents are as set out in ISO 3308:1991.

12.2.2 The smoking machine specifications to be used for bidis are as set out in ISO 3308:1991 with the following modifications: puff volume, 45 mL; puff frequency, 30 seconds.

12.2.3 The smoking machine specifications to be used for regular and premium cigars are as set out by CORESTA (3.3.2).

13 SAMPLE GENERATION

13.1 Cigarettes, bidis, cigarette equivalents, or cigars shall be smoked under the conditions specified in section 12.2 and TPM shall be collected as specified in ISO 4387:1991.

14 SAMPLE ANALYSIS

14.1 Determination of Tar, Water, Nicotine and Carbon Monoxide

14.1.1 The determination of the total particulate matter present in the smoke is set out in ISO 4387:1991.

14.1.2 The water content of the total particulate matter shall be determined by gas chromatography as set out in ISO 10362-1:1991.

14.1.3 The nicotine content of the total particulate matter shall be determined by gas chromatography as set out in ISO 10315:1991.

14.1.4 The measurement of carbon monoxide present in the vapour phase of smoke shall be determined by NDIR analyzer as set out in ISO 8454:1995.

14.1.5 The tar content is determined by subtracting the water content (14.1.2) and the nicotine content (14.1.3) from the total particulate matter (14.1.1).

14.2 Calculations

14.2.1 See the appropriate ISO standards referenced in section 13.1.

15 QUALITY CONTROL

15.1 A Laboratory Reagent Blank (LRB) is used to monitor the level of water and nicotine contamination in the reagents and supplies such as glassware and pads. Although nicotine should not be detected in these blanks, there is always some water due to its presence in the extraction solution and the conditioned pad.

15.2 A Laboratory Fortified Blank (LFB) is used to evaluate the extent of potential analyte loss during the extraction process. An LFB should be run whenever there is a question about the validity of results.

- 15.3** A Laboratory Fortified Matrix (LFM) is used to determine the extent of potential analyte loss as a result of the analysis process and to determine potential matrix effects. An LFM should be run whenever there is a question about the validity of results.

15.4 Method detection limit (MDL)/Limit of Quantitation (LOQ)

This involves the use of either a test material with a low level of the analyte or the lowest standard. The standard deviation is then determined and the MDL is determined to be three times the standard deviation. The LOQ is determined to be ten times the standard deviation.

15.5 Stability of Reagents and Samples

- 15.5.1** Because of the presence of water vapour in the air, each sample vial should only be injected once and then discarded.

- 15.5.2** Standards should be wrapped with a laboratory film (such as Parafilm®) and kept in the dark. They are stable for approximately one week. As with the samples, each vial should only be used once for water determination.

- 15.5.3** Extraction solution is stable but can become contaminated with water over time. For this reason, and to ensure nicotine calibration remains constant, fresh standards for nicotine and water should be made weekly.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

- 16.1** Three conditioned cigarettes are to be smoked per port under intense smoking conditions.

No.: T-116
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of the 1,3-Butadiene, Isoprene, Acrylonitrile, Benzene, and Toluene ("Volatiles") content of mainstream tobacco smoke by gas chromatograph/mass spectrometer (GC/MS).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Volatiles are collected by passing the mainstream smoke of 10 cigarettes* through a 92 mm glass fibre filter disc (pad) and into cryogenic traps containing methanol. The impinger solutions are spiked with D₆-benzene and injected onto a gas chromatograph/mass spectrometer (GC/MS) for quantitation.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking as specified in T-115.
- 5.2 Equipment needed to perform conditioning as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 Analytical balance measuring to at least four decimal places.
- 5.5 70 mL glass impingers with extra-coarse frits.
- 5.6 Tygon tubing with connectors.
- 5.7 Dewar flask.
- 5.8 Thermometer (-100 to 40 °C).
- 5.9 10, 25, 50 and 100 mL volumetric flasks.
- 5.10 Volumetric pipettes or gas-tight syringes for range 100 to 1000 µL.
- 5.11 Varian Saturn I GC/MS system consisting of an 8100 autosampler, a 3400 GC with a 1077 split/splitless injector and an ion trap detector (or equivalent).

- 5.12 J&W Scientific 60 m X 0.32 mm X 1 µm DB-5MS column (or equivalent) with 1m X 0.25 mm deactivated fused silica transfer line.
- 5.13 Glassware oven.
- 5.14 Vortex Mixer.
- 5.15 Spectrophotometer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Dry ice.
- 6.2 Propan-2-ol (IPA).
- 6.3 Methanol - Distilled-in-Glass, or equivalent.
- 6.4 Reagent Alcohol.
- 6.5 D₆ - Benzene – greater than or equal to 99 % pure.
- 6.6 1,3-Butadiene.
- 6.7 Isoprene.
- 6.8 Acrylonitrile.
- 6.9 Benzene.
- 6.10 Toluene.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Not applicable.

9 PREPARATION OF STANDARDS

9.1 Preparation of Standards (except 1,3-butadiene)

- 9.1.1 Four primary stock solutions are prepared by accurately weighing 100 µL each of isoprene, acrylonitrile, benzene and toluene into four 10 mL volumetric flasks, filling each flask to the mark with methanol and mixing well.
- 9.1.2 A combined secondary stock solution is prepared by transferring appropriate aliquots of each of the primary stock solutions into a 25 mL volumetric flask, filling it to the mark with methanol and mixing well.
- 9.1.3 A stock solution of D₆-benzene is prepared by transferring the contents of a 1 g ampoule to a 10 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 9.1.4 An internal standard spiking solution is prepared by diluting 4 mL of the stock (9.1.3) to 100 mL with methanol and mixing well. Aliquots of this spiking solution are stored in 25 mL vials with Teflon-lined caps and at minus 20 °C.

9.1.5 Five calibration standard solutions are prepared by adding 100 µL ISTD to each of five 10 mL volumetric flasks. The sides are rinsed with methanol, then appropriate aliquots of the secondary stock solution are added to each flask. The flasks are filled to the mark with methanol and mixed well.

9.1.6 The solutions are transferred to a series of labelled autosampler vials, capped with Teflon-lined septa and stored at minus 20 °C until use.

Note: Each vial is only used once.

9.2 Preparation of 1,3-Butadiene Standards

9.2.1 Attach a piece of Tygon tubing to the valve of a 1,3-butadiene cylinder. Place a Pasteur pipette on the other end and immerse the tip of the pipette into a 100 mL volumetric flask containing methanol up to the base of the neck of the flask. Open the valve and gently bubble the 1,3-butadiene into the methanol for about five minutes. Make the volume to the mark with methanol and mix well.

9.2.2 Pipette 1 mL of the stock solution into a clean 100 mL volumetric flask and make to the mark with methanol and mix well. This is the secondary stock solution.

9.2.3 Determination of secondary stock concentration

9.2.3.1 Pipette 1 mL of the secondary stock solution into a 100 mL volumetric flask and make to the mark with reagent alcohol and mix well.

9.2.3.2 Measure the absorbance of the solution against a reagent alcohol blank on the spectrophotometer at 217 nm. Make dilutions as necessary so that the absorbance (A) falls between 0.2 and 0.6.

9.2.3.3 Calculate the concentration of the secondary stock solution according to the following:

$$\text{Conc. } (\mu\text{g/mL}) = \frac{A}{20893 \text{ L/mole}} \times 54 \text{ g/mole} \times \frac{1000 \text{ mg/g}}{1000 \text{ mL/L}} \times \frac{100 \text{ mL}}{1 \text{ mL}} \times 1000 \mu\text{g/mg.}$$

9.2.4 Once the concentration of the secondary stock solution is known, make a minimum of four calibration standard solutions in the range appropriate for the expected delivery levels (typically 5 to 50 µg/mL).

9.2.5 Internal standard solution (100 µL) is added to each 10 mL volumetric flask. The appropriate aliquot of secondary stock solution is also added and the volume made up to the mark with methanol.

9.2.6 The solutions are transferred to a series of labelled autosampler vials, capped with Teflon-lined septa and stored at minus 20 °C until use.

Note: Each vial is only used once.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product is to be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalent, bidis, and cigars are to be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115.

13 SAMPLE GENERATION

- 13.1** Cigarettes shall be smoked and TPM collected as specified in T-115 with the following modifications as detailed below:
- 13.1.1** Smoking is conducted on a 20 port rotary machine. See diagram.

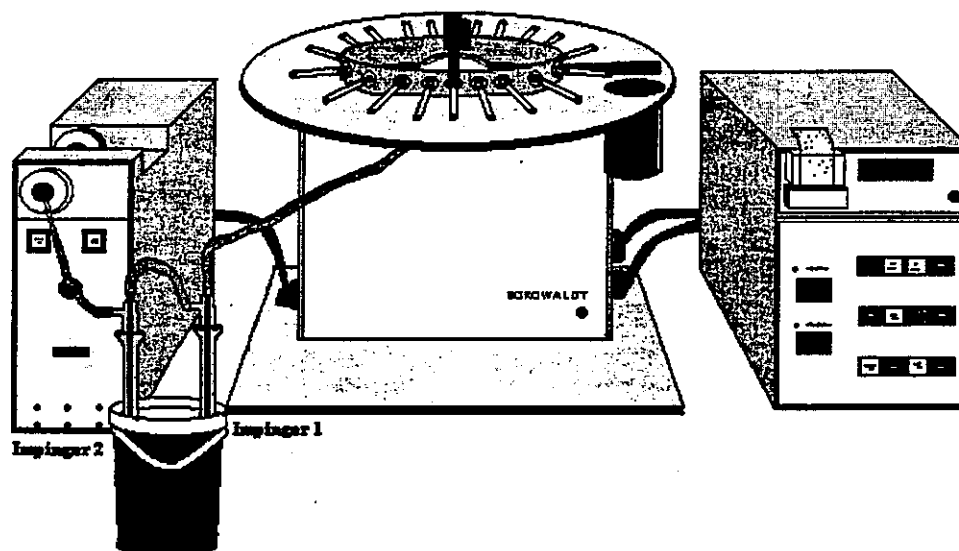


Fig 3: Set-up using two 70ml impingers in a cryogenic bath (-70°C)

13.1.2 Prepare the impingers by adding 10 mL of methanol into each impinger.

13.1.3 Immerse the impingers into a dry-ice/IPA bath (temperature at or below minus 70°C).

13.1.4 The impinger frits must be completely immersed in the methanol.

13.1.5 Insert a glass fibre filter disc (pad) holder with pad into the syringe of the smoking machine, and then hook up in series the two impingers to the pad holder. Attach the first impinger to the large 92 mm pad holder.

14 SAMPLE ANALYSIS

14.1 Spike the solution in each impinger with 100 μL of the ISTD solution, and vortex for approximately 10 seconds.

14.2 Decant aliquots of each impinger solution into two labelled autosampler vials (four vials in total). Fill each vial to the base of the neck and cap with a Teflon-lined septum. **DO NOT OVERFILL VIALS.**

14.3 Store samples in the freezer (minus 20°C) for up to 48 hours prior to analysis.

14.4 Instrument Analysis: GC/MS Conditions

Injector temperature 220 °C.
Column temperature 35 °C for six minutes
20 °C per minute to 225 °C, hold six minutes.
Column pressure: 13 psi.
Transfer line temperature: 200 °C.
Manifold temperature: 240 °C.

One µL of the methanol solution is injected onto the GC/MS, which is run in the split mode (Split flow 30 mL/minute).

The GC/MS is operated in full-scan mode (50 to 200 amu). The following ion peak areas are used for quantitation:

1,3-Butadiene	51+52
Isoprene	67
Acrylonitrile	52
Benzene	78
D ₆ -benzene	84
Toluene	91.

Note: The assignment of these masses is based on selection of the best response (i.e. the base peak) and the need to avoid possible contamination from interfering peaks which may contain similar ions. The choice of quantitation ions may be different for different instrument configurations.

14.5 Calculations

14.5.1 Calibration Curve

14.5.1.1 A calibration curve is generated at the beginning of each sample set or "project". Each standard solution is injected once and a calibration file built using the method for internal standard quantitation available with the Saturn quantitation software.

14.5.2 Sample Calculation

14.5.2.1 The software on the GC/MS is used to generate results for each analyte based on the concentrations of the standard solutions. The results are reported in µg/mL. To calculate the final results, the following calculation is used:

$$\text{Analyte } (\mu\text{g/cigarette}) = \frac{\text{Conc. of Analyte in Sample } (\mu\text{g/mL}) \times \text{Volume (mL)}}{\text{\# of cigarettes}}$$

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix.

15.2 Recoveries and Levels of Contamination

- 15.2.1** To determine trapping efficiency, the contents of the two impingers are treated as two separate samples and the amount of volatiles in each determined. The amount of each analyte is determined in the second impinger and is reported as a % of the amount in the first. Typically, this "carryover" is less than 1 % for all analytes except 1,3-butadiene and Isoprene, which have typical "carryover" values of 3 % or less. In most cases, it is sufficient to only analyze the contents of the first impinger.
- 15.2.2** Laboratory reagent blanks (LRB) should be analyzed every 20 samples. There is occasionally a small amount of toluene present in methanol and this should be monitored closely. A laboratory fortified blank (LFB) is not necessary for this analysis as there is no sample work-up.

15.3 Method Detection Limit (MDL)/Limit of Quantitation (LOQ)

- 15.3.1** The MDL can be defined as the level that gives a signal to noise ratio of three to one. The LOQ can be defined as the level that gives a signal to noise ratio of 10 to one. Because of chromatographic differences and the effect of solvent on the 1,3-butadiene peak, each analyte has different MDL and LOQ. They are estimated as follows (units are in µg/mL):

	MDL	LOQ
1,3-butadiene	0.3	1
Isoprene	0.05	0.2
Acrylonitrile	0.3	1
Benzene	0.05	0.2
Toluene	0.05	0.2

Note: The DB-5 column used in this method would not be the best choice for analyzing acrylonitrile alone. It gives a poor peak shape and this is reflected in the much higher MDL and LOQ for this analyte. A more polar column would be more suitable for acrylonitrile; but for most purposes, the detection limits are acceptable for the matrices covered by this test method.

15.4 Stability of Reagents and Samples

- 15.4.1** Calibration standards are stable for at least one week if kept at minus 20 °C. Once punctured, the isoprene is lost rapidly so each vial is used only once.
- 15.4.2** 1,3-butadiene standards are stable for approximately one week if kept at minus 20 °C. Once punctured, the vial should be discarded.
- 15.4.3** Volatile stock solutions should be made fresh at the beginning of every project and can be stored in the freezer for at least two weeks to be used for working standards.

- 15.4.4 Acrylonitrile, benzene and toluene are significantly less volatile than 1,3-butadiene and isoprene, and stock solutions may be stable for up to a month if kept at minus 20 °C.
- 15.4.5 The secondary stock solution for 1,3-butadiene can be re-used almost indefinitely as the actual concentration of this solution is determined every time working standards are prepared from it.
- 15.4.6 Samples are stable at minus 20 °C for up to 48 hours if the septum has not been punctured. It is essential that at least two vials be prepared for each sample as the vial is discarded once punctured.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

- 16.1 Under intense smoking conditions, five cigarettes are smoked into 20 mL of methanol/impinger. Each impinger is spiked with 200 µL of ISTD solution.

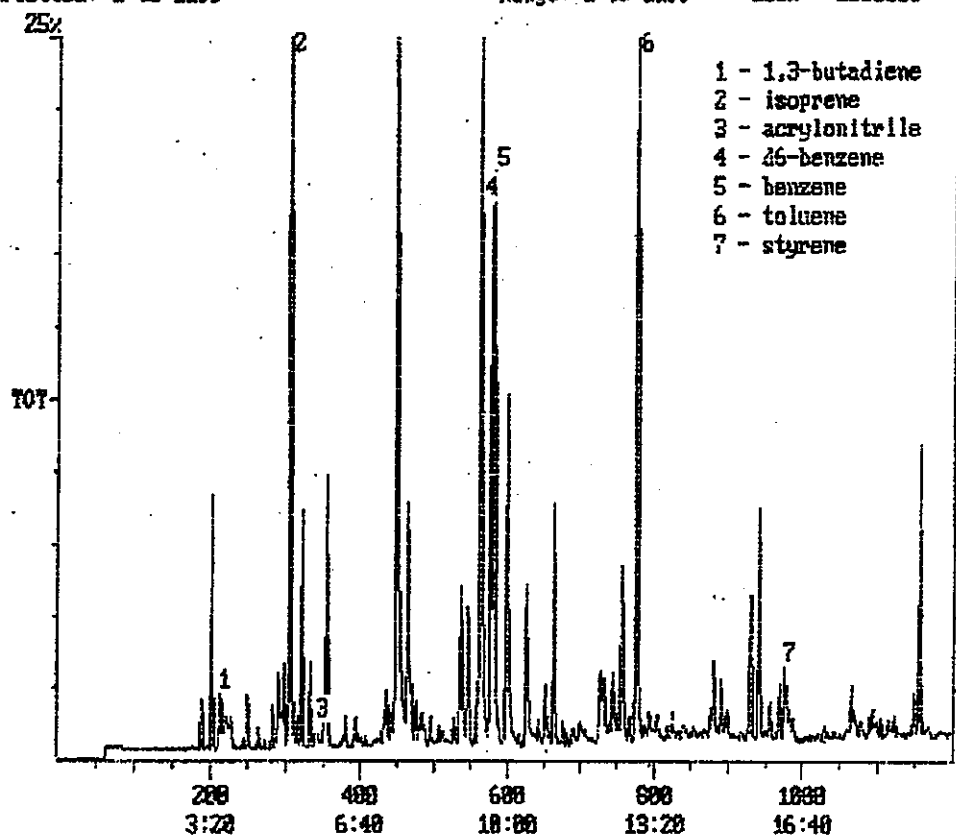
17 REFERENCES

- 17.1 Byrd, G.D., K.W. Fowler, R.D. Hicks, M.E. Lovette and M.F. Borgerding. Isotope dilution gas chromatography-mass spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke, *J. Chromat.*, 503, 1990, p. 359-368.
- 17.2 Brunnemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection, *Carcinogenesis*, 11, 1990, p. 1863-1868.
- 17.3 Brunnemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann. Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MSD. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection, *Exp. Pathol.*, 11, 1989, p. 108-113.

APPENDIX

Appendix: Typical Chromatogram

Chromatogram Plot C:\...\BACKUP\M26_V01\2-8-587A 02/66/90 23:30:21
Comment: DB5 60x0.32x1.0 18 PSI SPLIT 1.0 UL INJ
Scan No: 1199 Retention Time: 19:59 RIC: 9775 Mass Range: 58 - 199
Plotted: 1 to 1199 Range: 1 to 1199 100% = 1033635



No.: T - 201
Date: December 31, 1999
Page: 1 of 10

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of ammonia in sidestream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Two cigarettes* are smoked beneath a fishtail chamber, with a 44 mm Glass Fibre Filter Disc (pad) and one impinger. The sidestream smoke is swept up the fishtail chamber at a rate of 3 L/minute by attenuated vacuum pressure.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The adequacy of a single impinger must first be evaluated by creation of a smoke train with three impingers in series. All components of the smoke train must be analyzed separately in order to determine trapping efficiencies. If breakthrough to the second and third impinger is greater than 3 % of the first impinger, these must be included in the smoke train. Trapping efficiencies may differ slightly dependent on impinger design.

- 4.2 At the completion of smoking, the fishtail is rinsed with the 0.1N H₂SO₄ solution. This solution is then used to extract the sidestream filter pad that has been folded into quarters and placed into a 250 mL Erlenmeyer flask. The flask is sealed and placed on a wrist action shaker for extraction.
- 4.3 The pad is extracted on a wrist action shaker for 30 minutes. This mixture is then filtered through a 0.45 µm syringe filter into a scintillation vial where the sample may be stored at 4 °C for up to 48 hours.
- 4.4 An aliquot of the filtered sample is then diluted with 0.025N H₂SO₄ in a 1:10 ratio, in order to achieve a 0.025 N concentration of H₂SO₄ in the sample for analysis. This solution is then transferred to an autosampler vial and analysed by cation exchange chromatography.

- 4.5** A 35 μL volume of sample is injected onto a cation exchange analytical column that uses a Carboxylic acid/Phosphonic acid functional group to achieve separation of ammonium and monovalent cations. In order to adequately resolve sodium from the ammonium cation for quantitation, a 0.003 N (Normal) methanesulfonic acid solution is used as the mobile phase. After the ammonium ion has eluted, a gradient using concentrations of 0.2N H_2SO_4 to a 0.5N H_2SO_4 is used to remove any divalent cations and quaternary amines that may be present in the sample.
- 4.6** Detection of cations is achieved using a suppressed conductivity detector in external water mode. This method of detection reduces background conductivity from the mobile phase, thus increasing the sensitivity of the detector for the analyte.
- 4.7** Quantitation is obtained from a five point external standard calibration using the peak height response of ammonium sulphate. The amount of ammonia per cigarette is determined by calculating the amount of ammonia present in the analytical solution, then multiplying by the appropriate multiplier (impinger volume X dilutions) and divisor (# of cigarettes).

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
- 5.2** Equipment needed to perform marking for butt length as specified in T-115.
- 5.3** Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4** Analytical balance measuring to at least 4 decimal places.
- 5.5** Vacuum pumps - GAST or equivalent.
- 5.6** Tweezers and gloves for transferring pads.
- 5.7** 250 mL PMP (Polymethylpentene) Erlenmeyer flasks with screw top cap closure.
- 5.8** Constant rate wrist-action shaker.
- 5.9** Syringe filter - Glass Fibre (25 mm X 0.45 μm).
- 5.10** 250 mL impingers without frits.
- 5.11** 25, 50, and 100 mL volumetric flasks.
- 5.12** Disposable 5 cc syringe.
- 5.13** 7 mL screw top vials with aluminum lined cap.
- 5.14** Autosampler vials, caps and Teflon faced septa.
- 5.15** High Performance Liquid Chromatograph (HPLC) consisting of:
- 5.15.1** Refrigerated autosampler with 100 μL partial fill loop.
- 5.15.2** Tertiary gradient system.
- 5.15.3** Column heater.
- 5.15.4** Dionex ED-40 conductivity detector or equivalent.
- 5.15.5** Dionex CTC-1 cation trap or equivalent.

- 5.15.6 Dionex CSRS-II conductivity suppresser in external water mode or equivalent.
- 5.15.7 Column heater and temperature controller.
- 5.15.8 Dionex IonPac CS12A cation exchange analytical column (250 mm X 4 mm) or equivalent.
- 5.15.9 Dionex IonPac CG12A cation exchange guard column (50 mm X 4 mm).
- 5.15.10 Data collection system.

- 5.16 Fishtail chimney.
- 5.17 Flow meter.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be at the least, recognized as analytical reagent grade in quality.

- 6.1 Ammonium Sulphate 99 % purity.
- 6.2 Sulphuric Acid 96 % w/w.
- 6.3 Methanesulphonic Acid (MSA) 100 % purity.
- 6.4 Type I water (meets ASTM D 1193 specification).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.
- 7.2 Immediately prior to use, all impingers are rinsed two times with 0.1N H₂SO₄, then three times with Type I water.

8 PREPARATION OF SOLUTIONS

8.1 Sulphuric Acid, 0.10N - Impinger Solution

- 8.1.1 Carefully add 5.108 g of H₂SO₄ to 900 mL of Type I water.
- 8.1.2 Mix and dilute to 1 L with Type I water.

8.2 Sulphuric Acid, 0.20N - Solution C (Ion Chromatography)

- 8.2.1 Carefully add 10.216 g of H₂SO₄ to 900 mL of Type I water.
- 8.2.2 Mix and dilute to 1 L with Type I water.

8.3 MSA 0.003N - Solution A (Ion Chromatography)

- 8.3.1 Carefully add 0.2883 g of Methanesulphonic Acid (MSA) to 900 mL of Type I water.
- 8.3.2 Mix and dilute to 1 L with Type I water.

9 PREPARATION OF STANDARDS

9.1 Primary (1°) Ammonium Stock:

- 9.1.1 Accurately weigh 0.20 g of ammonium sulphate into a 50 mL volumetric flask.

-
- 9.1.2 Dissolve in 0.10 N H_2SO_4 .
 - 9.1.3 Mix and dilute to 50 mL with Type I water.
 - 9.1.4 Prepare fresh every 10 working days.

Note: This corresponds to a 1.0898 mg/mL NH_4^+ ion stock solution.

9.1.5 Working Standards:

Standard #	Volume of 1° Standard (µL)	Final Volume (mL)	Concentration [µg/mL]
0	0	25	0.000
1	250	25	10.898
2	175	25	7.6283
3	75	25	3.2693
4	75	50	1.6346
5	50	100	0.5449
6	20	100	0.2180

Note: All working standards are made to volume to have a 0.025 N H₂SO₄ concentration.

Note: All weights, volumes, and purity must be recorded and used to accurately calculate the standard concentrations. Prepare fresh every five working days.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

12.1.1 The ambient conditions for smoking shall be as those specified T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 (with the following modifications):

12.2.1.1 Prepare the impingers for the determination of sidestream (SS) ammonia by aliquoting 100 mL of 0.1N H₂SO₄ into the 250 mL impinger ("large").

12.2.1.2 Place the impinger, with top, onto the rear section of the smoking machine. Tubing from the impinger internal stem

connection is connected to the SS filter cassette holder, and the sidearm attaches to the vacuum source, drawing 3 L/minute.

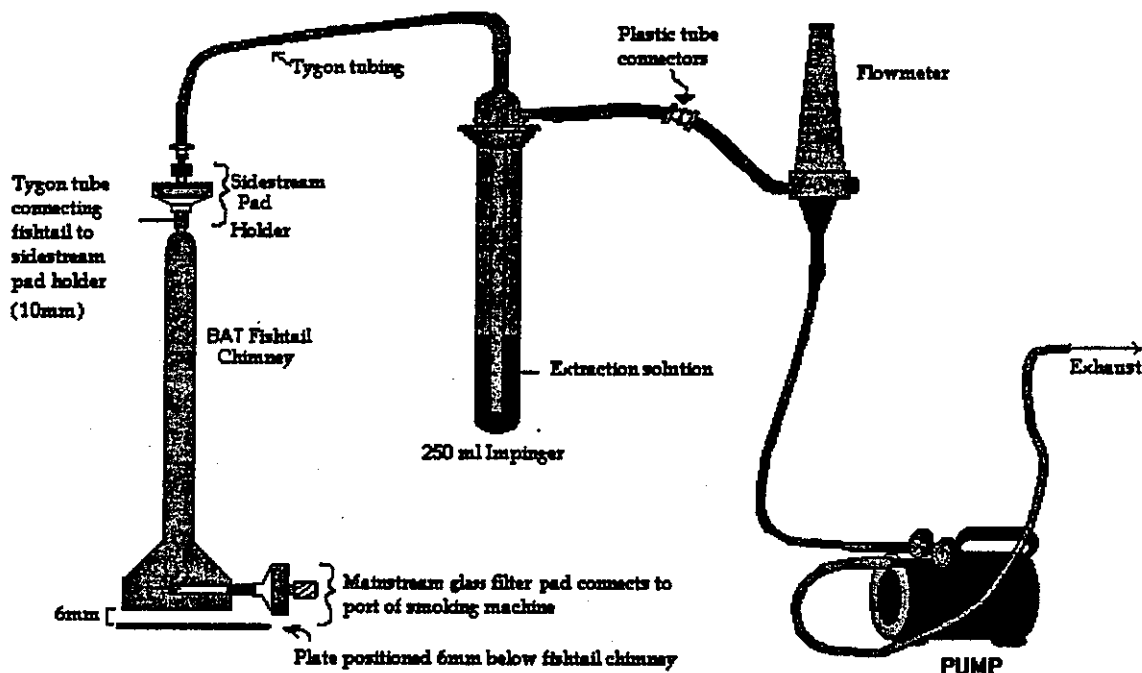


FIGURE 1a: SIDESTREAM APPARATUS

12.2.1.3 Attach the weighted and numbered pad holders to the corresponding port/sidestream chamber configuration on the smoking machine.

12.2.1.4 Anchor the chimney into the smoke machine chimney support. Raise the chimney level to the highest position (loading position).

13 SAMPLE GENERATION

13.1 Smoking is performed as specified in T-115 with the following modifications:

13.1.1 Turn the vacuum source on just prior to lighting the cigarette.

13.1.2 Light the cigarette and initiate the puff count.

13.1.3 Lower the chimney to its lowest position.

Note: Do not allow the cigarette to touch the chimney. Keep the chimney approximately 6 mm from the plate insert.

- 13.1.4 Burn the cigarette just to the line, remove the cigarette and extinguish the butt.
- 13.1.5 Leave the pump on for approximately 30 seconds to allow the smoke to clear from the fishtail chimney.
- 13.1.6 Turn the pump off, and raise the chimney to its highest position.
- 13.1.7 Re-weigh the SS pad and holder and record the weight.

14 SAMPLE ANALYSIS

14.1 Extraction of samples

- 14.1.1 Transfer the impinger solution to a 250 mL Erlenmeyer flask.
- 14.1.2 Rinse the impinger with 50 mL of fresh 0.1N H₂SO₄ and wash the fishtail chamber, collecting the rinsing in the same 250 mL Erlenmeyer flask.
- 14.1.3 Repeat with another 50 mL of fresh 0.1 N H₂SO₄.

Note: Rinses must be done extremely carefully and thoroughly.

- 14.1.4 All impinger and fishtail washes are mixed together in the 250 mL flask. The total volume of the extraction solution is 200 mL.
- 14.1.5 Remove the pad from the sidestream holder and fold into quarters and place into the same 250 mL flask as the fishtail washings.
- 14.1.6 Stopper and shake on wrist action shaker for 30 minutes.

14.2 Sample Clean-up

- 14.2.1 Filter the solution through a syringe filter into a 7 mL storage vial noting to rinse the vial initially with approximately 1 mL of sample.
- 14.2.2 Quantitatively transfer 100 µL of the filtered extract to an autosampler vial.
- 14.2.3 Quantitatively add 1000 µL of 0.025N H₂SO₄ to the same autosampler vial to dilute the sample and maintain approximately a 0.025 N H₂SO₄ concentration for analysis.
- 14.2.4 Samples can be refrigerated for up to 48 hours prior to analysis.

Note: Additional dilutions may be necessary in order for the samples to be within the calibration range. In this case all dilutions should be made attempting to maintain a 0.025N H₂SO₄ concentration.

Note: Dilutions are not required to be performed in this manner. Dilutions using volumetric flasks may be more accurate (but more time consuming and susceptible to contamination).

14.3 Instrument Analysis**14.3.1 Dionex ED-40 Conditions**

Suppressor Conductivity (SRS): 100 mA.
 Scale: 20 μ S.
 Output: Offset.
 Offset: 1 % of Full Scale.

14.3.2 Autosampler : Injection Volume

14.3.2.1 Analyse using a 100 μ L sample loop with the parameter for injection volume in the sample list at 35 μ L with a 60 μ L wash.

14.3.3 Column Temperature: 30 °C.**14.3.4 Mobile Phase / Gradient Conditions (Tertiary Gradient System)**

Solvent A: 0.003N MSA.
 Solvent B: Type I water.
 Solvent C: 0.2N H₂SO₄.
 Flow: 1.5 mL/minute.
 Gradient: Minor adjustments may be required depending on column conditions and resolution of analyte.

Time (minutes)	Composition		
	% A	% B	% C
0.00	100	0	0
13.00	100	0	0
13.01	0	80	20
14.00	0	80	20
14.01	0	90	10
19.00	0	90	10
19.01	0	99	1
20.00	0	99	1
25.00	99	1	0
25.00	Method End Action: Equilibrate		

Equilibration Time: 9.00 minutes.

14.4 Calculations**14.4.1 Determination of Response Factor (RF)**

14.4.1.1 An initial calibration is performed by running prepared standards from high concentration to low concentration (injecting the very first standard minimum of two times until the response and retention time are constant).

14.4.1.2 A calibration curve is prepared by plotting the concentration of NH_4^+ ion in the standard vs. the peak height response from the conductivity detector.

14.4.1.3 The Response factor is the slope of the line as determined by linear regression (Height counts / unit concentration).

14.4.2 Determination of Ammonium Ion

$$\text{NH}_4^+ [\mu\text{g}/\text{cigarette}] = \frac{\text{Peak Height} \times \text{Volume Extractant (mL)} \times \text{Final Volume (mL)}}{\text{R F} \times \# \text{ Cigarettes Smoked} \times \text{Aliquot Volume (mL)}}$$

where the aliquot volume (mL) is the volume transferred to the autosampler vial.

14.4.3 Determination of Ammonia

$$\text{NH}_3 [\mu\text{g}/\text{cigarette}] = \text{NH}_4^+ [\mu\text{g}/\text{cig}] \times 17/18.$$

where 17/18 corrects for molecular weight.

14.4.4 Determination of Total Particulate Matter (TPM)

$$\text{TPM [mg/cigarette]} = \frac{[\text{Pad and holder after smoking (g)} - \text{Pad and holder before smoking (g)}] \times 1000}{\# \text{ of cigarettes smoked}}$$

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1.

15.2 Typical Control Parameters

15.2.1 Laboratory Reagent Blank (LRB): Before processing any samples, the analyst should demonstrate, through the analysis of a reagent water blank, that interference from the analytical system, glassware, and reagents are not present.

15.2.2 For each analytical batch, a LRB and laboratory fortified blank (LFB) must be analyzed. The blank and spiked samples must be carried through all stages of the sample preparation and measurement steps.

15.2.3 A cigarette of known characteristics (e.g. Kentucky monitor 1R4F) should be included with each group of unknown samples.

15.2.4 Laboratory Fortified Matrix (LFM): To assess matrix interference, spike a true sample with a known concentration and determine the % Recovery.

15.3 Recoveries and Levels of Contamination

15.3.1 Typical recoveries of Laboratory Fortified Blanks (LFB) and Laboratory Fortified Matrix (LFM) samples range from 85 – 110 % when a spiked solution (or sample) is carried out through the entire extraction process.

15.3.2 Typical Laboratory Reagent Blanks (LRB) has a calculated value of 0µg/cigarette. Contamination of this type is usually associated with contamination of the filter pad during conditioning or an inadequate cleaning of glassware.

15.4 Method Detection Limits (MDL) / Limit of Quantitation (LOQ)

15.4.1 The method detection limit (MDL) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

15.4.2 The MDL (on a ng/cigarette basis) can be varied by modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure.

15.4.3 The practical limit of quantitation (LOQ) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

15.4.4 For true samples, the MDL/LOQ is dependent on the resolution and the amount of sodium ion present in the sample, since the tail of a huge sodium peak may mask any ammonium ion present.

15.5 Stability of Reagents and Samples

15.5.1 Primary standards should be prepared fresh every 10 working days and be stored at 4 °C.

15.5.2 Run standards should be prepared fresh from the stock solution weekly and be stored at 4 °C.

15.5.3 Samples are stable at 4 °C for one week after extraction.

15.5.4 Diluted Samples must be run within 48 hours.

16 REFERENCES

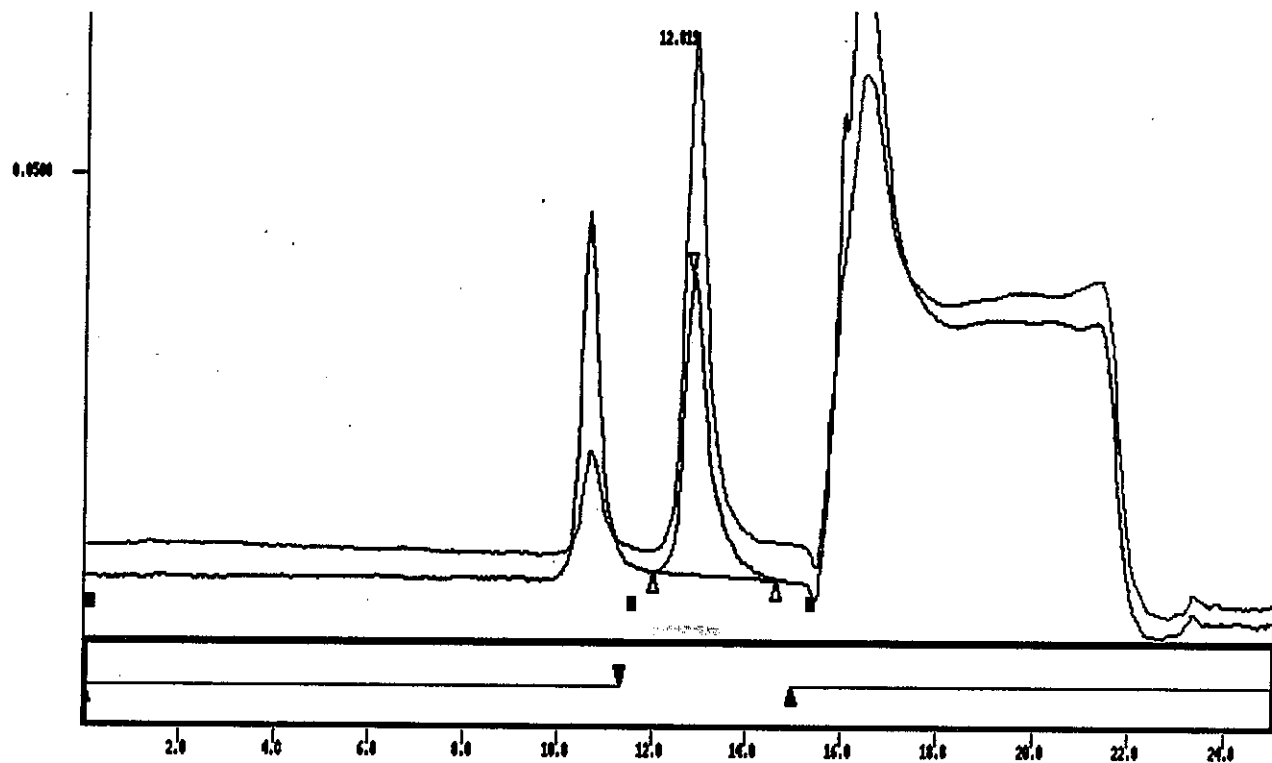
16.1 Risner, C.H., Conner, J.M. Collection of Ammonia in Indoor Air by Means of a Weak Cation Exchange Cartridge. *Environmental Toxicology and Chemistry*, Vol. 10, p. 1417-1423, 1991.

16.2 Nanni, E.J., Lovette, M.e., Hicks, R.D., Fowler, K.W. and Borgerding, M.F. Separation and Quantitation of Monovalent and Cationic Species in mainstream Cigarette Smoke Aerosols by High-Performance Ion Chromatography. *Journal of Chromatographic Science*, Vol. 28, August 1990.

-
- 16.3** IonPac CS12A Analytical Column, Installation Instructions and Troubleshooting Guide, Document No. 031132, Revision 01, Dionex Corporation, 1995.

APPENDIX

Appendix 1: Typical Chromatogram



An overlay of a standard and a Reference cigarette with a 5 % offset.

No: T - 202
Date: December 31, 1999
Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of the aromatic amine content (1- and 2-aminonaphthalene and 3- and 4-aminobiphenyl) of sidestream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specifications for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 SUMMARY OF METHOD

- 4.1 This method is used for the analysis of sidestream (SS) tobacco smoke using a fishtail chimney configuration. Sidestream smoke is all the smoke emitted from the lit end of a burning cigarette during the smoulder process. The glass fishtail chimney sits over a burning cigarette and allows the smoke to be directed in a controlled manner for the determination of sidestream tobacco constituents.
- 4.2 Aromatic amines of sidestream tobacco smoke are collected by passing the smoke from two cigarettes through a glass fibre filter disc (pad). The pad is placed in an Erlenmeyer flask with 100 mL of 5 % hydrochloric acid solution. The flask is shaken for 30 minutes on a wrist-action shaker and the contents filtered into a 500 mL separatory funnel. The internal standard (²H₉-4-aminobiphenyl) is spiked into the solution. The extract is washed with dichloromethane, made basic with sodium hydroxide solution and extracted with hexane. The hexane extracts are dried with sodium sulfate, derivatized with pentafluoropropionic acid anhydride and trimethylamine, passed through a florisil column, and quantitated by GC/MS.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Volumetric Flasks - Class A - 10 mL, 25 mL, 100 mL.
- 5.5 Pipettes - Class A - 20 µL, 50 µL, 100 µL, 250 µL, 500 µL, 1 mL or equivalent gas-tight syringe covering the range required.
- 5.6 Graduated Cylinder - 50 mL, 100 mL.
- 5.7 Fish-tail Chamber (mounted on a retort stand).
- 5.8 125 mL Polymethylpentene (PMP) Erlenmeyer flasks with screw-cap (or equivalent).
- 5.9 Separatory Funnels - 500 mL with glass stoppers.
- 5.10 Filter funnels - 10 cm internal diameter.
- 5.11 Round-bottom flasks - 500 mL.
- 5.12 Pasteur pipettes (disposable) with rubber bulbs.
- 5.13 Conical tubes - 15 mL.
- 5.14 Autosampler vials and caps with Teflon-lined septa (disposable).
- 5.15 Analytical balance measuring to at least four decimal places.
- 5.16 Vacuum pump (GAST or equivalent).
- 5.17 Victor flow meter calibrated at three litres per minute.
- 5.18 Tygon tubing.
- 5.19 Glass fibre filter pad holders and glass fibre filter pads.
- 5.20 Rotary Evaporator - with water bath set at 40 °C.
- 5.21 Turbo Evaporator - with water bath set at 40 °C.
- 5.22 Supelco SPE-system or equivalent.
- 5.23 GC/MS System - Autosampler, SPI Injector with high-performance insert, GC, Ion Trap Detector or equivalent.
- 5.24 GC Column - FS-capillary DB-5MS, 30 m X 0.25 mm ID X 0.25 µm or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

- 6.1 D₉-4-aminobiphenyl.
- 6.2 1-Aminonaphthalene - 95 % purity or better.
- 6.3 2-Aminonaphthalene - 95 % purity or better.
- 6.4 4-Aminobiphenyl - 98 % purity or better.

Note: 3-aminobiphenyl is not available.

- 6.5 Hydrochloric Acid - 32 %.
- 6.6 Hexane - Distilled in Glass (DIG).
- 6.7 Dichloromethane - (DIG).
- 6.8 Diethylether - (DIG).
- 6.9 Benzene - (DIG).
- 6.10 Acetone - (DIG).
- 6.11 Water - Type I (meets ASTM D 1193 specifications).
- 6.12 Sodium Hydroxide Solution - 50 %.
- 6.13 Sodium Sulfate - Granular.

- 6.14 Florisil SPE Columns (Supelco) – 6 mL X 1 g packing or equivalent.
- 6.15 Florisil (J.T.Baker) - deactivated 60-100 mesh or equivalent.
- 6.16 Pentafluoropropionic Acid Anhydride (PFPA).
- 6.17 Trimethylamine – 40 % wt solution in water.
- 6.18 pH Paper - high range.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 5 % Hydrochloric Acid - Add 312 mL of 32 % HCl solution to 1 L of Type I water, dilute to 2 L with Type I water. Mix very well.
- 8.2 TMA Solution - Add 2 mL of 40 % Trimethylamine Solution to a conical tube containing 2 mL of hexane. Vortex for one minute, let settle and transfer hexane to a 1.5 mL autosampler vial. Cap and refrigerate when not in use.
- 8.3 Florisil Elution Solution – 500 mL hexane, 400 mL benzene and 100 mL acetone. Mix well.

9 PREPARATION OF STANDARDS

9.1 Stock Solutions

- 9.1.1 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 4-aminobiphenyl (4-amb) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.2 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 1-aminonaphthalene (1-amn) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.3 A primary stock solution (1 mg/mL) is prepared by accurately weighing 25 mg of pure 2-aminonaphthalene (2-amn) into a 25 mL volumetric flask and diluting to volume with diethylether.
- 9.1.4 A mixed secondary stock solution is prepared by diluting the 100 µL of primary 4-amb solution, 500 µL of the primary 1-amn solution and 500 µL of primary 2-amn solution to 10 mL with hexane.
- 9.1.5 A tertiary stock solution is prepared by diluting 500 µL of the secondary stock solution to 25 mL with hexane. This solution is approximately 200 ng/mL in 4-amb and 1000 ng/mL in the aminonaphthalenes.

9.2 Internal Standard Solution (D₅-4-aminobiphenyl)

9.2.1 A primary stock solution (100 µg/mL) is prepared by accurately weighing 10 mg of pure D₉-4-aminobiphenyl (D₉-4amb) into a 100 mL volumetric flask and diluting to volume with hexane.

9.3 Internal Standard Spiking Solution (200ng/mL D₉-4amb) (ISTD)

9.3.1 The internal standard spiking solution is prepared by diluting 100 µL of the primary D₉-4-aminobiphenyl stock solution to 50 mL with hexane.

9.4 Working Standards

- 9.4.1** Standard 1 (80 ng/mL 4-amb & 400 ng/mL 1- and 2-amn) - Add 4 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFFA, vortex and let sit a minimum of 30 minutes. Proceed as under sample preparation from 14.2.2.
- 9.4.2** Standard 2 (40 ng/mL 4-amb & 200 ng/mL 1- and 2-amn) - Add 2 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFFA, vortex and let sit a minimum of 30 minutes. Proceed as under sample preparation from 14.2.2.
- 9.4.3** Standard 3 (20 ng/mL 4-amb & 100 ng/mL 1- and 2-amn) - Add 1 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFFA, vortex and let sit a minimum of 30 minutes. Proceed as under sample preparation from 14.2.2.
- 9.4.4** Standard 4 (10 ng/mL 4-amb & 50 ng/mL 1- and 2-amn) - Add 0.5 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFFA, vortex and let sit a minimum of 30 minutes. Proceed as under sample preparation from 14.2.2.
- 9.4.5** Standard 5 (5 ng/mL 4-amb & 25 ng/mL 1- and 2-amn) - Add 0.25 mL of tertiary stock solution and 1 mL of internal standard spiking solution to a 10 mL volumetric flask and make to the mark with hexane. Mix well. Transfer 1 mL of this solution to a conical tube, add 50 µL of TMA, 50 µL of PFFA, vortex and let sit a minimum of 30 minutes. Proceed as under sample preparation from 14.2.2.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:)

12.2.1.1 Smoking is conducted on four to eight ports of a linear 20 port smoking machine.

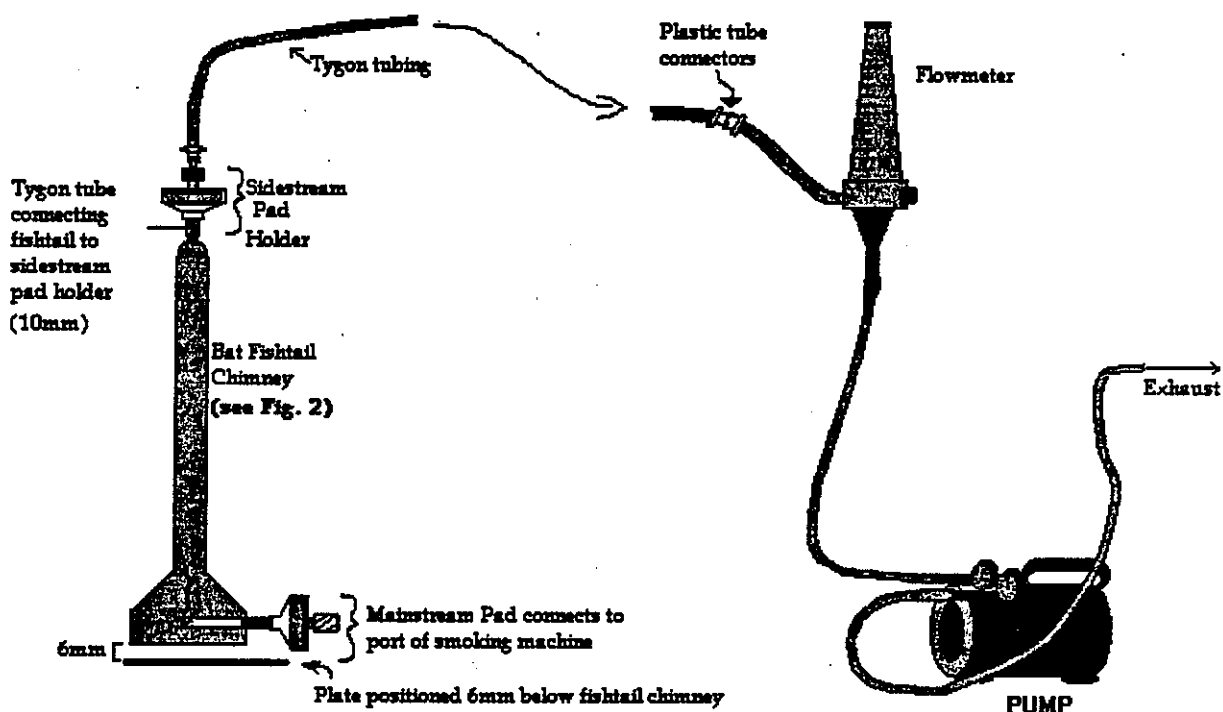


FIGURE 1e: SIDESTREAM APPARATUS

13 SAMPLE GENERATION

13.1 The mainstream smoke of two cigarettes* is passed through a pad attached to the port and the Total Particulate Matter (TPM) is determined.

13.2 The sidestream smoke is drawn up from the cigarettes, through the fishtail chamber and a cassette holding a pre-weighed glass fibre filter pad.

- 13.3** After smoking, weigh the SS filter holder for TPM, and place pad in an Erlenmeyer flask. Rinse the fishtail chimney into the flask with 100 mL of 5 % HCl, and cap. Store, until extracted, in a refrigerator at 4 °C.

*For other tobacco products, select a number such that breakthrough does not occur.

14 SAMPLE ANALYSIS

14.1 Liquid-Liquid Extraction

- 14.1.1** Mix sample well on a wrist-action shaker for 30 minutes.
- 14.1.2** Filter the contents of the flask through a filter funnel with a glass wool plug into a separatory funnel. Rinse the flask three times with 5 % HCl and transfer rinsing into the separatory funnel.
- 14.1.3** Add 100 µL of the ISTD solution to the separatory funnel. Stopper and shake.
- 14.1.4** Add 50 mL of dichloromethane to the separatory funnel. Shake with careful venting until there is no more pressure and let contents settle.
- 14.1.5** Draw off the dichloromethane layer into a beaker and discard as chlorinated waste.
- 14.1.6** Repeat 14.1.4 and 14.1.5 twice (total of three rinses).
- 14.1.7** Slowly add 10 to 15 mL of 50 % NaOH solution to the aqueous phase in the separatory funnel. Mix gently with careful venting, until venting no longer releases pressure, and check that the pH is above 12. If it is not, add 5 mL more NaOH. If more than 20 mL of NaOH need to be added, check that the HCl solution was made up properly.
- 14.1.8** Add 50 mL of hexane to the separatory funnel and shake VERY, VERY carefully with venting into the fume-hood. Shake until there is no more pressure and let contents settle.
- 14.1.9** Prepare a filter funnel with a plug of glass wool and approximately 100 g of sodium sulphate. Rinse the sodium sulphate with approximately 50 mL of hexane into a 500 mL round-bottom flask. Discard the rinse.
- 14.1.10** Draw bottom (aqueous) layer of the contents of the separatory funnel into a beaker and retain.
- 14.1.11** Pass the top (hexane) layer through the sodium sulphate into the 500 mL round bottom, spreading the hexane over the entire surface of the sodium sulphate.

14.1.12 Pour the aqueous layer in the beaker back into the separatory funnel and add 50 mL of hexane. Shake very carefully and let settle.

14.1.13 Repeat 14.1.10 to 14.1.12.

14.1.14 After the third extraction with hexane, the aqueous layer can be discarded.

14.1.15 Repeat 14.1.11.

14.1.16 Rinse the sodium sulphate with approximately 50 mL hexane into the round-bottom flask.

14.1.17 Add 50 µL of the TMA solution and 50 µL of the PFPA. Swirl and observe that a fine mist forms in the flask. Let sit for a minimum of 30 minutes (or overnight).

14.2 Solid Phase Extraction (SPE)

14.2.1 Evaporate the sample in the round bottom flask on the rotary evaporator to near dryness.

14.2.2 Prepare a florisil column by adding 2 g of florisil to a 6 mL X1 g florisil tube. Add a small amount of sodium sulfate to the top of the florisil.

14.2.3 Pre-wash with 5 mL of hexane: benzene: acetone (removing all air bubbles from the column).

14.2.4 Add approximately 1 mL of the hexane: benzene: acetone solution to the round-bottom flask (rinsing the solution down the sides of the flask).

14.2.5 Transfer the solution to the top of the florisil column and let drain to the top of the florisil into a 15 mL conical tube.

14.2.6 Repeat 14.2.4 and 14.2.5 two more times.

14.2.7 Rinse the florisil tube with hexane: benzene: acetone until approximately 15 mL have been collected in the conical tube.

14.2.8 Reduce the volume of the eluate in the tube in the TurboVap Evaporator under a gentle stream of nitrogen (3 to 5psi) until approximately 1 mL remains.

14.2.9 Make the volume in the tube up to approximately 1 mL if necessary with hexane and vortex briefly (approximately 10 seconds).

14.2.10 Transfer the contents to an autosampler vial, cap and store at 4°C until injected on the GC/MS.

14.3 GC/MS Operating Conditions

- 14.3.1** Injector Temperature: 60 °C for 0.5 minute.
180 °C per minute to 280 °C, hold to end of run.
- 14.3.2** Column Temperature: 80 °C for two minutes.
10 °C per minute to 210 °C.
20 °C per minute to 280 °C, hold at 280 °C for 3.5 minutes.
- 14.3.3** Transfer Line Temperature: 270 °C.
- 14.3.4** Manifold Temperature: 240 °C.
- 14.3.5** Column Head Pressure: 12 psi.
- 14.3.6** Injection Volume: 1 µL.
- 14.3.7** Scan Range: 100 to 330 amu .
- 14.3.8** Ion Peaks Used: m/z 315 for analyte (3- and 4-aminobiphenyl).
m/z 324 for internal standard (D₆-4aminobiphenyl).
m/z 289 for analyte (1- and 2-aminonaphthalene).
- 14.4** A calibration curve (ratio of each analyte's response to the ISTD response versus the amount of the analyte in ng/mL) is generated at the beginning of analysis from the five working standards. Quantitation is performed using the internal standard method available with the GC/MS software. A calibration curve for 3-aminobiphenyl is generated from the calibration for 4-aminobiphenyl. The spectra and retention times for these two analytes are established with the analysis of a control cigarette.
- 14.5** A Check Standard is run every 20 injections and is analyzed as a sample to confirm that the calibration is still valid. If the result differs by more than 10 % of the expected value for that standard, the calibration process must be repeated and a new calibration curve generated.
- 14.6** The amount of each analyte is reported in ng/cigarette and is calculated as follows:

$$\text{Analyte (ng/cigarette)} = \frac{\text{Amount of Analyte from Curve (ng/mL)} \times \text{Final Volume (1mL)}}{\text{Number of Cigarettes}}$$

Note: There is no volume dilution factor because all of the samples are concentrated to the final stage. The samples and standards are quantitated in the same manner.

15 QUALITY CONTROL

15.1 Typical Chromatograms

15.1.1 See Appendix.

15.2 Recoveries and Levels of Contamination

15.2.1 With every set of approximately 20 samples, include a laboratory reagent blank (LRB). The LRB is the pad plus extraction solution (100 mL of 5 % HCl) spiked with ISTD solution and taken through the sample preparation to determine if any of the reagents contribute to the aromatic amines. LRB results are consistently ND (not detected) for all analytes.

15.2.2 With every set of approximately 20 samples, include a laboratory fortified blank (LFB). A known amount of the tertiary stock solution is added to solution in a separatory funnel along with the ISTD and the solution is taken through the entire procedure, to determine if any aromatic amine is lost during the different stages. Recoveries of 2-aminonaphthalene and 4-aminobiphenyl are typically 90 ± 10 %. Recoveries of 1-aminonaphthalene are somewhat lower (70 to 80 %) due to the more volatile nature of the derivatized 1-aminonaphthalene.

15.2.3 To assess potential matrix interference, a laboratory fortified matrix (LFM) can be analyzed. A sample of the control cigarette can be split before extraction and each half treated as a separate sample. One of the split halves should be spiked with a known amount of the tertiary stock solution at approximately the level expected in the sample. Recoveries should be very close to 100 %. Typical recoveries are:

4-aminobiphenyl - 98.4 %.
2-aminonaphthalene - 96.8 %.

15.3 Method Detection Limit (MDL) and Limit of Quantitation(LOQ)

15.3.1 For GC/MS analysis, the detection limit can be defined as a peak whose signal to noise ratio (S/N) is three to one. The limit of quantitation can be defined as a S/N of 10 to one. The lowest standard typically run for 4-aminobiphenyl is 1 ng. This peak gives a S/N of approximately 20 to one and a corresponding MDL of approximately 0.08ng/cigarette and an LOQ of approximately 0.25 ng/cigarette for a smoking run of two cigarettes.

15.4 Stability of Reagents and Samples

15.4.1 Store ISTD spiking solution in a 25 mL amber vial with open cap and Teflon-lined septum. Use a 100 μ L gas-tight syringe to transfer the ISTD from the vial to the separatory funnel. Do not touch the sides of the separatory funnel with the tip of the syringe (to avoid contaminating the ISTD). Wash the syringe with hexane between samples and change the septum daily. Store the ISTD at 4 °C when not in use.

15.4.2 There is no apparent problem with the stability of the stock or underivatized working standards. Derivatize fresh 1 mL aliquots of the working standards weekly and replace the red Teflon-lined septa after each injection to minimize contamination from the septa.

15.4.3 Samples should be extracted within one week of being produced.

15.4.4 The area responses of the analytes (including the internal standards) in the calibration standards and the LFBs are occasionally lower than in the samples and LFMs. Investigation has suggested that analyte loss occurs if the standards or LFBs are taken to complete dryness during rotary evaporation or turbo evaporation. The samples do not appear to be affected in the same way, possibly due to the matrix acting as a "keeper" during the solvent removal stages. As a cautionary measure, no samples or standards are taken to complete dryness at any stage of the process.

16 REFERENCES

- 16.1** Pieraccini, G., F. Luceri, and G. Moneti, 1992. New Gas-Chromatographic/Mass-Spectrometric Method for the Quantitative Analysis of Primary Amines in Main- and Sidestream Cigarette Smoke. I. *Rapid Communications in Mass Spectrometry*. 6, p. 406-409.

APPENDICES

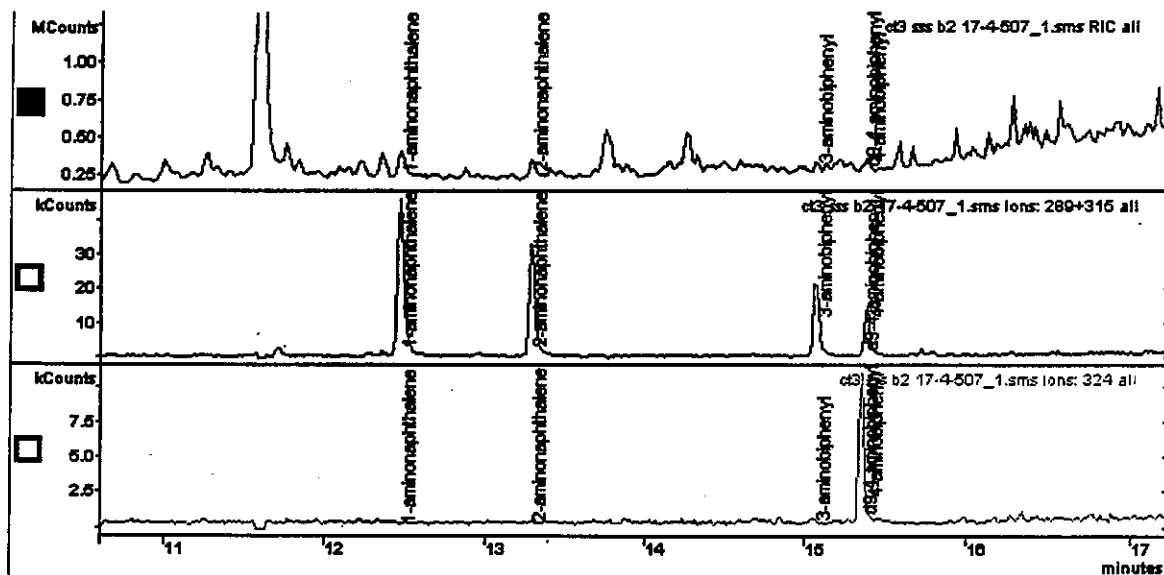


Figure 1: Total Ion Chromatogram (TIC) for a Control Cigarette and ion chromatograms for the aminonaphthalenes (m/z 289), aminobiphenyls (m/z 315) and D_9 -4-aminobiphenyl (m/z 324).

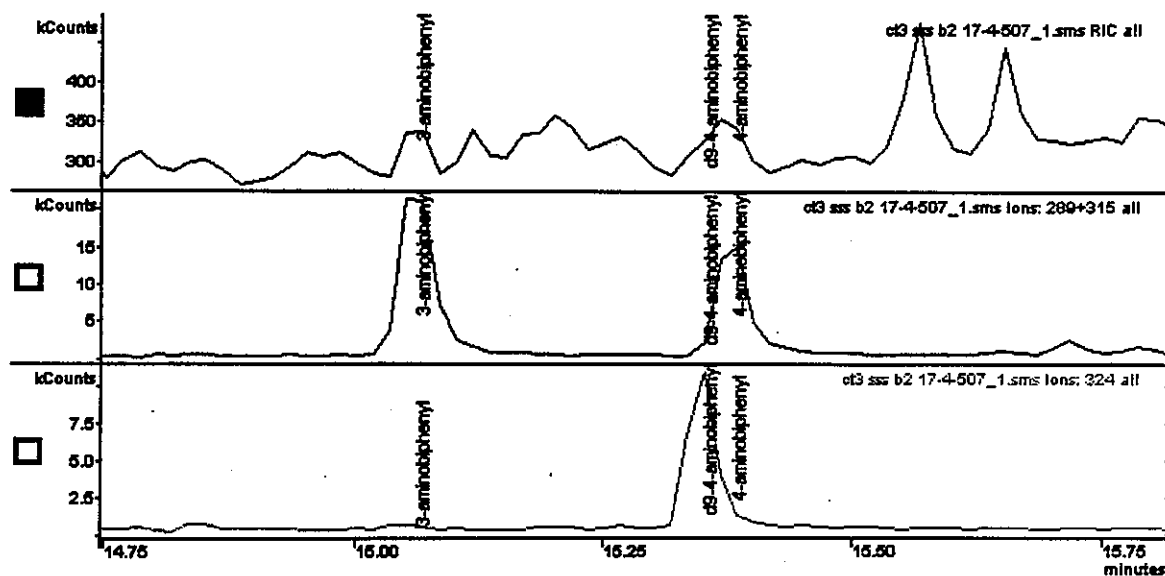


Figure 2: Expanded view of Figure 1, showing resolution of D_9 -4-aminobiphenyl and 4-aminobiphenyl.

No.: T - 203
Date: December 31, 1999
Page: 1 of 12

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the quantitation of Benzo[a]pyrene B[a]P content in total particulate matter (TPM) of sidestream (SS) tobacco smoke by reversed phase high performance liquid chromatography (HPLC) via fluorescence detection.

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193 -77 Standard Specification for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 - Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.3 Methods of Sampling and Testing Tobacco - Determination of Benzo[a]pyrene in Total Particulate Matter of Tobacco Smoke. *National Standard of Canada*, Canadian General Standards Board CAN/CGSB-176.2 No. 1-96, March 1996.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Cigarettes are smoked beneath a British American Tobacco (BAT) fishtail chamber with a 3 L/minute flow rate in order to capture the sidestream smoke.
- 4.2 Total particulate matter (TPM) of the sidestream smoke, collected on a glass fibre filter disc (pad), is extracted with a sufficient amount of acetone to create a homogeneous solution of tar in acetone. A portion of this solution is filtered through a 0.45 µm syringe filter to a 7 mL glass vial for storage.
- 4.3 A 2 mL aliquot of this extract is evaporated under a constant stream of nitrogen to dryness and reconstituted in 2 mL of cyclohexane. This cyclohexane solution is passed through a 1 g (6 mL) silica cartridge and 360 mg NH₂ plus cartridge mounted in series. The B[a]P is eluted with hexane, evaporated to dryness, and made to a 1 mL volume with acetonitrile.
- 4.4 The sample is subjected to reversed phase liquid chromatography and quantitated via fluorescence detection.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.

- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Supelco Visi-Prep Solid Phase Extraction unit (24 cartridge unit) or equivalent.
- 5.5 2 mL glass pipettes.
- 5.6 Brinkman Dispensette (10-50 mL) or equivalent.
- 5.7 Micro-pipettes (5, 1000 μ L).
- 5.8 16 X 125 mm culture tubes (20 mL).
- 5.9 125 mL Erlenmeyer flasks with ground glass joints or PMP Erlenmeyers with screw top caps.
- 5.10 Zymark turbo-vap or equivalent.
- 5.11 GAST Pumps or equivalent.
- 5.12 Flowmeters.
- 5.13 BAT Fishtail Chambers.
- 5.14 Glass fibre filter discs (pads) and holders (45 mm).
- 5.15 Disposable 5 cc syringe.
- 5.16 Auto sampler vials, caps and septa.
- 5.17 Pasteur Pipettes.
- 5.18 1 g silica Sep-Pak cartridges (6 mL capacity).
- 5.19 360 mg NH_2 Plus Sep-Pak cartridge.
- 5.20 7 mL screw top vials with aluminum lined cap.
- 5.21 0.45 μ m glass fibre syringe filters 25 mm.
- 5.22 Merck 250 X 4 mm, RP-18e, 5 μ m packing, HPLC column with a Lichrocart 4-4 Lichrosphere 100 RP-18 endcapped, 5 μ m guard column or equivalent.
- 5.23 High Performance Liquid Chromatograph with:
 - 5.23.1 Fluorescence detector.
 - 5.23.2 Autosampler.
 - 5.23.3 Tertiary pump.
 - 5.23.4 Data collection system.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

- 6.1 Benzo[a]pyrene (B[a]P).
- 6.2 Cyclohexane.
- 6.3 Hexane.
- 6.4 Acetonitrile.
- 6.5 Methanol.
- 6.6 Isopropanol (IPA).
- 6.7 Anhydrous Sodium Sulfate.
- 6.8 Acetone.
- 6.9 Tetrahydrofuran (THF).
- 6.10 Type I water (meets ASTM D 1193 specifications).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Prepare solutions required for analysis, as specified in T-115, in accordance with good laboratory practice.

9 PREPARATION OF STANDARDS

9.1 Preparation of Spiking Solution for Laboratory Fortified Samples

9.1.1 Primary B[a]P Stock: Dissolve 10 mg B[a]P solid to 50 mL Cyclohexane.

9.1.2 Secondary Stock: Pipette 50 μ L of 1° Stock to 50 mL Cyclohexane.

9.1.3 A 10 μ L volume of spiking solution is added to a second 2 mL aliquot of a control brand cigarette extract solution prior to solvent substitution and clean-up through the SPE cartridges (LFM*). Another 10 μ L volume of spiking solution is added to a second 2 mL aliquot of the LRB* prior to solvent substitution and clean-up through the SPE cartridges (LFB*). The spiking analytical concentration is approximately 2 ng/mL (dependent on stock concentration).

*See section on Quality Control for explanations of these initialisms.

9.2 Preparation of Working Standards

9.2.1 Primary (1°) B[a]P Standard: Dissolve 10 mg B[a]P to 50 mL Acetonitrile.

9.2.2 Secondary (2°) Standard: Pipette 100 μ L of 1° Standard to 50 mL Acetonitrile.

9.3 Working Standards:

Standard #	Volume of (2°) Standard (μ L)	Final Volume (mL)	Concentration [ng/mL]
1	40	25	0.6400
2	175	25	2.800
3	350	25	5.600
4	600	25	9.600
5	900	25	14.4
6	2 mL of Std # 1	10	0.1280
7	4 mL of Std # 1	10	0.2560

9.3.1 All weights, volumes, and purity must be recorded and used to accurately calculate the standard concentrations. These concentrations are only representations of standards used in a calibration curve.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product is to be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks, and cigars are to be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

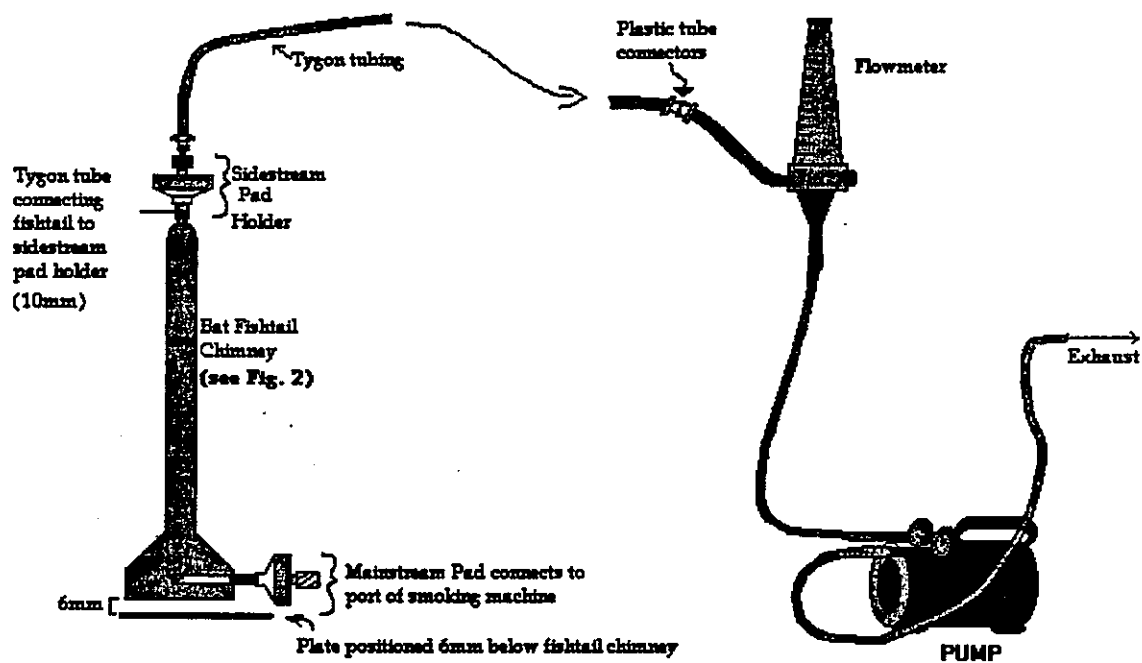
12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:
 - 12.2.1.1** The B[a]P sidestream apparatus (smoke train) is assembled as per diagram.
 - 12.2.1.2** The flowmeters are calibrated so that a measured flow rate of 3 L/minute could be monitored with the sidestream filter pad present. Calibrate all ports recording the flowmeter setting.
 - 12.2.1.3** Weigh conditioned cigarettes (three cigarettes* / observation) and the sidestream filter holder prior to smoking.

*For other tobacco products, select a number such that breakthrough does not occur.
 - 12.2.1.4** Place the conditioned cigarettes in position using the calibrated ports.

**FIGURE 1a: SIDESTREAM APPARATUS**

13 SAMPLE GENERATION

13.1 Turn on the sidestream pumps 30 seconds prior to the first puff taken by the smoking machine.

13.2 Light the cigarette on the first puff with the cigarette below the fishtail.

Note: Special consideration of lighting procedures for specific types of cigarettes must be followed.

13.3 Lower the fishtail directly over the cigarette positioning a plate 6 mm below the fishtail to create a uniform air flow up the chimney.

13.4 When the cigarette is smoked to the end mark, completely remove the butt from the chamber as to no longer contribute any more sidestream smoke. Turn off the pump 30 seconds after the cigarette has been extinguished in order to pull all smoke present in the chimney through the sidestream filter pad.

13.5 Repeat steps 13.1 through 13.4 with the second and third cigarette.

13.6 After the smoking is complete, disassemble the sidestream apparatus and weigh the sidestream filter holder to determine TPM.

13.7 Record weights of TPM for sidestream pads.

14 SAMPLE ANALYSIS**14.1 Extraction of Sidestream Pads**

14.1.1 Remove the sidestream pad from its holder, folding it into quarters and wiping the inside of the holder with the clean side of the pad.

14.1.2 Add this sidestream pad to a 125 mL Erlenmeyer flask and cap.

14.1.3 Rinse the fishtail chimney with a volume of acetone, such that the final concentration of TPM in the flask is approximately 1 mg/mL, collecting the rinse in the 125 mL Erlenmeyer flask containing the pad.

For example: The volume of acetone used to rinse the fishtail is numerically equivalent to the total TPM in mg (rounded to the nearest 10 mL) yielding a concentration of approximately 1 mg TPM/mL of acetone.

Example: 1. If TPM = 70 mg (total), then acetone volume = 70 mL.

Example: 2. If TPM = 83 mg (total), then acetone volume = 80 mL.

Example: 3. If TPM = 57 mg (total), then acetone volume = 60 mL.

Note : The maximum amount of acetone to be added is 100 mL.

-
- 14.1.4** Record the volume of acetone used to rinse the fishtail and subsequently extract the pad.

14.1.4.1 Shake the Erlenmeyer containing the sidestream pad and acetone vigorously on a wrist action shaker for 30 minutes, until there appears to be a homogeneous solution and there is no longer any evidence of localized colour on the pad.

14.1.4.2 Place the flasks in the dark to allow some of the broken-up pad to settle.

14.2 Sample Clean-up

14.2.1 Filter approximately an 8 mL portion of the acetone extract through a 0.45 µm disposable filter into a 7 mL vial with foil lined cap (samples may be stored at 4 °C at this point).

14.2.2 Pipette a 2 mL aliquot of the acetone extract to a 16 X 125 mm culture tube.

14.2.3 Place the tubes containing the 2 mL sample into the turbo evaporator.

Note: Turbo-vap conditions are to be set at 40 °C with a Nitrogen pressure of 7.5 psi.

14.2.4 Evaporate the samples to complete dryness.

14.2.5 Redissolve the sample by pipetting 2 mL of cyclohexane to the tube and vortex for 15 seconds (vortex each sample twice).

14.2.6 Pre-condition both (the silica and the NH₂ plus) cartridges using hexane as recommended by manufacturer.

Note: All air has to be removed from the packing thus exposing sorbent material to solution.

14.2.7 Place the pre-conditioned cartridges on the Visi-prep unit, add approximately 1 g anhydrous sodium sulfate to the silica cartridge, and wash the cartridges with 10 mL hexane allowing the hexane to flow through the cartridge by gravity.

14.2.8 Transfer the 2 mL cyclohexane solution onto the packing of the Silica cartridge.

Note: High tar samples containing high amounts of B[a]P may require only 250-500 µL to be transferred.

14.2.9 Allow the cyclohexane to pass through the SPE cartridges (by gravity) at a rate of approximately one drop/second. Discard the eluant.

14.2.10 Pipette 4 mL hexane to the cartridge allowing the eluant to gravimetrically pass through the cartridge. Discard the eluant.

Note: If the entire sample has been transferred, use these volumes of hexane to further wash the culture tube to ensure the sample has been quantitatively transferred.

Note: Elution volumes must initially be determined with the use of a different manufacturer's cartridge and should be checked between lot numbers.

14.2.11 Place 20 mL disposable glass culture tubes beneath each of the cartridges.

14.2.12 Gravimetrically elute the B[a]P from the cartridges with 4 X 4 mL additions of hexane.

14.2.13 Add 1 mL of THF to each tube.

14.2.14 Place the tubes containing the 17 mL of collected eluant into the Zymark Turbo-vap.

Note: Turbo-evaporation conditions are to be set at 40 °C with a Nitrogen pressure of 7.5 psi.

14.2.15 Evaporate the samples to complete dryness.

Note: This will require an initial 20 minutes of evaporation in which the nitrogen pressure may be slowly increase to a maximum of 10 psi in such a manner as to prevent any loss of sample from splattering.

14.2.16 Remove samples that are completely dry. If some samples are not completely dry, evaporate the samples in additional five minutes intervals.

14.2.17 Pipette 1000 µL of Acetonitrile into each of the dried tubes to dissolve the analyte and any residue that may be present.

14.2.18 Vortex the sample at high speed for approximately 15 seconds.

14.2.19 Using a glass transfer pipette, wash down the sides of the tube five times with the sample, and transfer the sample to a 2 mL autosampler vial with a screw cap and Teflon faced septa.

14.2.20 The samples are ready for HPLC analysis and may be stored at 4 °C until they are analyzed.

14.3 Instrument Analysis: Reversed Phase High Performance Liquid Chromatography (HPLC) Analysis

14.3.1 Jasco Fluorescence Detector Conditions

Excitation Wavelength:	365 nm.
Emission Wavelength:	425 nm.
Gain:	X 1000.
Attenuation:	32.

Note: A different manufacturer's fluorescence detector may need to be programmed differently to maintain the full calibration range. A slight change in excitation and emission wavelength may be required dependent on manufacturer (i.e. 366 and 424 for the wavelengths).

14.3.2 Autosampler : Injection Volume

14.3.2.1 Analyze using a 50 μ L sample loop with the parameter for injection volume in the sample list at 75 μ L to ensure a thorough flushing of the sample loop with the sample.

14.3.3 Mobile Phase / Gradient Conditions (Tertiary Gradient System)

Solvent A: 55: 45 Acetonitrile: 1 % IPA in Type I water (degassed and filtered with a 0.45 μ m nylon filter).
Solvent B: Methanol.
Solvent C: Acetonitrile.
Flow: 1.5 mL/minute.
Gradient: Adjustments to the gradient may be required depending on column conditions and resolution of analyte.

Time (minutes)	% A	Composition % B	% C
0.00	55	0	45
20.00	75	0	25
25.00	100	0	0
28.00	100	0	0
30.00	0	100	0
32.00	0	100	0
34.00	100	0	0
35.00	100	0	0
35.00	Method End Action:		Equilibrate

Equilibration Time: 8.00 minutes.

14.4 Calculations

14.4.1 Determination of Response Factor (RF)

14.4.1.1 An initial calibration is performed by running prepared standards from high concentration to low concentration (injecting the very first standard a minimum of two times until the response and retention time are constant).

14.4.1.2 A calibration curve is prepared by plotting the concentration of B[a]P in the standard vs. the peak height response from the fluorescence detector.

14.4.1.3 The Response factor is the slope of the line as determined by linear regression (Height counts / unit concentration).

14.5 Determination of B[a]P Delivery [ng/cig]

$$\text{14.5.1 } \text{B[a]P [ng/cig]} = \frac{\text{Peak Height} \times \text{Volume Extractant (mL)} \times \text{Final Volume (mL)}}{\text{RF} \times \# \text{ Cigarettes Smoked} \times \text{Aliquot Volume (mL)}}$$

where the aliquot volume (mL) is the volume transferred to the Sep-pak cartridge correcting for any potential previous dilutions in the solvent substitution step. The Response Factor is to be determined from the calibration curve.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1a and 1b.

15.2 Typical Control Parameters

15.2.1 Each set of analysis should contain at least one of each of the following per day of smoking or batch of up to 16 samples (four sets of four samples).

15.2.1.1 Laboratory Reagent Blank (LRB): To determine background contamination from solutions, glassware, or materials used in the analysis process.

15.2.1.2 Laboratory Fortified Blank (LFB): To determine whether there is any loss of analyte as a result of the analysis process.

15.2.1.3 Laboratory Fortified Matrix (LFM): By spiking of the control brand cigarettes: To determine whether there is any loss of analyte as a result of the analysis process and to determine potential matrix effects.

15.2.1.4 Reference Sample: To determine the inter-experimental reproducibility of the entire method of analysis.

15.2.1.5 Duplicate Sample: To determine the reproducibility of the procedure within the same experiment or batch on analysis.

15.3 Recoveries and Levels of Contamination

15.3.1 Typical recoveries of Laboratory Fortified Blanks (LFB) and Laboratory Fortified Matrix (LFM) samples range from 85 – 110 % when a spiked solution (or sample) is carried out through the entire extraction process.

15.3.2 Recoveries lower than 85 % indicate either an insufficient elution of B[a]P from the solid phase extraction cartridges or a change in response factor (RF) of the fluorescence detector. A change in RF must first be investigated before re-processing of samples is initiated.

15.3.3 Typical Laboratory Reagent Blanks (LRB) range from a calculated value of 0 - 0.3 ng/cigarette. Contamination of this type is usually associated with contamination of the filter pad during conditioning or an inadequate cleaning of glassware.

15.4 Method Detection Limits (MDL) / Limit of Quantitation (LOQ)

15.4.1 The method detection limit (MDL) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.

15.4.2 The MDL (on a ng/cig basis) can be modified by varying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure.

15.4.3 The practical limit of quantitation (LOQ) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.

15.5 Stability of Reagents and Samples

15.5.1 Analytical stocks and standards should be stored at -20 °C.

15.5.2 Stock standards and stock spike solutions remain stable for up to six months. Although there is no loss of analyte, evaporation (loss) of solvent may be an issue.

15.5.3 Analytical run standards should be freshly prepared every two months.

15.5.4 Samples are stable at 4 °C for three weeks after extraction.

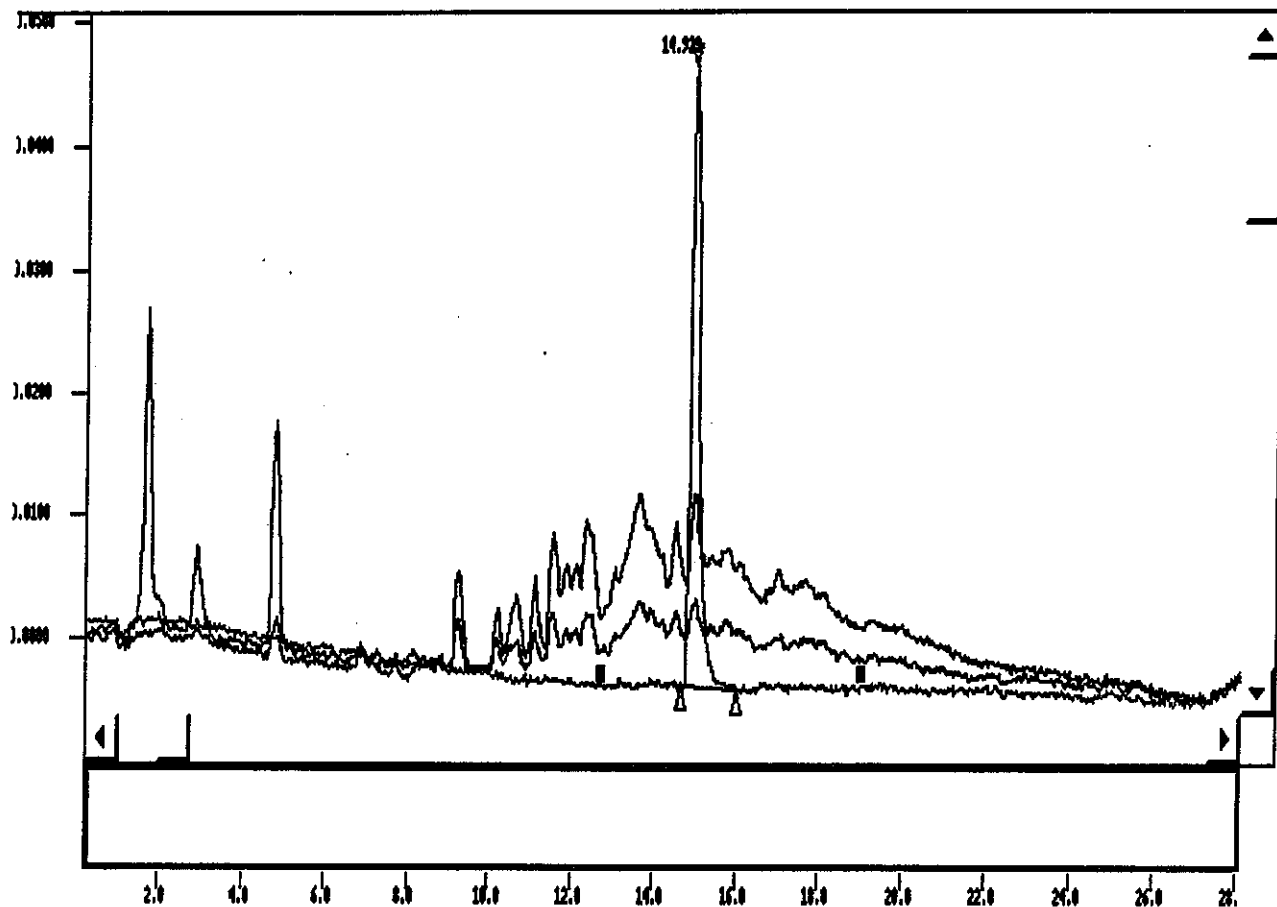
16 REFERENCES

16.1 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

16.2 Dumont, J., Larocque-Lazure, F., and Iorio, C., 1993. An Alternative Isolation Procedure for the Subsequent Determination of Benzo[a]pyrene in Total Particulate Matter of Cigarette Smoke. *Journal of Chromatographic Science*, Vol. 31. September 1993. p. 371-374.

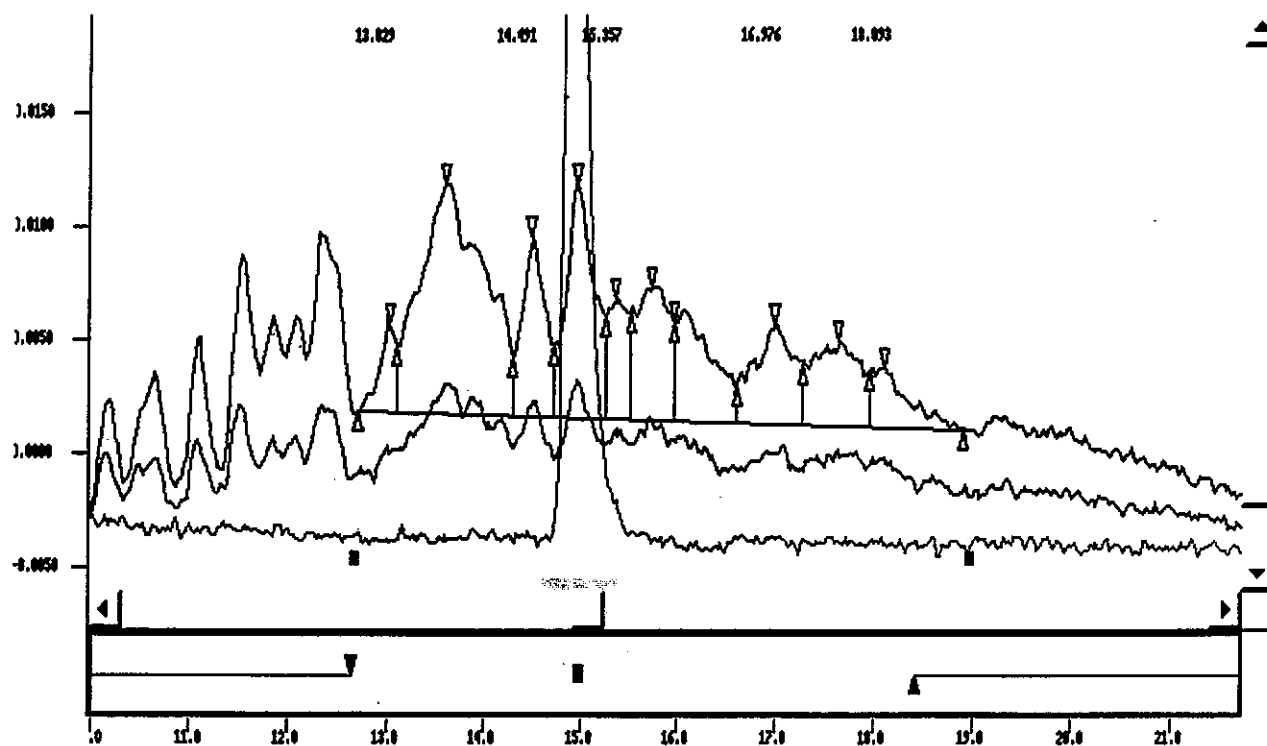
APPENDICES

Appendix 1a: Typical Chromatogram



An overlay of a standard, a high tar reference cigarette and a low tar reference cigarette.

Appendix 1b: Typical Chromatogram



An expanded view of Appendix 1a to show the integrating baseline of a true sample.

No.: T-204{PRIVATE}
Date: December 31, 1999
Page: 1 of 14

1 SCOPE OF APPLICATIONS

- 1.1 This method describes the collection of major volatile carbonyls (as their 2,4-dinitrophenylhydrazones [DNPH]) in sidestream tobacco smoke using a fishtail chimney assembly with separation and quantification by reversed phase high performance liquid chromatography (HPLC). The carbonyls determined are formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, and butyraldehyde and the method can be applied to cigarettes, cigarette equivalents, bidis, kreteks and cigars.
- 1.2 This method is applicable to the carbonyl compounds extracted from the sidestream (SS) vapour phase (including SS filter pad) and quantitated from the DNPH trapping solution only.

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.2 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specification for Reagent Water, Version 1977.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Four equidistant ports of a standard 20 port linear smoking machine are reconfigured with the British American Tobacco Company (BAT) fishtail chambers and flow-controlled vacuum pumps.
- 4.2 Cigarettes are smoked beneath the fishtail chambers and the smoke is drawn up the chimney by vacuum at the rate of 2 L/minute.
- 4.3 The total particulate matter (TPM) of the sidestream smoke is collected on a glass fibre filter pad at the top of the chimney and the filtered sidestream vapour phase is then bubbled through an impinger containing 100 mL of 2,4-dinitrophenylhydrazine (DNPH) in aqueous acetonitrile.
- 4.4 After smoking two cigarettes*, the fishtail chimney is rinsed with 2 X 20 mL aliquots of fresh DNPH solution which is collected in a glass-stoppered Erlenmeyer flask.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.5 The sidestream glass fibre filter disc (pad) is placed in the flask that contains its corresponding 40 mL fishtail rinsing and is extracted by wrist-action shaking.
- 4.6 The impinger solution is then added to the Erlenmeyer flask and well mixed.
- 4.7 An aliquot of this combined TPM-vapour phase extract is then syringe filtered and diluted with 1 % Trizma in aqueous acetonitrile.
- 4.8 The samples are subjected to reversed phase high performance liquid chromatography (HPLC) and quantitated via ultra violet detection

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

The sample preparation and analysis should be completed in one day and the solvent waste generated by the HPLC must be stored for disposal by a registered chemical-recycling agency.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-15.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Analytical Balance capable of measuring to four decimal places.
- 5.5 Eight Glass Drechsel Type traps (cap 250 mL) with impingers.
- 5.6 Nalgene Tubing - 1/4" ID X 3/8" OD.
- 5.7 Vacuum Pumps - GAST or equivalent.
- 5.8 Flow meters.
- 5.9 Fishtail Chambers - BAT.
- 5.10 Volumetric flasks 10 mL, 25 mL, 50 mL, 1 L, and 2 L.
- 5.11 Erlenmeyer flasks with ground glass joints 250 mL.
- 5.12 Glass Micropipettes - assorted volumes (100, 150, 300, 400, 500, 800, 1000, and 2000 µL).
- 5.13 Glass Transfer Pipettes - 1, 2, 5, 6, 7, 8, and 20 mL.
- 5.14 Syringe Filters - 0.45 µm PVDF.
- 5.15 Disposable syringes - 5 cc.
- 5.16 Glass Graduated Measuring Cylinders - 25 mL and 50 mL.
- 5.17 Disposable Glass Pasteur Pipettes.
- 5.18 Rubber Bulbs.
- 5.19 Autosampler vials (amber), screw caps and Teflon-faced septa.
- 5.20 Wrist Action Shaker.
- 5.21 Mini Hot Plate / Stirrer.
- 5.22 PC controlled High Pressure Liquid Chromatography System (or equivalent) consisting of:
 - 5.22.1 Tertiary gradient pump.
 - 5.22.2 Autosampler with 50 µL sampling loop.
 - 5.22.3 UV Detector.
 - 5.22.4 Work Station.

5.22.5 Column: Merck Lichrosphere 250 X 4 mm, 100, RP 18e (5 μ m) or equivalent.

5.22.6 Disposable Guard Column: Lichrocart 4 X 4 mm, Lichrosphere RP 18e (5 μ m) or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Methanol - Distilled-in-glass (DIG).
- 6.2 Acetonitrile (MeCN) - (DIG).
- 6.3 Isopropanol (IPA) - (DIG).
- 6.4 Ethyl Acetate - (DIG).
- 6.5 Tetrahydrofuran (THF) - (DIG).
- 6.6 Reagent Alcohol HPLC Grade.
- 6.7 Perchloric Acid (60 %).
- 6.8 Hydrochloric Acid (35 %).
- 6.9 Concentrated Sulphuric Acid (H₂SO₄).
- 6.10 Type I water (meets ASTM D 1193 specification).
- 6.11 Formaldehyde Solution 37-41 % (w/v).
- 6.12 Acetaldehyde > 99 % purity.
- 6.13 Acetone - (DIG).
- 6.14 Acrolein > 99 % purity.
- 6.15 Propionaldehyde > 97 % purity.
- 6.16 Crotonaldehyde > 99+ % purity.
- 6.17 Methyl Ethyl Ketone > 99+ % purity.
- 6.18 Isobutyraldehyde > 99 % purity.
- 6.19 Butyraldehyde > 99+ % purity.
- 6.20 Trizma Base.
- 6.21 Helium - UHP grade.
- 6.22 Parafilm® or equivalent.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

- 7.1.1 It is extremely important that all possible sources of contamination are removed from the work area: e.g. acetone solvent wash bottles.

8 PREPARATION OF SOLUTIONS

8.1 Preparation of DNPH Solution

- 8.1.1 Weigh 6.792 g (24.0 mmol) of commercially available 2,4 dinitrophenylhydrazine (DNPH). Add to 1 L of fresh acetonitrile in a 2 L volumetric flask. Dissolve DNPH by alternating: gently swirling and warming the flask. Make sure there are no crystals remaining before proceeding. (Warning! Do not sonicate.)
 - 8.1.2 After the DNPH is dissolved, add 5.6 mL 60 % perchloric acid with gentle mixing. The solution will turn yellow at this point.
 - 8.1.3 Dilute to volume with Type I water. The solution will turn to a bright orange upon addition of the water.

8.1.4 Store the solution in a 4 L amber bottle at room temperature in the dark to reduce the chances of DNPH precipitation. This solution, if properly sealed, will remain stable for one week under these conditions.

8.2 Preparation of Trizma Base Dilution Solution (80:20, MeCN:1 % aqueous Trizma)

8.2.1 Dissolve 2.00 g of Trizma Base in 200 mL of Type I water in a 1 L volumetric flask.

8.2.2 Dilute to volume with acetonitrile.

8.2.3 Store in a 1 L amber bottle with Teflon lined cap at room temperature. This solution should remain stable for several weeks under these conditions.

9 PREPARATION OF STANDARDS

9.1 Preparation of Dinitrophenylhydrazone Derivatised Carbonyls

9.1.1 Dissolve 600 mg commercially available DNPH in 2 mL concentrated H_2SO_4 in a 50 mL Erlenmeyer flask.

9.1.2 Stir with a glass rod while adding 3 mL of Type I water (clear solution). Then add 10 mL of reagent alcohol.

9.1.3 Add the DNPH solution to a solution of the appropriate aldehyde or ketone containing (each as an individual preparation):

- 120 mg formaldehyde
- 50 mg acetaldehyde
- 40 mg acetone
- 40 mg acrolein
- 40 mg propionaldehyde
- 35 mg crotonaldehyde
- 33 mg methyl ethyl ketone
- 33 mg butyraldehyde.

Crystallisation generally occurs rapidly.

9.1.4 Filter crystals (hydrazones) using vacuum filter and rinse the crystals with cold (4 °C) reagent alcohol.

9.1.5 Recrystallization of hydrazones: Add about 10 mL reagent alcohol to the crystals in a small Erlenmeyer flask, heat and then add 3 mL ethyl acetate dropwise to dissolve crystals. Cool to room temperature.

9.1.6 Filter crystals under vacuum, rinse with cold (4 °C) reagent alcohol, air dry and then store in vials in desiccator at -20 °C.

9.2 HPLC Calibration Standards and Working Solutions

9.2.1 Primary (1°) Carbonyl Standards

9.2.1.1 Weigh purified hydrazones as described in **Appendix 1(a)**. Put into individual 25 mL volumetric flasks and dissolve in acetonitrile. Concentration is of the free aldehyde.

9.2.1.2 Seal volumetric flask with parafilm and refrigerate at 4 °C. When properly stored, solutions are stable for up to one year.

9.2.2 Secondary (2°) Carbonyl Standards

9.2.2.1 Pipette predetermined volumes of each primary hydrazone stock standard into a single 25 mL volumetric flask and dilute up the mark with acetonitrile. See **Appendix 1(a)**.

9.2.2.2 Seal volumetric flask with parafilm and store at 4 °C. Prepare fresh every 20 days.

9.2.3 Carbonyl Working Standards

9.2.3.1 Take appropriate volumes (0.050 to 7.5 mL) of the 2° carbonyl standard and dilute to 10 mL with acetonitrile to give calibration standards with approximate carbonyl concentrations in the ranges noted in **Appendix 1(b)**.

9.2.3.2 Transfer to autosampler vials.

9.2.3.3 Carbonyl calibration standards should be prepared fresh every 20 days.

9.2.4 Carbonyl Spiking Solution

9.2.4.1 Pipette predetermined volumes of each primary hydrazone stock standard into a single 25 mL volumetric flask and dilute up the mark with acetonitrile (See **Appendix 1(c)**).

9.2.4.2 Prepare fresh every 20 days.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

-
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.1.1 The carbonyl sidestream fishtail assembly with vacuum pumps is assembled as per the following diagram:

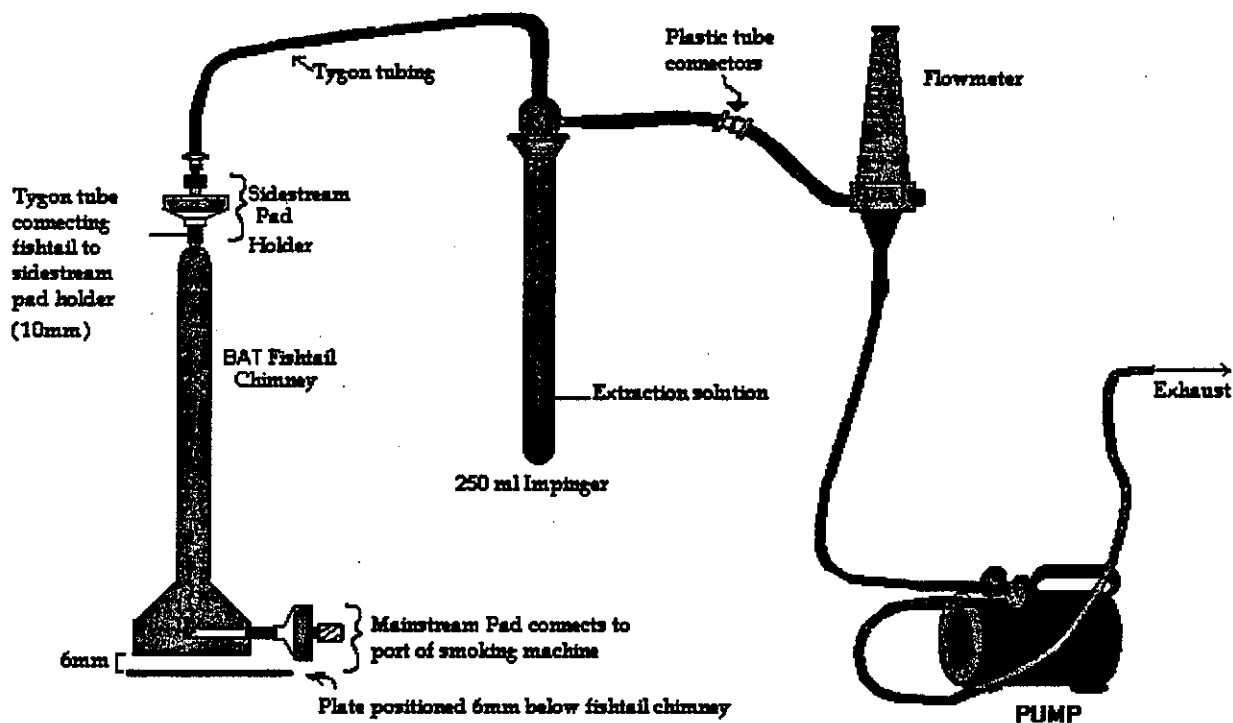


FIGURE 1a: SIDESTREAM APPARATUS

12.2.2 Add 100 mL of DNPH solution to the 250 mL impinger.

12.2.3 Install the sidestream filter holder at the top of the fishtail chamber and place the impinger onto rear section of smoke machine.

12.2.4 Tubing from impinger front (internal stem connection) attaches to SS pad holder and from impinger rear (bulb) to flow meter and to the vacuum pump.

12.2.5 Calibrate the vacuum pumps to deliver a flow rate of 2 L/minute with the impinger solution present. Record the settings.

12.2.6 Dispose of the original solution and replace with fresh DNPH solution once the impinger and trap has been calibrated and flushed. (This is to avoid contamination from room air or the flow meter itself).

12.2.7 Raise chimney level to highest position (loading position).

12.2.8 Attach the mainstream filter pad holders to the corresponding port on the smoking machine.

12.2.9 Using the vacuum bar, install the first test cigarette to be smoked in position below the fishtail in the four pre-calibrated ports. Gently insert the cigarette into the cigarette holder to the 9 mm mark.

12.2.10 Using forceps and disposable latex gloves, fit a pre-conditioned pad into each of the numbered pad holders with the rough side towards the incoming smoke.

13 SAMPLE GENERATION

Note: It is important to ensure that at least 25 to 100 mg of TPM has been deposited on the sidestream glass fibre disc (pad) before proceeding with the analysis. This can be accomplished if the sidestream TPM is determined in accordance with T-115 (i.e. the net difference in the weight of the pad before and after smoking).

13.1 Turn on sidestream vacuum pump 30 seconds before the first puff is taken.

13.2 Light the cigarette (on the first puff) and initiate the puff count according to the following schedule.

13.3 Normal lighting procedure is 15 second warm-up beginning at t-18 seconds followed by five second ignition (three seconds prior to puff plus the two second puff).

13.4 Lower the fishtail chimney over the cigarette to a position 6 mm above a plate that is beneath the cigarette. This is to create a uniform air flow around the cigarette and up the chimney. **Do not allow the cigarette to touch the chimney.**

13.5 The test cigarettes are smoked to the previously marked standard butt length. Extinguish and remove from beneath the BAT fishtail chamber.

13.6 The pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream filter.

13.7 The smoking process is repeated for the second cigarette (13.1 – 13.6).

13.8 Smoking is terminated and the butt is extinguished and removed when the final test cigarette has been consumed to the predetermined end mark.

13.9 At the end of the smoking process raise the chimney and disassemble the sidestream apparatus.

13.10 Re-weigh the sidestream filter holders and record the "after smoking" weights of the sidestream filter holders.

14 SAMPLE ANALYSIS

14.1 Sidestream Smoke Extract Solution

-
- 14.1.1** One run consists of eight samples. Process eight samples at a time but not more than five runs or 40 samples per day. Do not smoke more than can be analysed in a 24 hour period.
- 14.1.2** Rinse the fishtail chimney with 2 X 20 mL aliquots of fresh DNPH solution. Add the rinse DNPH to a clean 250 mL Erlenmeyer flask with a ground glass joint and glass stopper.
- 14.1.3** Remove the sidestream pad, fold in quarters with the "clean" side facing out. Grasp with a pair of clean tweezers, and wipe the holder. Place the pad into the 250 mL Erlenmeyer flask containing the fishtail rinse.
- 14.1.4** Place a piece of masking tape over the ground glass stopper to hold it in place.
- 14.1.5** Place the 250 mL Erlenmeyer flasks on the wrist action shaker for a period of 30 minutes.
- 14.1.6** Rinse the tubing of the impinger by forcing the impinger solution back up the impinger as far as the connection to the cassette holder using positive air pressure and then with negative air pressure until air is forced back through the solution. Repeat this rinsing procedure at least three times for each impinger.
- 14.1.7** After shaking, the contents of the impinger are transferred into the Erlenmeyer flask (a total of 140 mL plus the pad).
- 14.1.8** Stopper the Erlenmeyer flask and mix well (invert at least 10 times).
- 14.1.9** Allow the Erlenmeyer to sit at least five minutes before continuing with sample preparation.
- 14.1.10** Pipette 6 mL of 1 % Trizma base solution into a 10 mL volumetric flask.
- 14.1.11** Add 4 mL of syringe-filtered DNPH smoke extract to the volumetric flask.
- 14.1.12** Mix the volumetric flask well. Transfer a portion of this solution by Pasteur pipette to autosampler vials in duplicate. (Rinse the vial first with a few drops discard and then fill to minimise head space).
- 14.1.13** Cap the vials with Teflon-faced septa and store at 4 °C until analysed.
- 14.2 Preparation of Controls and Blanks**
- 14.2.1** Prepare a laboratory reagent blank (LRB), laboratory fortified blank (LFB) and laboratory fortified matrix (LFM) with set of analyses as follows to demonstrate that interference from the analytical system, glassware, and reagents are not present.
- 14.3 Laboratory Reagent Blank (LRB)**
-

- 14.3.1 Add one blank conditioned pad to a clean 250 mL Erlenmeyer flask, add 40 mL of fresh DNPH solution, stopper, and shake 30 minutes on wrist action shaker. Add an additional 100 mL fresh DNPH to the flask and mix well.
- 14.3.2 Pipette 6 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.
- 14.3.3 Add 4 mL of fresh filtered DNPH solution to the volumetric flask. Cap the flask and mix well.
- 14.3.4 Transfer to two autosampler vials, cap and store until ready to analyze.

14.4 Laboratory Fortified Blank (LFB)

- 14.4.1 Add one blank conditioned pad to a clean 250 mL Erlenmeyer flask; add 39 mL of fresh DNPH solution plus 1 mL of Carbonyl Spiking Solution. Shake 30 minutes on wrist action shaker. Add an additional 100 mL fresh DNPH to the flask and mix well.
- 14.4.2 Pipette 6 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.
- 14.4.3 Add 4 mL of the filtered, mixed DNPH/Spiking solution (section 14.4.1) to a 10 mL volumetric flask.
- 14.4.4 Cap the volumetric flask and mix well.
- 14.4.5 Transfer to two autosampler vials, cap and store until ready to analyze.

14.5 Laboratory Fortified Matrix (LFM)

- 14.5.1 After shaking the samples, prepare an LFM using a control brand with set of analyses.
- 14.5.2 Pipette 5 mL of the 1 % Trizma base dilution solution into a 10 mL volumetric flask.
- 14.5.3 Add 1 mL of the Carbonyl Spiking Solution to the 10 mL volumetric flask.
- 14.5.4 Add 4 mL of filtered DNPH/smoke extract solution from a control brand to the volumetric flask.
- 14.5.5 Transfer to two autosampler vials, cap and store until ready to analyze.
- 14.5.6 Compare to results of the same control sample used for preparing the LFM solution.

14.6 Instrument analysis: Reversed Phase High Performance Liquid Chromatography

14.6.1 Column Temperature: 30 °C.

14.6.2 Injection volume: 20 µL.

14.6.3 UV detection at 365 nm.

14.6.4 Mobile Phase: Reagents.

Solvent A: Prepare 2 L of 30 % Acetonitrile, 10 % THF, 1 % IPA in Type I water, filter and degas. (UHP Helium sparged).

Solvent B: Prepare 2 L of 65 % Acetonitrile, 1 % THF, 1 % IPA in Type I water, filter and degas. (UHP Helium sparged).

Solvent C: Acetonitrile (UHP Helium sparged).

14.6.5 Sample Wash: Solvent A.

14.6.6 Mobile Phase: Gradient.

Flowrate Time (minutes)	1.5mL/minute Composition		
0.0	100 % A	0 % B	0 % C
8.0	70 % A	30 % B	0 % C
20.0	47 % A	53 % B	0 % C
27.0	0 % A	100 % B	0 % C
30.0	0 % A	0 % B	100 % C
32.0	0 % A	0 % B	100 % C
34.0	95 % A	5 % B	0 % C
Method End (Equilibrate 10 minutes).	100 % A	0 % B	0 % C

14.7 Sample Analysis

14.7.1 Sample vials are loaded onto the autosampler such that every eighth vial is a standard solution and in such quantities that the total analysis does not exceed 24 hours.

14.7.2 Inject 20 µL of one vial of each sample onto the HPLC column and analyse as per the chromatographic conditions. The other vial is the backup sample in the event of a problem.

14.7.3 Elution pattern should be similar to **Figure 1**.

14.8 Calculations

14.8.1 Construct a Calibration Curve:

14.8.1.1 Twenty µL of each calibration standard is injected onto the HPLC column and analysed. Do in duplicate. Elution pattern should be similar to Figure 2.

14.9 Determination of Response Factor

14.9.1 A calibration curve for each individual carbonyl is prepared by plotting the concentration of the standards versus their respective peak areas.

14.9.2 Response factors are calculated for each individual carbonyl compound from the calibration curves.

14.10 Sample Quantification

14.10.1 The amount of the various carbonyl compounds in smoke samples is quantified by the external standard method.

14.10.2 Carbonyl concentrations are given in µg/mL basis.

14.10.3 Determination of Sidestream Carbonyl Deliveries in [µg/cigarette]

$$\text{e.g. Carbonyl } [\mu\text{g/cigarette}] = \frac{\text{Peak Area}}{\text{Resp. Factor}} \times \frac{\text{DF}}{\text{No. of Cigarettes}}$$

where DF is the dilution factor. The response factor shall be determined from the calibration curve.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Figures No. 1 and 2.

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

15.2.1.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, filter pads, and analyzer effects.

15.2.1.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

15.2.2 Each analytical run should include a standard run as a sample to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary stock Carbonyl standards are prepared as required.

15.4.2 All work standards, and reagents are prepared fresh every 20 days.

15.4.3 All samples are analysed as soon as they are available from sample preparation and within 24 hours.

16 MODIFICATIONS FOR INTENSE SMOKING

16.1 No modifications for intense smoking required.

17 REFERENCES

- 17.1** Risner, C.H. and Martin, P. "Quantitation of Formaldehyde, Acetaldehyde, and Acetone in Sidestream Cigarette Smoke By High Performance Liquid Chromatography", *Journal of Chromatographic Science*, 32, 1994.
- 17.2** Adams, J.D. and Parent-Ermini, A.J. "Volatile Carbonyls in Sidestream Tobacco Smoke" American Health Foundation Method 4, p. 205-212.
- 17.3** Manning, D.L., Maskerinec, M.P., Jenkins, R.A., and Marshall, A.H. "High Performance Liquid Chromatographic Determinations of Selected Gas Phase Carbonyls in Tobacco Smoke" *Journal of Assoc of Anal Chem.*, 66, p. 8-12.

APPENDICES**Appendix 1: Typical Calibration Standards****(a): Stock Standards ***

Carbonyl Hydrazone	Primary Stock Standard						Working Stock Standard *		
	Formula Wt e	Formula Wt	Weight (mg)	Purity (%)	Volume (mL)	Stock [µg/mL]	Vol (ml) Primary Stock	Dilute to Vol (mL)	Stock [µg/mL]
Formaldehyde	211.20	30.03	39.72	100.0	25.0	225.879	0.5	25.0	4.51758
Acetaldehyde	225.14	44.05	53.90	100.0	25.0	421.834	0.5	25.0	8.43669
Acetone	239.17	58.08	31.20	100.0	25.0	303.064	0.5	25.0	6.06128
Acrolein	237.15	56.06	32.27	100.0	25.0	305.133	0.5	25.0	6.10266
Propionaldehyde	239.17	58.08	31.18	100.0	25.0	302.870	0.5	25.0	6.05740
Crotonaldehyde	251.18	70.09	27.37	100.0	25.0	305.496	0.5	25.0	6.10992
MEK	253.20	72.11	27.43	100.0	25.0	312.477	0.5	25.0	6.24953
Butyraldehyde	253.20	72.11	23.28	100.0	25.0	265.201	0.5	25.0	5.30402

*In a single 25 ml volumetric flask and made up to volume with acetonitrile.

(b): Carbonyl Running Standards **

Label	5	50	100	250	500	750	1000
Vol (mL) W/S	0.050	0.500	1.000	2.500	5.000	7.500	10.000
Carbonyl	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]	[µg/mL]
Formaldehyde	0.0226	0.2259	0.4518	1.1294	2.2588	3.3882	4.5176
Acetaldehyde	0.0422	0.4218	0.8437	2.1092	4.2183	6.3275	8.4367
Acetone	0.0303	0.3031	0.6061	1.5153	3.0306	4.5460	6.0613
Acrolein	0.0305	0.3051	0.6103	1.5257	3.0513	4.5770	6.1027
Propionaldehyde	0.0303	0.3029	0.6057	1.5143	3.0287	4.5430	6.0574
Crotonaldehyde	0.0305	0.3055	0.6110	1.5275	3.0550	4.5824	6.1099
MEK	0.0312	0.3125	0.6250	1.5624	3.1248	4.6872	6.2495
Butyraldehyde	0.0265	0.2652	0.5304	1.3260	2.6520	3.9780	5.3040

**Prepared in single 10mL volumetric flasks and made up to volume with acetonitrile.

(c): Spiking Solutions ***

Carbonyl	LFB Spiking Solution ***					
	Stock Level	Stock [mg/mL]	Volume (mL)	Dilute to Vol (mL)	Spike [µg/mL]	as Analyzed [µg/mL]
Formaldehyde	Primary	225.879	2.8		63.24614	0.22588
Acetone	Primary	303.064	2.0	10.0	60.61282	0.21647
Butyraldehyde	Primary	265.201	2.0		26.52008	0.09471
Total Butyraldehyde					47.68601	0.1703

***In a single 10mL volumetric flask and made up to volume with acetonitrile.

Figure 1: Analytical Chromatogram of Volatile Carbonyls in DNPH Extract of Sidestream Tobacco Smoke

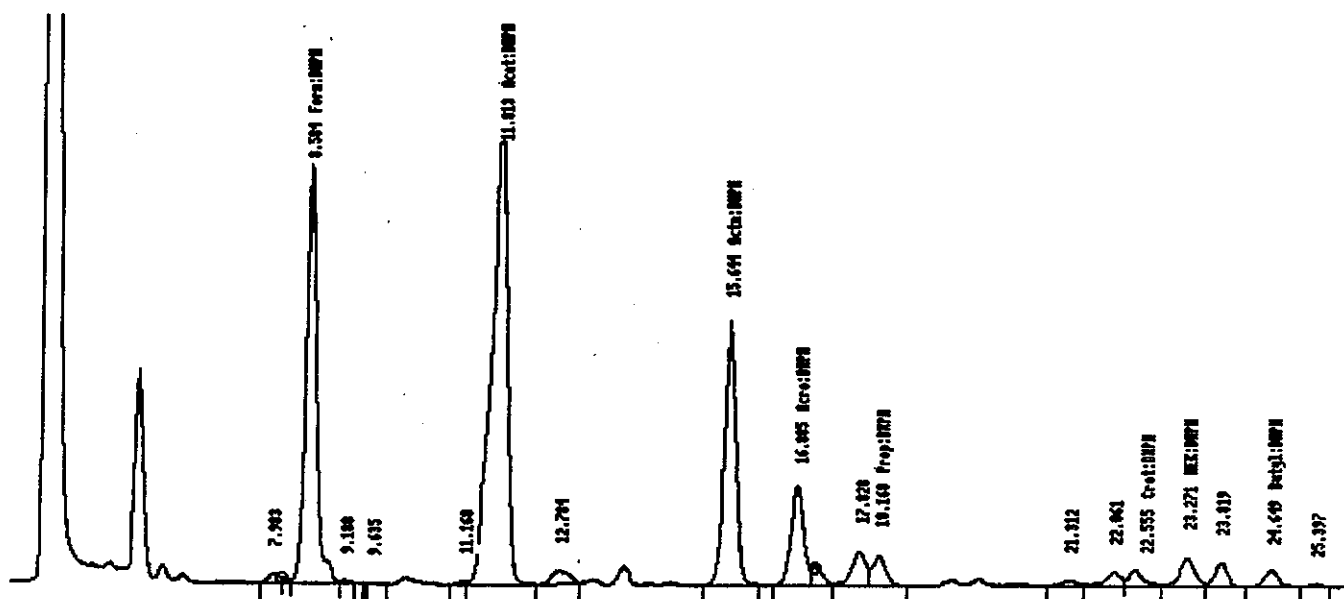
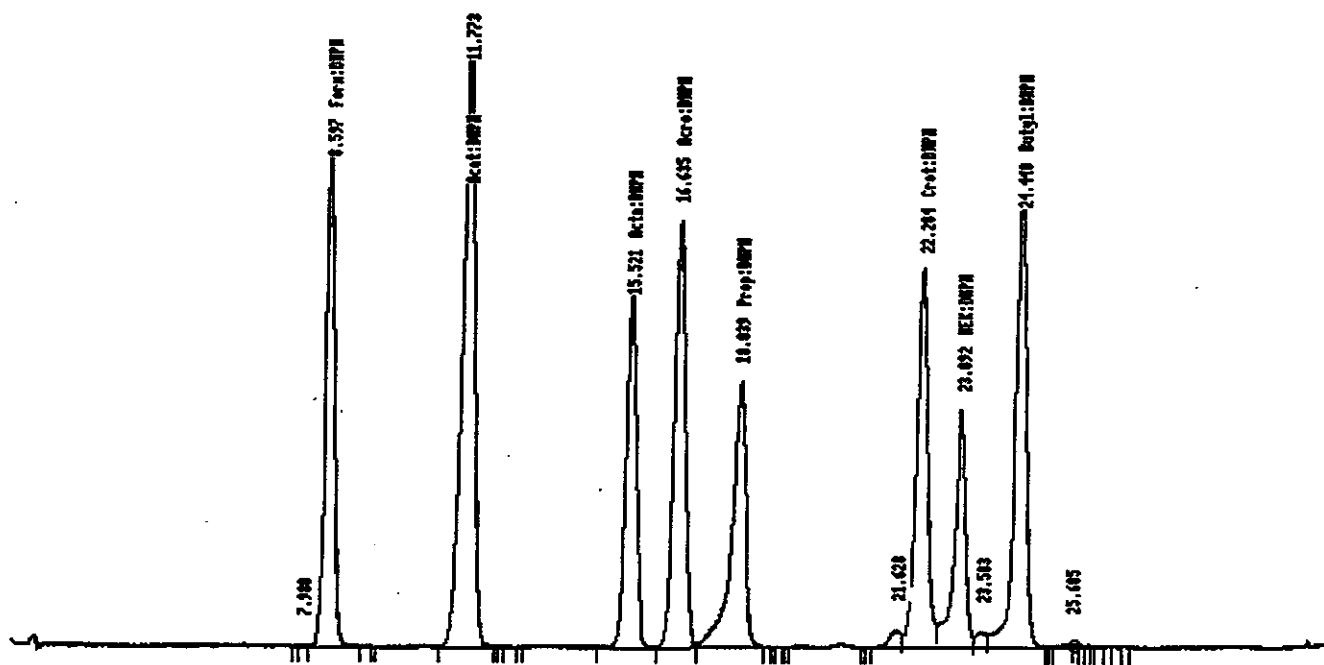


Figure 2: Analytical Chromatogram of Calibration Standard

No.: T - 205
Date: December 31, 1999
Page: 1 of 8

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the extraction and determination of hydrogen cyanide (HCN) in the total particulate matter (TPM) and gaseous phase of sidestream (SS) tobacco smoke by an automated continuous flow analyzer. This method is applicable to the testing of cigarettes and cigars.

2 NORMATIVE REFERENCES

- 2.1** American Society for Testing and Materials (ASTM) D1193-77 – Standard Specifications for Reagent Water, Version 1977.
- 2.2** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Between four and eight equidistant ports of a standard linear smoking machine are reconfigured with the British American Tobacco (BAT) fishtail chambers and flow-controlled vacuum pumps. Two cigarettes* are smoked per port beneath the fishtail chambers and the smoke is swept up the chimney at the rate of 3 L/minute. The TPM of the sidestream smoke is collected on a Cambridge filter pad (CFP) at the top of the chimney. The filtered puff is then bubbled through an impinger containing 90 mL of 0.1N NaOH.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2** After smoking two cigarettes, the sidestream filter pad is placed in an Erlenmeyer flask that contains the impinger solution and 2 x 15 mL rinsings of the BAT fishtail chamber and extracted by wrist-action shaking.
- 4.3** An aliquot of the extract is then syringe-filtered and subjected to automated continuous flow analysis where the hydrogen cyanide is reacted with choramine-T to form cyanogen chloride. This then reacts with pyridine to give glutaconic aldehyde, which produces a coloured complex when mixed with a pyrazolone reagent. A single channel colorimeter monitors the complex, which is quantified by comparison to external calibration standards.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

The analysis should be completed in one day, and the waste potassium cyanide solutions generated must be stored for disposal by registered chemical recycling agencies. All pipetting must be done with mechanical devices.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to condition tobacco product as per ISO 3402:1991.
- 5.2** Equipment needed to perform smoking analyses as per ISO 3308:1991; ISO 4387:1991.
- 5.3** Analytical balance, capable of reading to four decimal places.
- 5.4** 125 mL polymethylpentene (PMP) Erlenmeyer flasks with screw caps.
- 5.5** Wrist-action shaker.
- 5.6** 5 cc Disposable Syringe.
- 5.7** Syringe Filter - Nalgene SFCA (25 mm) (or equivalent).
- 5.8** PC Controlled Continuous Flow AutoAnalyzer consisting of:
 - 5.8.1** Technicon IV Autosampler or equivalent.
 - 5.8.2** Technicon II Peristaltic Pump or equivalent.
 - 5.8.3** HCN Manifold.
 - 5.8.4** Single Channel Colorimeter equipped with 15 mm flow cell and 540nm filter.
 - 5.8.5** IBM compatible PC with Pentium Processor, 133 Mhz, 32 meg ram 1.5GB hard-drive and 14" monitor, preloaded with Win 95 and Labtronics NAP 2.4 data handling software.
- 5.9** 250 mL Impingers with no frits.
- 5.10** 50 mL volumetric flasks with ground glass joints.
- 5.11** 100 mL graduated cylinders.
- 5.12** Glass filter funnel.
- 5.13** Magnetic Stirrer and stir bars.
- 5.14** 1000 μ L Eppendorf (or equivalent) variable adjusting volume pipettor.
- 5.15** Sample cups for autoanalyzer.
- 5.16** Vacuum Pumps – GAST.
- 5.17** Flowmeters - Ace Glass Inc. or equivalent.
- 5.18** Fishtail Chambers – BAT.
- 5.19** Retort stands (one per fishtail chimney).

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1** Potassium cyanide.

- 6.2 Chloramine T.
- 6.3 Pyridine.
- 6.4 Sodium Hydroxide (NaOH).
- 6.5 3-methyl-1-phenyl-2-pyrazolin-5-one.
- 6.6 Bispyrazolone.
- 6.7 Potassium dihydrogen phosphate.
- 6.8 Disodium hydrogen phosphate.
- 6.9 Brij-35 solution (30 %).
- 6.10 Type I water (as per ASTM D1193).

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

8.1 Chloramine-T Solution

- 8.1.1 Add 2 g of chloramine-T to 500 mL of Type I water. Mix well. Prepare fresh weekly.

8.2 Saturated Pyrazolone Solution

- 8.2.1 Stir 5 g of 3-methyl-1-phenyl-2-pyrazolin-5-one with 2 L of water for five hours, using a magnetic stirrer and stir bar.

8.3 Pyridine-Pyrazolone Solution

- 8.3.1 Dissolve 0.080 g of bispyrazolone in 80 mL of pyridine in an amber bottle and mix on magnetic stirrer for 30 minutes. After complete solution is obtained, add 400 mL of filtered saturated pyrazolone solution and mix.

8.4 Buffer solution

- 8.4.1 Dissolve 13.6 g of potassium dihydrogen phosphate and 0.28 g of disodium hydrogen phosphate in Type I water and dilute to 1 L. Add 0.5 mL of Brij-35 and mix.

8.5 Sodium Hydroxide (0.1N)

- 8.5.1 Add 8 g of NaOH pellets to 2 L of Type I water. Stir until completely dissolved.

9 PREPARATION OF STANDARDS

- 9.1 Prepare a primary stock solution equivalent to 500 ppm HCN (60.2 mg of KCN to 50 mL with 0.1 N NaOH).

- 9.2 Then dilute 0.05, 0.1, 0.3, 0.5, and 0.7 mL aliquots of this solution each to 50 mL with 0.1 N NaOH. These standards are equivalent to 0.5, 1.0, 3, 5 and 7 ppm ($\mu\text{g/mL}$) HCN. These standards should be prepared fresh weekly.

10 SAMPLING

- 10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1 Product shall be conditioned as specified in T-115.
- 11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

- 12.2.1.1 Assemble the HCN sidestream apparatus as per the diagram. Attach BAT fishtail chimney (*Proctor et. al 1988*) to SS holder via vacuum tubing and anchor chimney into smoke machine chimney support. Raise chimney level to highest position (loading position).

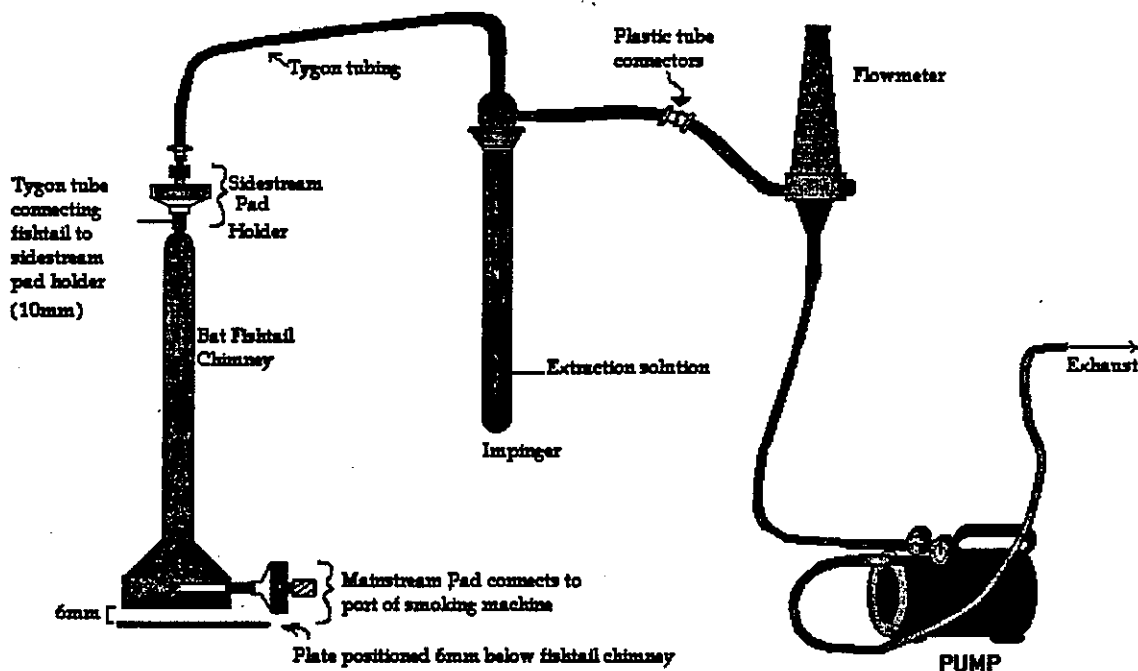


FIGURE 1a: SIDESTREAM APPARATUS

12.2.1.2 Add 90 mL of 0.1 N NaOH to each impinger.

12.2.1.3 Fit a pre-conditioned pad into each of the numbered sidestream pad holders with the rough side towards the incoming smoke. Record the before smoking weight of the sidestream pad holder.

12.2.1.4 Install the sidestream filter pad assembly at the top of the fishtail chamber and calibrate the vacuum pumps to draw at the rate of 3 L/minute. Record the flowmeter settings.

13 SAMPLE GENERATION

13.1 Turn on the sidestream pumps (3 L/minute).

13.2 At t minus 30 seconds, light the cigarette according to the following procedure and initiate the puff count.

13.2.1 Normal lighting procedure is 15 second warm-up beginning at $t-18$ seconds followed by a five second ignition. (Three seconds prior to puff plus the two second puff).

13.2.2 Lower the chimney to its lowest position. Do not allow the cigarette to touch the chimney. Keep the chimney approximately 6 mm from the

plate insert. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.

- 13.3 Smoke the cigarette to the previously marked standard butt length. Extinguish and remove from beneath the BAT fishtail chamber.
- 13.4 The smoking process is repeated for the second cigarette.
- 13.5 Smoking is terminated and the butt is extinguished and removed when the final test cigarette has been consumed to the predetermined end mark.
- 13.6 The sidestream pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream filter.
- 13.7 At the end of the smoking process raise the chimney and disassemble the sidestream apparatus.
- 13.8 Re-weigh the sidestream pad and record the "after smoking" weights of the sidestream filter holders.

14 SAMPLE ANALYSIS

14.1 Sample Preparation

- 14.1.1 Remove the sidestream pad. Fold it in half and in half again with the "clean" side facing out. Grasp it with a pair of clean tweezers, and wipe the holder. Place the pad into a 125 mL PMP Erlenmeyer flask.
- 14.1.2 Add the 90 mL of impinger solution to the Erlenmeyer flask.
- 14.1.3 Rinse the fishtail chimney with 2 x 15 mL of fresh 0.1 N NaOH. Use a glass rod to free up any debris on the chimney. Add the chimney washings to the Erlenmeyer flask for a total volume of 120mL.
- 14.1.4 Screw the cap on firmly.
- 14.1.5 Clamp flasks onto armature of wrist action shaker and agitate 30 minutes. (Pad should be disintegrated).
- 14.1.6 Filter the extract directly into appropriately labelled vials or sample cups using a syringe filter disc attached to a 5 cc disposable syringe.
- 14.1.7 Analyse the extract immediately for HCN.

14.2 Instrument Analysis

- 14.2.1 The Autosampler is operated at a sampling rate of 20 per hour with a 2:1 sample to wash ratio. Sufficient time should be allowed for the system to become stable with the reagents being pumped.

14.2.2 Samples are only rerun if out of range or there was a problem with the analysis.

14.2.3 Samples undergo on-line dilution.

14.2.4 Sampling cups containing only 0.1 N NaOH are placed at regular intervals to allow for baseline correction.

14.3 Calculations

14.3.1 Construct a calibration curve relating ppm of HCN to peak height with the data obtained from the standards.

14.3.2 Obtain ppm ($\mu\text{g/mL}$) of HCN for each extract and calculate micrograms per cigarette of HCN in sidestream smoke:

$$\text{HCN } (\mu\text{g/cigarette}) = [\text{amount } (\mu\text{g/mL}) \times 120 \text{ (mL)}] / \text{Cigarettes smoked.}$$

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix.

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

15.2.1.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, pads, and analyzer effects.

LRB: Add one conditioned filter pad to a clean 125 mL Erlenmeyer flask, add 120 mL of 0.1 N NaOH solution and cap.

15.2.1.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

LFB: Add one conditioned filter pad to a clean 125 mL Erlenmeyer flask, add 119 mL of 0.1N NaOH plus 1.0 mL of the 500 ppm HCN stock solution and cap.

15.2.1.3 A Laboratory Fortified Matrix (LFM) to evaluate any potential matrix effects.

LFM: After shaking the flasks, prepare a laboratory fortified matrix (LFM) daily using a control brand:

LFMA – Dilute 5 mL of a control pad extract to 10 mL with 0.1 N NaOH.

LFMB – Dilute 5 mL of a control pad extract with 0.1 mL of the 500 ppm. KCN stock solution and make to 10 mL with 0.1 N NaOH.

15.2.1.4 Check standards run as samples to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) & Limit of Quantification (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations. Typical values are: 0.36 µg/mL which gives an MDL of 2.2 µg/cigarette.

15.3.2 Limit of Quantification (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations. Typical values are: 0.12 µg/mL which gives an LOQ of 7.2 µg/cigarette.

15.4 Stability of Reagents and Samples

15.4.1 All primary stock and working KCN standards are prepared fresh weekly.

15.4.2 All autoanalyzer reagents are prepared fresh weekly or as needed.

15.4.3 All samples are analyzed within 24 hours.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

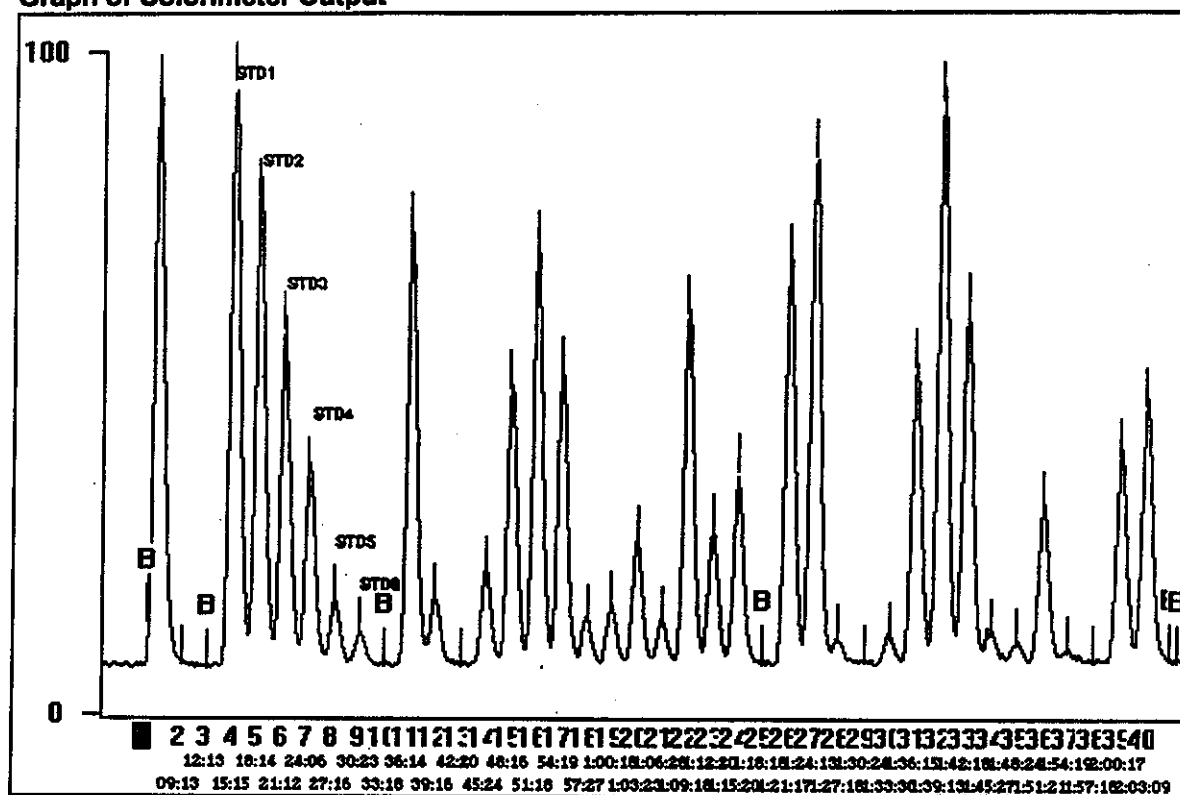
16.1 There are no modifications for intense smoking conditions.

17 REFERENCES

- 17.1 Collins, P.F. et al. 1973. A Trapping System for the Combined Determination of Total HCN and Total Gas Phase Aldehydes in Cigarette Smoke. *Beitrage zur Tabakforschung*, Vol. 7, No.2.
- 17.2 Rickert, W. S., and P. B. Stockwell, 1979. Automated determination of hydrogen cyanide, acrolein, and total aldehydes in the gas phase of tobacco smoke. *J. Autom. Chem.* 1: p. 152-154.
- 17.3 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

APPENDIX

Graph of Colorimeter Output



No.: T - 206
Date: December 31, 1999
Page: 1 of 11

1 SCOPE OF APPLICATIONS

- 1.1** This method is to be used to determine the amount of Hg in sidestream tobacco smoke. The method is designed to trap and quantitate both the particulate phase and gaseous phase components of the smoke together in the same impinger solution as it is smoked on a linear smoking machine.
- 1.2** Sidestream smoke is, effectively, all the smoke emitted from a cigarette other than the mainstream smoke. This is collected using a glass fishtail chimney assembly to direct the smoke to various trapping devices.
- 1.3** Particulate phase mercury can not be separated from gaseous phase mercury using this type of trapping and analysis system.
- 1.4** Mercury compounds are analysed by automated cold vapour atomic absorption spectroscopy : EPA Stannous Chloride Methodology

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.2** American Society for Testing and Materials (ASTM) – D1193-77 Standard Specifications for Reagent Water, Version 1977.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Equidistant ports of a standard linear smoking machine are reconfigured with the BAT (British American Tobacco) fishtail chambers and flow controlled vacuum pumps. Five cigarettes* per port are smoked beneath the fishtail chambers. The smoke is swept up the chimney at the rate of 2 L/minute.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2** The analyte is collected by passing the whole tobacco smoke through two impingers in series containing an acidified potassium permanganate solution. The particulate phase trapped on the wall of the fishtail is washed off using 2 X 5 mL additions of acidified potassium permanganate solution, 1 X 5 mL addition of hydrogen peroxide and 1 X 5 mL addition of Type I water. This rinse is added to a microwave digestion vessel already containing the two impinger solutions combined. The samples are then subjected to microwave digestion.

- 4.3** When digestion is complete, the vessels are removed from the digester, allowed to cool. Excess potassium permanganate is reduced with hydroxylamine hydrochloride, and transferred to a volumetric flask where they are made to volume with Type I water.
- 4.4** The digestate is analysed by using cold vapour atomic absorption spectroscopy at 253.7 nm. This method uses a continuous flow vapour generator to reduce the divalent mercury to its atomic state with stannous chloride. A peristaltic pump pushes the reducing agent and sample through a mixing coil to a gas liquid separator. Nitrogen gas carries the mercury vapour into a flow cell positioned in the burner compartment.

Note: The reaction is very sensitive to fluctuations in temperature so the response must be checked frequently against standards.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
- 5.2** Equipment needed to perform marking for butt length as specified in T-115.
- 5.3** Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4** 70 mL impingers without frits.
- 5.5** 1/4" ID X 3/8" OD ester grade Tygon tubing.
- 5.6** 1/4" Nalgene connectors.
- 5.7** 44 mm glass fibre filter discs (pads) and cassettes.
- 5.8** 20 X 150 mm disposable borosilicate culture tubes.
- 5.9** Linear Smoking Machine - Filtrona SM300 20 port smoking machine or equivalent.
- 5.10** Vacuum Pumps - GAST (4) or equivalent.
- 5.11** Flowmeters.
- 5.12** Fishtail Chambers - BAT (8).
- 5.13** Analytical Balance measuring to at least four decimal places.
- 5.14** Mini Hot Plate / Stirrer.
- 5.15** 50 mL, 100 mL, 1000 mL volumetric flasks.
- 5.16** Eppendorf or micro-pipettes for the preparation of analytical run standards.
- 5.17** Eppendorf pipette (1-5 mL adjustable volume) or equivalent.
- 5.18** 125 mL HDPE storage bottles.
- 5.19** Varian 400P Atomic Absorption Spectrophotometer or equivalent.
- 5.20** Varian PSC-56 Programmable Sample Changer or equivalent.
- 5.21** Varian VGA-76 Vapour Generation Assembly or equivalent.
- 5.22** Varian Mercury Flow Through Cell or equivalent.
- 5.23** Hollow Cathode Lamp for Hg.
- 5.24** CEM MDS-2100 Microwave Digestion System or equivalent.
- 5.25** CEM ACV-12 Digestion Vessel Assembly (X 2) or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical grade in quality.

- 6.1 Concentrated hydrochloric acid (HCl).
- 6.2 Concentrated sulphuric acid (H₂SO₄).
- 6.3 Concentrated nitric acid (HNO₃).
- 6.4 Type I water (meets ASTM D 1193 specifications).
- 6.5 Potassium Permanganate (KMnO₄).
- 6.6 Hydrogen peroxide (H₂O₂) 30-32 %.
- 6.7 Stannous Chloride.
- 6.8 Hydroxylamine Hydrochloride.
- 6.9 Atomic Absorption Reference Standards - Mercury standard solution at 1000 µg/mL in 10 % HNO₃.

Note: Reference standards must:

- 1. Come with a certificate of analysis.
- 2. Be NIST traceable.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

Important: The cleaning of glassware and the cleanliness of the environment in which the analysis is performed, has a direct effect on the accuracy and precision of the method. In order to achieve accurate results, all glassware must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

8 PREPARATION OF SOLUTIONS

- 8.1 Sulphuric Acid / Potassium Permanganate Impinger Solution (20 % H₂SO₄ v/v, 4 % KMnO₄ w/v)
 - 8.1.1 Add approximately 500 mL of Type I water to a 1000 mL volumetric flask.
 - 8.1.2 Carefully add 200 mL of concentrated H₂SO₄ to the flask and gently swirl and allow the flask to cool completely to room temperature before proceeding.
 - 8.1.3 Add 40 g of potassium permanganate to the flask and continue to mix until it appears that all the permanganate is dissolved.
 - 8.1.4 Make solution to volume with Type I water.

Note: When diluting a concentrated acid solution, it is always important to add the acid to water.

Note: This solution is stable for a maximum of one day due to potential contamination and precipitation of the permanganate.

8.2 Hydroxylamine Hydrochloride Solution (10 % w/v)

8.2.1 Add 10 g of hydroxylamine hydrochloride to a 100 mL volumetric flask.

8.2.2 Add approximately 70 mL of Type I water to dissolve the solid.

8.2.3 Make solution to volume with Type I water.

8.3 Stannous Chloride Solution (25 % w/v SnCl₂ in 20 % v/v HCl)

8.3.1 Weigh 125 g of Stannous Chloride into an acid washed 500 mL volumetric flask.

8.3.2 Add 100 mL of concentrated HCl to completely dissolve the solid material.

Note: Gentle heating may be applied in order to speed up this process.

8.3.3 Allow the solution to cool before carefully adding Type I water to make to the 500 mL volume.

8.3.4 Mix well and transfer the contents to the 500 mL bottle for the reducing agent channel of the Vapour Generation Assembly.

Note: If any precipitate appears in the bottle or flask, discard the solution and prepare fresh. It is necessary to keep the stannous chloride in solution as well as contaminant free as possible.

9 PREPARATION OF SOLUTIONS AND STANDARDS

9.1 Analytical Standards Stocks and Required Dilutions

9.1.1 All analytical standards are made to a 12 % (v/v) H₂SO₄ acid solution immediately prior to analysis, and are to be considered stable for only two days.

9.1.2 The purchased stock standard is in a 10 % (v/v) HNO₃ acid solution at a concentration of 1000 µg/mL for stability purposes.

9.1.3 In order to make the proper dilutions, it is necessary to prepare a secondary stock standard at a concentration of 1 µg/mL also in a 10 % (v/v) HNO₃ acid solution. This secondary stock solution is considered to be stable for one week.

9.1.4 Representative dilutions are as follows:

Primary Stock = 1000 µg/mL.

Secondary Stock = 100 µL of Primary Stock to 100 mL = 1 µg/mL.

Standard Concentration = 0.300 ng/mL = 30 µL Secondary Stock to 100 mL.

Standard Concentration = 0.500 ng/mL = 50 µL Secondary Stock to 100 mL.

Standard Concentration = 1.500 ng/mL = 150 µL Secondary Stock to 100 mL.

Standard Concentration = 3.000 ng/mL = 300 µL Secondary Stock to 100 mL.

Standard Concentration = 5.000 ng/mL = 500 µL Secondary Stock to 100 mL.

10 SAMPLING

- 10.1** The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1** Product shall be conditioned as specified in T-115.
- 11.2** Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 11.3** Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

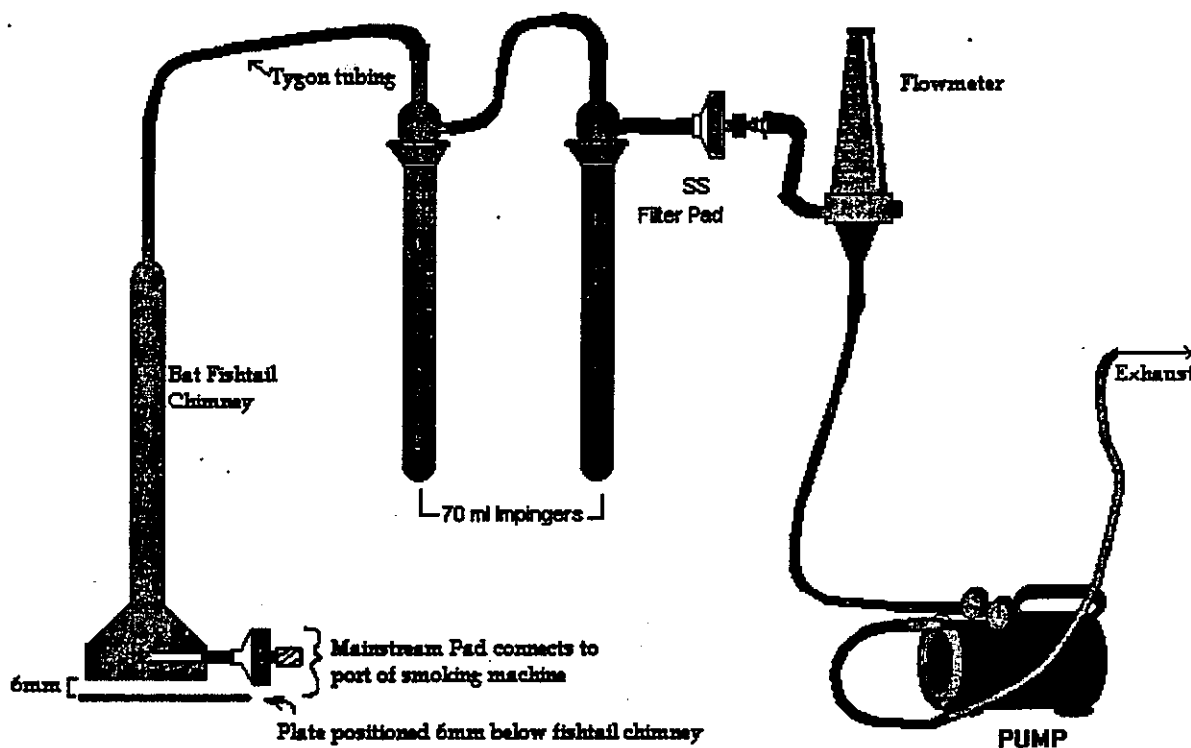
12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1** The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1** The machine conditions shall be as those specified in T-115 (with the following modifications as detailed below):
- 12.2.2** Assemble the sidestream smoke train such that the smoke of cigarettes smoked beneath the fishtail chambers is drawn up the chimney at a rate of 2 L/minute. The whole sidestream smoke is bubbled through two impingers each containing 25 mL of impinger solution (20 % H_2SO_4 v/v, 4 % KMnO_4 w/v). Any particles (or acid) that pass through the impinger, are then trapped onto a standard glass fibre filter pad and holder before it goes through the flowmeter to the vacuum pump. This filter pad is not used in the analysis, but is only there to protect the vacuum pump from permanganate particles. See Diagram.



12.2.3 The sidestream impingers are loaded with 25 mL of impinger solution (20 % H_2SO_4 v/v, 4 % KMnO_4 w/v).

12.2.4 Calibrate the vacuum pumps to draw at the rate of 2 L/minute. Record the flowmeter settings.

12.2.5 Connect two clean 70 mL impingers, in series, containing 25 mL of fresh impinger solution (20 % H_2SO_4 v/v, 4 % KMnO_4 w/v).

12.2.6 Place a new filter pad into the back-up holder in line between the second impinger and the flowmeter.

13 SAMPLE GENERATION

13.1 Install the first test cigarette to be smoked in position below the fishtail of the calibrated ports.

13.2 Turn on the sidestream pumps and begin the lighting procedure at 30 seconds prior to lighting the cigarette.

13.3 Light the cigarette on the first puff and then lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.

- 13.4 Smoking is terminated and the butt is extinguished and removed when the cigarette has been consumed to the predetermined end mark.
- 13.5 The pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream filter.
- 13.6 The smoking process is repeated for the second through fifth cigarette.

Note: When smoking under intense smoking conditions, the mainstream filter pad must be replaced after three cigarettes to prevent breakthrough.
- 13.7 At the end of the smoking process, disassemble the sidestream apparatus.

14 SAMPLE ANALYSIS

- 14.1 Using positive pressure backwash the Tygon tubing for each of the impingers with the impinger solution.
- 14.2 Both impinger solutions are transferred to the same digestion vessel.
- 14.3 The particulate matter trapped on the wall of the fishtail is washed using 2 X 5 mL additions of fresh impinger solution.
- 14.4 This rinse is transferred to the second of the two impingers for rinsing.
- 14.5 This rinse is then transferred to the first impinger for rinsing and then carefully added to the digestion vessel containing the original impinger solutions.
- 14.6 The fishtail is then rinsed using 1 X 5 mL of H₂O₂ that is transferred to the second of the two impingers for rinsing.
- 14.7 This rinse is then transferred to the first impinger for rinsing and then carefully added to the digestion vessel containing the original impinger solutions.
- 14.8 This rinsing process (14.1.6 to 14.1.7) is then repeated using a 1 X 5 mL of Type I water.
- 14.9 Install the rupture membrane and cap the digestion vessel.
- 14.10 Place the digestion vessel into the turntable and lock into position.
- 14.11 Choose the sample that appears to be the most reactive sample as the reference vessel for monitoring pressure and temperature to control the digestion.
- 14.12 Load the turntable of samples into the microwave digester, and start the digestion program. See Appendix: Microwave Digestion Parameters.
- 14.13 When the digestion is complete, remove the turntable from the microwave and allow the samples to cool to room temperature before opening.

- 14.14** Add 5 mL of the hydroxylamine hydrochloride solution dropwise to react with the excess permanganate in the samples.

Note: If the digestion appears to be incomplete, by evidence of particulate matter in the digestate, carefully add one to two more mL of fresh impinger solution and/or hydrogen peroxide and repeat the original digestion procedure.

- 14.15** When the digestion is complete, remove the turntable from the microwave and allow the samples to cool to room temperature before opening.

- 14.16** Transfer the digestate to a 100 mL volumetric flask and make to volume using the washing of the digestion vessel with Type I water.

Note: Samples must be analyzed within 48 hours of completing the digestion (24 hours recommended) and taking to volume for stability purposes. Manual dilutions (if necessary) of the digestate should only take place at the time of analysis.

14.17 Sample Dilutions Required for Elemental Analysis

- 14.17.1** No further dilutions of the sample are required, and may be analysed as is.

- 14.17.2** A portion of the diluted sample is transferred to a 20 mm X 150 mm disposable borosilicate culture tubes for analysis. The remainder of the solution is stored in the 125 mL HDPE storage bottle to prevent possible contamination.

Note 1: Any dilutions (if necessary) must be accounted for when calculating the results in a ng/cigarette basis.

Note 2: Sample volumes are based on "average" literature values. These dilutions may need to be modified depending on: 1. The samples country of origin, 2. The year in which the sample was grown (environmental factors), 3. The soil type and conditions which the sample was grown, 4. The type of tobacco used for the sample, 5. The stalk position of the tobacco used for analysis (if not a blended, finished product).

14.18 Analysis of Hg by Cold Vapour Atomic Absorption

- 14.18.1** Samples are analysed using the parameters established for the instrument at a wavelength of 253.7 nm and slit width of 0.5 nm.

- 14.18.2** It is important to analyse the samples for Hg within 48 hours of completing the digestion.

- 14.18.3** If samples are not analysed within this time frame, the digestate should be returned to the digestion vessel and the secondary digestion procedure performed.

Note: Parameters may slightly differ between instruments. .

14.19 Calculations

14.19.1 Results reported by the computer controlled software are expressed as [ng/mL] in solution. This result, multiplied by the dilution of the sample and divided by the number of cigarettes smoked, will calculate the result in a [ng/cigarette] basis.

14.19.2 The [ng/cigarette] results can be converted to [µg/cigarette] by dividing this result by 1000.

Analytical Result (on a "per cigarette" basis):

Analyte [ng/cigarette] = (Analytical result [ng/mL] X 100 mL X Additional Dilution factor) / No. of Cigarettes (5).

15 QUALITY CONTROL

15.1 Each set analysis should contain one of each of the following per day of smoking or batch of up to 24 analysis (20-22 true samples):

15.2 Laboratory Reagent Blank (LRB): to determine background contamination from solutions or glassware used in the analysis process.

15.3 Laboratory Fortified Blank (LFB): to determine whether there is any loss of analyte as a result of the analysis process.

15.4 Reference Sample: to determine the inter-experimental reproducibility of the entire method of analysis

15.5 Duplicate Sample: to determine the reproducibility of the procedure within the same experiment or batch on analysis.

Note: As an initial evaluation of the method and materials used, it is recommended that a minimum of five blanks be analysed using the method in order to establish control parameters for expected levels of background contamination before any samples are analysed. An LRB outside these control limits is an indicator of possible contamination problems or the use of materials and reagents of different lot numbers.

Note: Room contamination should also be analyzed by analyzing the background as a sample with no smoking occurring. This should be done once per day. If background is too high (most likely due to the existence of broken Hg thermometers), the entire room must be scrubbed down and filters changed in the ventilation system to remove existing contaminated dust. If a high background remains, the cleaning procedure must be repeated.

15.6 Recoveries and Levels of Contamination

15.6.1 Contamination must be monitored with each individual set of samples that are digested and is dependent on the laboratory environment. This ultimately effects the precision of the analysis.

15.7 Method Detection Limit (MDL) / Limit of Quantitation (LOQ)

Note: Individual instruments will have different MDL's and LOQ's depending on the optimization of the instrument.

The MDL is defined as either:

1. The concentration of analyte that yields an absorbance of 0.004 units (the characteristic mass); or
2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations; or
3. Same as number two using a blank solution.

The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The MDL (on a ng/cigarette basis) can be enhanced by varying the amount of cigarettes smoked, however this may affect the amount of background contamination observed.

The practical limit of quantitation (LOQ) may be defined as either:

1. The lowest level of standard other than a blank used to construct a calibration curve; or
2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations; or
3. Same as number two using a blank solution.

The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

The effect of modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

15.8 Stability of Reagents and Samples

15.8.1 As stated earlier, all samples and analytical run standards must be analyzed within 48 hours of the digestion (24 hours recommended).

15.8.2 All solutions for the analysis (other than the impinger solution) are stable for only two weeks because of probable contamination problems.

15.8.3 Impinger solutions are stable for a maximum of one day because of precipitation of permanganate and the possibility of contamination.

16 REFERENCES

- 16.1 Varian Instruments at Work: Automated Cold Vapor Determination of Mercury: EPA Stannous Chloride Methodology, No. AA-51, September 1985.
- 16.2 Van Delft, W. & Vos G., 1988. Comparison of Digestion Procedures for the Determination of Mercury in Soils by Cold-Vapour Atomic Absorption Spectrometry, *Analytica Chimica Acta* 209, 1988, p.147-156.
- 16.3 Determination of ultratrace-level mercury in sediment and tissue by microwave digestion and atomic fluorescence detection. CEM reference R105.
- 16.4 Comparison of a Microwave Digestion System to Other Digestion Methods for Plant Tissue Analysis. CEM reference RO 26.
- 16.5 The Determination of Total Mercury (Hg) in Air Sampling Solutions, Regulation respecting Mercury (made under the Occupational Health and Safety Act, O. Reg. 23/87, 1987, p. 47-55.

APPENDIX

Appendix : Microwave Digestion Parameters

Manufacturer: CEM
 Model: MDS 2100
 Digestion Vessel Type: ACV – Advanced Composite Vessel

Pressure/Temperature/Time Program for the Digestion of MS Smoke Samples

Stage:	1	2	3	4	5
Power %:	70	70	70	0	0
Pressure (psi):	50	125	175	20	150
Run Time (min):	20	15	20	20	20
Time at Parameter:	8	8	15	20	10
Temperature:	95	125	165	20	190
Fan Speed	50	50	50	80	

Note: The temperature and pressure parameters are set as the controlling parameters in this digestion program one of which will define the maximum reached. If either preset is not reached, the microwave oven delivers the designated power for the time programmed in the Run Time function.

Note: These are only suggested parameters as a starting point. The digestion procedure must be optimized for the specific application and instrument used.

No: T - 207
Date: December 31, 1999
Page: 1 of 16

1 SCOPE OF APPLICATIONS

- 1.1** This method is applicable to the determination of nickel (Ni), lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and selenium (Se) in sidestream tobacco smoke by Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Argon Plasma - Atomic Emission Spectroscopy (ICP-AES). The method is designed to quantitate these toxic trace metals in both the particulate phase and gaseous phase of sidestream smoke from cigarettes, cigarette equivalents, kreteks, bidis and cigars smoked on a linear smoking machine.
- 1.2** Sidestream smoke is, effectively, all the smoke emitted from a cigarette other than the mainstream smoke. This is collected using a glass fishtail chimney assembly to direct the smoke to various trapping devices.
- 1.3** Particulate phase metals are determined as those metals that become part of the sidestream smoke particulate matter, trapped on a glass fibre filter disc (pad), as well as metals bound to the particulate matter remaining on the glass wall of the fishtail chimney assembly.
- 1.4** Gaseous phase metals are determined as those metals that may have reacted to form a gaseous species or particulate matter that is not retained in the normal Total Particulate Matter (TPM) condensate.

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 - Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.2** American Society for Testing and Materials (ASTM) D1193-77 - Standard Specifications for Reagent Water, Version 1977.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Equidistant ports of a standard linear smoking machine are reconfigured with the British American Tobacco (BAT) fishtail chambers and flow-controlled vacuum pumps. Four cigarettes* per port are smoked beneath the fishtail chambers as specified by T-115. The smoke is swept up the chimney at the rate of 3 L/minute.

*For other tobacco products, select a number such that breakthrough does not occur.

- 4.2** The TPM of the sidestream smoke is collected on a 44 mm pad at the top of the chimney. The particulate matter trapped on the wall of the fishtail is washed off

using the solution from the impingers (20 mL) followed by 10 mL of hydrogen peroxide plus 10 mL of fresh impinger solution. This rinse is added to a PMP Erlenmeyer flask with screw top closure. The pad is also added to the flask where it is extracted on a wrist action shaker. After 30 minutes of shaking, the mixture is transferred to a microwave digestion vessel by pouring it through a funnel with a plug of glass wool. The Erlenmeyer is then rinsed with an additional 2 X 10 mL of fresh impinger solution. These solutions are also added to the digestion vessel.

Note: Tar is extracted from the pad, rather than attempting to digest the entire pad, in order to reduce the amount of background metals from the pad. The magnitude of the variability in the pad from its digestion could be substantial in comparison to the amount of metals to be analyzed.

- 4.3 The gaseous phase metals are trapped by placing two impingers of a 10 % v/v nitric acid solution between the pad and the vacuum pump. Both impinger solutions are added to the same digestion vessel and subjected to microwave digestion.
- 4.4 When the digestion is complete, the vessels are removed from the digester, allowed to cool, and transferred to a volumetric flask where they are made to volume with Type I water.
- 4.5 The digestates are then analyzed by flameless atomic absorption spectroscopy (or graphite furnace atomic absorption). This method uses pyrolytic coated partition tubes for increased resistivity toward acid therefore increasing the lifetime of the tube and sensitivity to the analyte.
- 4.6 Quantitation is achieved by interpolating the relevant calibration curves prepared from standard metal solutions of aqueous standards in the same acid concentration to minimize matrix effects. For some metals the use of a matrix modifier is required to prevent loss of analyte during the analysis.

Note: Arsenic and selenium may also be analyzed by hydride generation using sodium borohydride. Extreme care, and a secondary digestion procedure, must be used to ensure these metals are in the proper oxidation state for the hydride reaction to quantitatively occur. This also requires the digestate to be further diluted resulting in a loss in sensitivity.

Note: The analysis of Cd, Pb, Ni, and Cr, can also be analyzed by ICP-AES in conjunction with an ultrasonic nebulizer in order to increase sensitivity.

Important: The cleaning of glassware and the cleanliness of the environment in which the analysis is performed, has a direct effect on the accuracy and precision of the method. In order to achieve accurate results, all glassware must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

Note: The testing and evaluation of certain products against this method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this standard has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction

with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 70 mL impingers without frit.
- 5.5 1/4" ID x 3/8" OD ester grade Tygon tubing.
- 5.6 1/4" Nalgene connectors.
- 5.7 Vacuum Pumps.
- 5.8 Flow meters.
- 5.9 Fishtail Chambers – BAT.
- 5.10 125 mL Polymethylpentene (PMP) Erlenmeyer flasks with screw caps.
- 5.11 Gass funnels.
- 5.12 Aid-washed glass wool.
- 5.13 Balance capable of reading to four decimal places.
- 5.14 Wrist Action Shaker.
- 5.15 10 mL, 50 mL, 100 mL, 1000 mL volumetric flasks.
- 5.16 Pipettor or micro-pipettes for the preparation of working standards.
- 5.17 Eppendorf pipettor (1-5 mL adjustable volume) or equivalent.
- 5.18 125 mL High Density Polyethylene (HDPE) storage bottles.
- 5.19 Atomic Absorption Spectrophotometer.
- 5.20 Graphite Tube Atomizer.
- 5.21 Varian Partition Tubes (Coated) or equivalent.
- 5.22 Hollow Cathode Lamps for: Ni, Pb, Cd, Cr, As, Se and Hg.
- 5.23 Microwave Digestion System with temperature and pressure controls or equivalent.
- 5.24 Advanced Composite Vessel (ACV) Digestion Vessel Assembly (X 2) or equivalent.
- 5.25 Alternatively: Varian Axial Vista Simultaneous ICP or equivalent.
- 5.26 Cetac U-5000AT⁺ Ultrasonic Nebulizer or equivalent.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Concentrated HCl – trace metals analysis grade or equivalent.
- 6.2 Concentrated HNO₃ – trace metals analysis grade or equivalent.
- 6.3 Type I water (as specified in ASTM D1193).
- 6.4 Methanol.
- 6.5 Hydrogen Peroxide (32 %).
- 6.6 Ortho-phosphoric Acid – trace metals analysis grade or equivalent.
- 6.7 Atomic Absorption Reference Standards - individual standards solutions at 1000 ug/mL.

Note: Reference standards must:

- 1. Come with a certificate of analysis.
- 2. Be NIST traceable.

7 PREPARATION OF GLASSWARE

-
- 7.1** Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

Important: The cleaning of glassware and the cleanliness of the environment in which the analysis is performed, directly effects the accuracy and precision of the method. In order to achieve accurate results, all glassware and digestion vessels must be cleaned immediately prior to use with dilute HCl (1+1) and then rinsed with Type I water.

8 PREPARATION OF SOLUTIONS

8.1 Nitric Acid Impinger Solution (10% HNO₃ v/v)

8.1.1 Add approximately 500 mL of Type I water to a 1000 mL volumetric flask.

8.1.2 Add 100 mL of conc. HNO₃.

8.1.3 Make solution to volume with Type I water.

Note: When diluting a concentrated acid solution, it is always important to add the acid to water.

9 PREPARATION OF STANDARDS

9.1 Elemental Standards and Required Dilutions

9.1.1 All standards for graphite furnace analysis are made to a 10 % HNO₃ (v/v) acid solution.

Note: For stability purposes, it is desired to dilute the analytical run standards in the same acid as the stock solution was purchased in.

9.1.2 All purchased stock standards are in 1000 µg/mL concentrations for stability purposes. The required standards for each analyte are found in the instrument parameters for each particular element in **Appendix 1: Instrument Parameters**.

9.1.3 Representative dilutions are as follows:

9.1.3.1 Primary Stock = 1000 µg/mL.

9.1.3.2 Secondary Stock (As/Se) = 1mL of Primary Stock to 10 mL = 100 µg/mL.

9.1.3.3 Mixed Stock :

= 100 µL of each Primary Stock (Pb, Ni, Cd) to 100 mL = 1 µg/mL each.

= 25 µL Cr Primary Stock to 100 mL = 0.25 µg/mL.

= 100 µL As/Se Secondary Stock to 100 mL = 0.10 µg/mL.

9.1.3.4 Standard 0 = 0 µL Mixed Stock to 100 mL.

9.1.3.5 Standard 1 = 250 µL Mixed Stock to 100 mL.

9.1.3.6 Standard 2 = 500 µL Mixed Stock to 100 mL.

9.1.3.7 Standard 3 = 1500 µL Mixed Stock to 100 mL.

9.1.3.8 Standard 4 = 3000 µL Mixed Stock to 100 mL.

9.1.3.9 Standard 5 = 5000 µL Mixed Stock to 100 mL.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.1.1 Assemble the sidestream smoke train as per the diagram:

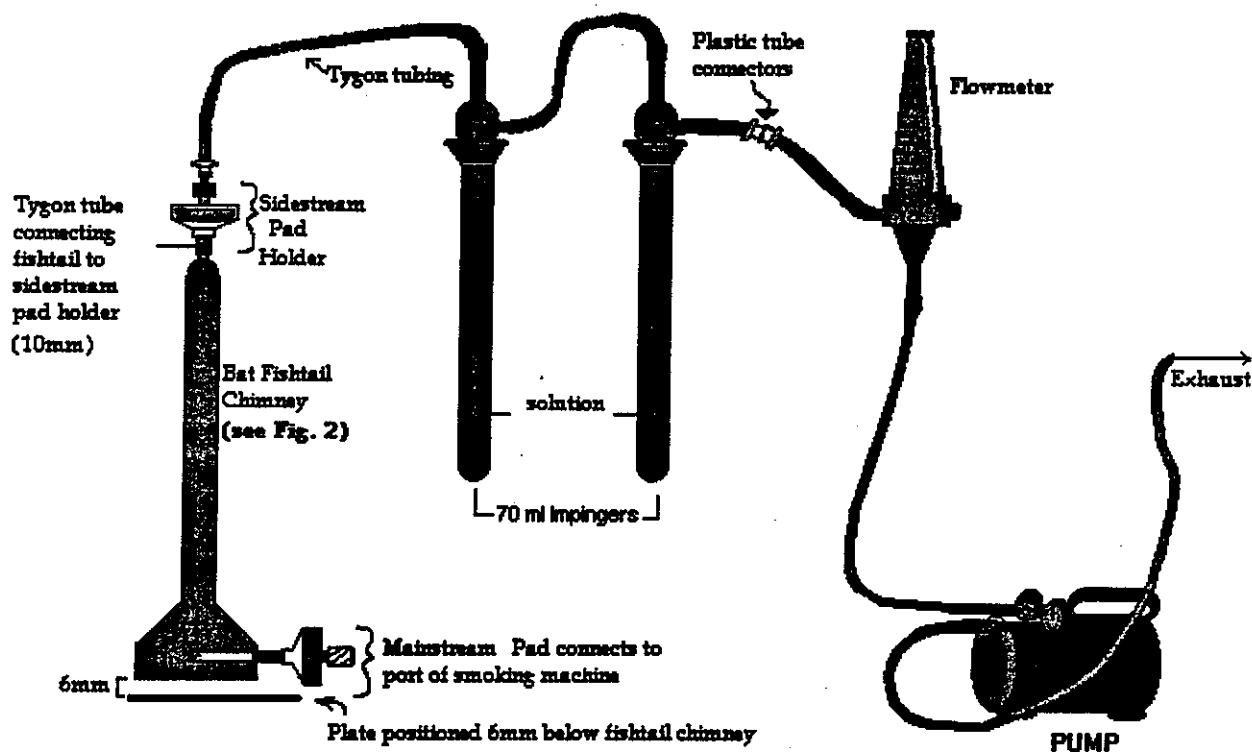


FIGURE 1b: SIDESTREAM APPARATUS USING TWO IMPINGERS

12.2.1.2 The sidestream impingers are each loaded with 10 mL of a 10 % v/v HNO_3 solution.

Note: A third empty impinger may be placed in series as an "overflow" to protect the flow meter.

- 12.2.1.3** Install the sidestream pad assembly at the top of the fishtail chamber and calibrate the vacuum pumps to draw at the rate of 3 L/minute. Record the flow meter settings.

13 SAMPLE GENERATION

- 13.1** Cigarettes shall be smoked and TPM collected as specified in T-115 with the following changes:
- 13.1.1** Record the weights of the sidestream pad holders.
 - 13.1.2** Connect two clean 70 mL impingers in series each containing 10 mL of fresh 10 % HNO_3 .
 - 13.1.3** Place a third impinger (empty) to be used as a trap in case the impinger solution begins to overflow due to the "soapyness" of the resulting smoke into the impingers.
 - 13.1.4** Insert the first test cigarette to be smoked in position below the fishtail of the calibrated ports.
 - 13.1.5** Turn on the sidestream pumps and begin the lighting procedure at 30 seconds prior to lighting the cigarette.
 - 13.1.6** Light the cigarette on the first puff and then lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.
 - 13.1.7** Smoking is terminated and the butt is extinguished and removed when the cigarette has been consumed to the predetermined end mark.
 - 13.1.8** The pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream filter.
 - 13.1.9** The smoking process is repeated for the second, third and fourth cigarette.
 - 13.1.10** At the end of the smoking process, disassemble the sidestream apparatus and record the "after smoking" weights of the sidestream pad holders.

14 SAMPLE ANALYSIS

14.1 Particulate Phase Sample Preparation and Digestion

- 14.1.1** The first impinger's contents are transferred directly to a 125 mL Erlenmeyer flask. The particulate matter, trapped on the wall of the fishtail, is washed off using the second impinger solution. This rinse is added to the same Erlenmeyer flask.

- 14.1.2 Rinse the second impinger with 10 mL of hydrogen peroxide, then transfer to the first impinger and subsequently the fishtail and Erlenmeyer.
- 14.1.3 Repeat 14.1.2 using 10 mL of fresh impinger solution.
- 14.1.4 The sidestream pad is added to the same Erlenmeyer flask.
- 14.1.5 Extract the particulate matter from the pad on a wrist action shaker for 30 minutes.
- 14.1.6 The mixture is transferred to a microwave digestion vessel by pouring it through a funnel with a plug of glass wool.
- 14.1.7 The Erlenmeyer is then rinsed with an additional 2 X 10 mL of fresh impinger solution. These solutions are also added to the digestion vessel.
- 14.1.8 Squeeze the residual solution trapped in the funnel using a glass rod so the liquid runs into the digestion vessel.
- 14.1.9 Add 6 mL of concentrated HCl to the sample in the digestion vessel.
- 14.1.10 Add 2 mL of concentrated HNO₃ to the sample, swirling in the acid, and allow to sit until the original frothing subsides and there is no longer evidence of orange/brown fumes (NO_x formation).
- 14.1.11 Allow samples to sit until the effervescence subsides (approximately 10 minutes).
- 14.1.12 Install the rupture membrane and cap the digestion vessel.
- 14.1.13 Place the digestion vessel into the 12 places turntable and lock into position.
- 14.1.14 Load the turntable of samples into the microwave digester, and start the digestion program as described in **Appendix 2: Microwave Program**.
- 14.1.15 When the digestion is complete, remove the turntable from the microwave and allow the samples to cool to room temperature before opening.
- 14.1.16 Inspect the digestate. If the digestion appears to be incomplete, carefully add 2 to 4 more mL of hydrogen peroxide and return to the microwave for a secondary digestion as described in **Appendix 2: Microwave Program**.
- 14.1.17 Transfer the digestate to a 100 mL volumetric flask and make to volume using the washings of the digestion vessel with Type I water.
- 14.1.18 Transfer the contents of the flask into a 125 mL HDPE storage bottle.

Note: Samples should be stored in the highest concentration of both analyte and acid for stability purposes. Manual dilutions of the digestate should only take place at the time of analysis.

14.2 Sample Dilutions required for Individual Elemental Analysis

14.2.1 Samples may be required to be diluted so their absorbances fall within the desired calibration range with a good signal-to-noise ratio and very little matrix effect. Because of little matrix effect, standard additions is not required and a standards calibration will suffice.

14.2.2 If samples must be diluted for analysis by graphite furnace atomic absorption, this dilution can be accomplished by adjusting the sample volume: blank ratio in the Sampler section of the method program.

Note: This dilution must be accounted for when calculating the results in a ng/cigarette basis.

14.2.3 The analysis of Cd and/or Pb may require a manual dilution prior to analysis by transferring 1000 μL of the digestate to a 10 mL volumetric flask, and making to volume with Type I water.

Note: When using ICP for quantitation, the samples may be analyzed without further dilution for Ni, Pb, Cd, and Cr.

Note: For As and Se, a multiple injection technique may be required for an adequate instrument response.

Note: These dilutions are based on "average" literature values that have been calculated in an indirect manner. These dilutions may need to be modified depending on: 1. the samples' country of origin, 2. the year in which the sample was grown (environmental factors), 3. the soil type and conditions which the sample was grown, 4. the type of tobacco used for the sample, 5. the stalk position of the tobacco used for analysis (if not a blended, finished product).

15 ATOMIC ABSORPTION ANALYSIS

15.1 A, PB, CD, CR, AS, AND SE BY GRAPHITE FURNACE ATOMIC ABSORPTION

15.1.1 Samples are analysed using the suggested parameters in Appendix 1: Instrument Parameters.

Note: Parameters may differ between instruments and must be optimized for the particular instrument used.

15.2 Analysis of Ni, Pb, Cd and Cr by ICP-AES

15.2.1 Samples are analysed using the suggested parameters in Appendix 3: ICP Parameters

Note: Parameters may differ between instruments and must be optimized for the particular instrument used.

15.3 Calculations

Results reported by the instrument software are expressed as [ng/mL] in solution. This result, multiplied by the dilution of the sample and divided by the number of cigarettes smoked, will calculate the result in a [ng/cigarette] BASIS.

15.3.1 Analytical Result (on a "per cigarette" basis) :

Analyte [ng/cig] = (Analytical result [ng/mL] X 100 mL X Additional Dilution factor) / No. of Cigarettes.

15.3.1.1 The [ng/cigarette] results can be converted to [µg/g] by dividing this result by 1000.

15.3.1.2 Total Particulate matter [mg/cigarette] is calculated using the difference in weight of the mainstream pad before and after smoking and dividing by the number of cigarettes smoked. This is used as a measure of reproducibility of the smoking procedure.

15.3.2 Determination of Total Particulate Matter (TPM)

TPM [mg/cigarette] = [Wt. of MS Pad after smoking (g) - Wt. of MS Pad before smoking (g)] X 1000 mg/g / 4.

Note: Particulate phase and gaseous phase metals are analyzed together.

16 Quality Control

16.1 Each set analysis should contain one of each of the following per day of smoking or batch of up to 24 analyses (20-22 true samples):

16.1.1 Laboratory Reagent Blank (LRB): to determine background contamination from solutions, glassware, or materials used in the analysis process.

16.1.2 Laboratory Fortified Blank (LFB): to determine whether there is any loss of analyte as a result of the analysis process.

16.1.3 Reference or Control Sample: to determine the inter-experimental reproducibility of the entire method of analysis

16.1.4 Duplicate Sample: to determine the reproducibility of the procedure within the same experiment or batch on analysis.

Note: As an initial evaluation of the method and materials used, it is recommended that a minimum of 10 blanks be analysed using the method in order to establish control parameters for expected levels of background contamination before any samples are analysed. An LRB outside these control limits is an indicator of possible contamination

problems or the use of materials and reagents of different lot-numbers. It may be required to pre-wash the SS filter pads with a dilute acid solution in an attempt to leach out background metal contamination. In doing so, the filtering properties of the filter pad may change resulting in differences in SS TPM compared to other analysis.

16.2 Recoveries and Levels of Contamination

16.2.1 Recoveries for a Laboratory Fortified Blank (LFB) for Ni, Pb, Cd, and Cr are normally between 85 and 115 %. Variability in this range is associated to differences in the blanks.

16.2.2 Recoveries for a Laboratory Fortified Blank (LFB) for As and Se range from 60 to 85 %. Lower recoveries result from over-heating of the sample while evaporating the methanol.

16.2.3 Contamination must be monitored with each individual set of samples that are digested and is dependent on the laboratory environment. This ultimately effects the precision of the analysis.

16.3 Method Detection Limit (MDL) / Limit of Quantitation (LOQ)

Note: Individual instruments will have different MDL's and LOQ's depending on the optimization of the instrument.

16.3.1 The MDL is defined as:

1. The concentration of analyte that yields an absorbance of 0.004 units (the characteristic mass); or
2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations; or
3. As per item no. 2 analysing a blank a minimum of 10 times.

16.3.2 The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

16.3.3 The MDL (on a ng/cigarette basis) can be enhanced by varying the amount of cigarettes smoked, however this may affect the amount of background contamination observed.

16.3.4 The LOQ is defined as:

1. The lowest standard used in the preparation of the calibration curve (excluding a blank); or
2. Determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as ten times the standard deviation of these determinations; or

3. Same as per item no.two., using a blank solution.

16.3.5 The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume and dividing this by the number of cigarettes smoked.

16.3.6 The effect of modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

16.4 Stability of Reagents and Samples

16.4.1 Secondary and Mixed Standards are stable for one week.

16.4.2 Working standards must be prepared every other day.

16.4.3 All samples must be analyzed within one week of the digestion or samples will have to be re-digested.

17 MODIFICATIONS FOR INTENSIVE SMOKING

17.1 No modifications are required for intense smoking conditions.

18 REFERENCES

- 18.1** Environmental Carcinogens - Selected Methods of Analysis, Vol. 8 - Some Metals: As, Be, Cd, Cr, Ni, Pb, Se, Zn. IARC Scientific Publication No. 71, 1986, p. 129-138.
- 18.2** Perinelli, M.A. & Carugno, N., 1978. Determination of Trace Metals in Cigarette Smoke by Flameless Atomic Absorption Spectrometry, *Beitrage zur Tabakforschung International*, Band 9, Heft 4, Juli 1978, p. 214-217.
- 18.3** Westcott, D.T. & Spincer, D., 1974. The Cadmium, Nickel and Lead Content of Tobacco and Cigarette Smoke, *Beitrage zur Tabakforschung International*, Band 7, Heft 4, April 1974, p. 217-221.
- 18.4** Nitsch, Alfred et al, 1991. Schwermetalle in Tabaken und in Tabakrauch II: Spurenelemente Cadmium, Blei, Kupfer, Kobalt, und Nickel in osterreichischen Zigaretten und deren Rauchkondensaten und Rauchgasen, *Beitrage zur Tabakforschung International*, Volume 15, No. 1, August 1991, p. 19-32.
- 18.5** Bell, Paul & Mulchi, Charles L., 1990. Heavy Metal Concentrations in Cigarette Blends, *Tobacco Science*, Vol. 34, 1990, p. 32-34.
- 18.6** Varian Analytical Methods for Graphite Tube Atomizers, Varian Australia Pty Ltd, Publication No. 85-100848-00, 1988.
- 18.7** Proctor, Christopher J. et al, Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* Vol. 113, 1988, p. 1509-1513.
- 18.8** Rhodes, Charles B and White, Ralph T., 1997. Mainstream Smoke Collection by Electrostatic Precipitation for Acid Dissolution in a Microwave Digestion System

Prior to Trace Metal Determination, *Journal of AOAC International*, Vol. 80, No. 6, 1997, p. 1320-1331.

- 18.9** Gawalco Et Al, 1997. Comparison of Closed-Vessel and Focused Open-Vessel Microwave Dissolution for Determination of Cadmium, Copper, Lead and Selenium in Wheat, Wheat Products, Corn Bran, and Rice Flour by Transverse-Heated Graphite Furnace Atomic Absorption Spectrometry, *Journal of AOAC International*, Vol. 80, No. 2, 1997, p. 379-387.

APPENDICES**Appendix 1: Typical Instrument Parameters****Graphite Furnace Atomic Absorption Analysis of: Ni****Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	4
Slit Width (nm):	0.2
Slit Height:	Normal
Wavelength:	232.0
Sample Introduction:	Sampler Premixed
Measurement Time :	3.1
Replicates:	1
BGD Correction:	On

Graphite Furnace Atomic Absorption Analysis of: Pb**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	5
Slit Width (nm):	0.5
Slit Height:	Normal
Wavelength:	283.3
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier: Ortho-phosphoric Acid (1000µg/mL)

Graphite Furnace Atomic Absorption Analysis of: Cd**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	4
Slit Width (nm):	0.5
Slit Height:	Normal
Wavelength:	228.8
Sample Introduction:	Sampler Premixed
Measurement Time :	3.1
Replicates:	1
BGD Correction:	On

Graphite Furnace Atomic Absorption Analysis of: Cr**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	7
Slit Width (nm):	0.2
Slit Height:	Reduced
Wavelength:	357.9
Sample Introduction:	Sampler Premixed
Measurement Time :	3.2
Replicates:	1
BGD Correction:	Off

Matrix Modifier: Ortho-phosphoric Acid (1000 µg/mL)

Graphite Furnace Atomic Absorption Analysis of: As**Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	5
Slit Width (nm):	0.2
Slit Height:	Normal
Wavelength:	193.7
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier : Nickel Nitrate (100 µg/mL)**Graphite Furnace Atomic Absorption Analysis of: Se****Method Parameters:**

Instrument Mode:	Absorbance
Calibration Mode:	Concentration
Measurement Mode:	Peak Height

Instrument Parameters:

Lamp Current (mA):	10
Slit Width (nm):	1
Slit Height:	Normal
Wavelength:	196.0
Sample Introduction:	Sampler Premixed
Measurement Time :	3.0
Replicates:	1
BGD Correction:	On

Matrix Modifier : Nickel Nitrate (100 µg/mL)

Appendix 2: Microwave Digestion Parameters**Microwave Digestion Parameters**

Manufacturer: CEM
Model: MDS 2100
Digestion Vessel Type: ACV - Advanced Composite Vessels

Pressure/Temperature/Time Program for the Digestion of MS Smoke Samples

Stage:	1	2	3	4	5
Power %:	70	70	70	0	100
Pressure (psi):	45	125	175	20	150
Run Time (min):	20	10	30	20	20
Time at Parameter:	8	8	25	20	10
Temperature:	95	135	190	25	190
Fan Speed:	50%	50%	50%	80	

Note: Both pressure and temperature are set as the controlling parameters in this digestion program. If the preset pressure or temperature is not reached, the microwave oven delivers the designated power for the time programmed in the Run Time function.

Pressure/Temperature/Time Program a Secondary Digestion

Stage:	1	2	3	4
Power %:	75	75	75	0
Pressure (psi):	95	125	185	20
Temperature:	105	130	160	25
Run Time (min):	15	20	20	20
Time at Parameter:	10	15	15	20
Fan Speed	50	50	50	80

Note: These are only suggested parameters as a starting point. The digestion procedure must be optimized for the specific application and instrument used.

Appendix 3: ICP-AES Parameters

Power (kw): 1.20
Plasma Flow (L/minute): 15.0
Auxiliary Flow (L/minute): 1.50
Nebulizer Flow (L/minute): 0.65

	Ni	Pb	Cd	Cr
Emmision Wavelength (nm)	221.648	220.353	214.439	267.716

Sample Introduction Settings

Sample Uptake Delay(s): 40
Pump rate (rpm): 20
Instrument Stabilization Delay(s): 15
Rinse Time(s): 10

General Settings

Replicates: 3
Replicate Read Time(s): 3.0
Number of Standards Defined: 5

Ultrasonic Nebulizer Set-up

Heater: 140
Cooler: 2

No: T-208
Date: December 31, 1999
Page: 1 of 11

1. SCOPE OF APPLICATIONS

- 1.1** This is applicable to the Nitric Oxide (NO) and Total Oxides of Nitrogen (NO_x) generated in the gas phase of filtered sidestream tobacco smoke on a continuous basis.
- 1.2** This method describes the collection of vapour phase NO and NO_x in sidestream tobacco smoke using a fishtail assembly and their quantification by a dual channel chemiluminescence analyzer. The method can be applied to both cigarettes and cigars.

2. NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3. DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4. METHOD SUMMARY

- 4.1** A single port smoking machine is configured with a fishtail chimney assembly and calibrated flow-controlled vacuum pumps.
- 4.2** One cigarette or cigar is smoked beneath the fishtail chamber following a set smoking protocol and the smoke is swept up the chimney by vacuum at the rate of 2 L/minute. The Total Particulate Matter (TPM) of the sidestream smoke is collected on a glass fibre filter disc (pad) at the top of the chimney and a subsample of the filtered sidestream vapour phase is dynamically diluted with N₂ and then pumped to a dual channel chemiluminescence nitrogen oxides analyzer.
 - 4.2.1** The gas stream is split immediately into two channels.
 - 4.2.2** In channel A, the sample stream is reacted with ozone and the resultant chemiluminescent emission is directly proportional to the NO concentration in the sample.
 - 4.2.3** In channel B, the sample stream is chemically reduced first by a catalytic converter and then mixed with ozone in the reaction cell where the resultant chemiluminescent emission is due to NO_x or NO + NO₂.
 - 4.2.4** The NO₂ concentration is then derived electronically by subtracting the NO signal from the NO_x signal.

- 4.3** Selective photomultiplier detection monitors the reaction cell gas stream and the NO and NO_x found in the vapour phase of sidestream tobacco smoke are quantified by external standard calibration procedures.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
- 5.2** Equipment needed to perform marking for butt length as specified in T-115.
- 5.3** Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4** Dual Channel Chart Recorder.
- 5.5** Dual Channel Chemiluminescence Nitrogen Oxides Analyzer with vacuum pump.
- 5.6** V/F Integrator.
- 5.7** Digital Multimeter.
- 5.8** Fishtail Chambers – British American Tobacco (BAT).
- 5.9** 2 X 1 L Round bottomed boiling flask 1 L (PEC1 and PEC2).
- 5.10** Gas Regulator CGA 660 - Inlet 0-3000, Delivery 0-100.
- 5.11** Gas Regulator CGA 580 - Inlet 0-4000, Delivery 0-100.
- 5.12** Flow meter FM1 (NO_x Analyzer Make-up).
- 5.13** Flow meter FM2 (Calibration Gas).
- 5.14** Flow meter FM3 (Sidestream Sub Sample).
- 5.15** Flow meter FM4 (Sidestream Makeup).
- 5.16** Flow meter FM5 (Sidestream Make up gas).
- 5.17** Flow meter FM6 (Fishtail total flow).
- 5.18** Glass fibre filter holders 44 mm (threaded, screw cartridge) see Diagram 2.
- 5.19** Nalgene Tubing 1/4" ID X 3/8" OD.
- 5.20** Teflon (TFE) Tubing 1/4" (6.35 mm) X 5.8 mm ID.
- 5.21** Inert Valves (or stop cocks) with three port housing and plug with 90 ° right angled flow (STC1 & STC2).
- 5.22** Balance capable of measuring to four decimal places.
- 5.23** Vacuum Pump (VP1) (NO_x Analyzer make-up).
- 5.24** Vacuum Pump (VP3) (Sidestream Sub-sample).
- 5.25** Vacuum pump (VP4) (Sidestream sub-sample dilution).
- 5.26** Vacuum pump (VP6) (Sidestream total flow).
- 5.27** Flow Meter/High capacity bubble meter to measure sidestream flow
- 5.28** Filter Cartridges (Silica gel cartridges).
- 5.29** Barometer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical grade in quality.

- 6.1** Compressed Primary Standard Gas Mixture – 127 ppm NO balance nitrogen (certified).
- 6.2** Compressed Primary Standard Gas Mixture – 70 ppm NO balance nitrogen (certified).

- 6.3 Compressed Primary Standard Gas Mixture – 35 ppm NO balance nitrogen (certified).
- 6.4 Compressed Primary Standard Gas Mixture – 9 ppm NO balance nitrogen (certified).
- 6.5 Nitrogen N₂ (Zero Gas) UHP.
- 6.6 Methanol.
- 6.7 Reagent Alcohol.
- 6.8 Chart Paper.
- 6.9 Tweezers.
- 6.10 Stop watch.
- 6.11 Paper towels.
- 6.12 Glass fibre filter discs (pads) 44 mm in diameter, with no more than 5 % acrylic type binder.

7 PREPARATION OF GLASSWARE

- 7.1 The BAT fishtail chamber is rinsed three times with reagent alcohol and once with methanol and then air-dried.
- 7.2 All Teflon three way stopcocks are rinsed with reagent alcohol to remove the tar and once with methanol and allowed to air dry.
- 7.3 The piston and chamber of the single port smoking machine is cleaned and rinsed with methanol and then allowed to air dry. The piston is then reassembled, free of any lubricants.

8 SAMPLING

- 8.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

9 TOBACCO PRODUCT PREPARATION

- 9.1 Product shall be conditioned as specified in T-115.
- 9.2 Cigarettes and cigars shall be marked for butt length as specified in T-115.
- 9.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

10 SMOKING MACHINE PREPARATION

10.1 Ambient Conditions

- 10.1.1 The ambient conditions for calibration shall be between 21 and 23 °C.

10.2 Machine Conditions

- 10.2.1 The machine conditions shall be as those specified in T-115.

11 SAMPLE GENERATION

- 11.1** Allow the smoking machine to warm up on the automatic cycling for several minutes and ensure that the piston is oil-free.
- 11.2** The smoking machine operation shall be carried out as specified in T-115.
 - 11.2.1** Because the analysis is dynamic and ongoing, it is important to ensure that the sidestream tobacco smoke generated was characteristic of the test sample. This can be accomplished if the mainstream and sidestream TPM are determined (i.e. net difference in the weight of the pad before and after smoking). The mainstream and sidestream TPM data and puff count information can then be used to characterize the vapour phase samples and monitor the smoking process.
- 11.3 Set-up of Sidestream Flow Detection Equipment**
 - 11.3.1** The NO/NO_x Analyzer is pumped down (left on) overnight, then the ozone generator and molycon catalytic converter are activated.
 - 11.3.2** The NO/NO_x Analyzer inlet filter is changed once per day.
 - 11.3.3** The sidestream NO/NO_x equipment and tubing are assembled as per Appendix: Diagram 1.

12 INSTRUMENT ANALYSIS

12.1 Performance Test of NO/NO_x Analyzer

- 12.1.1** The NO/NO_x Analyzer electronic output is synchronized to both the two pen chart recorder and to the V/F Integrator to be in the range of 10 mV (ppm).
- 12.1.2** Various NO/NO_x Analyzer instrument tests are performed prior to any smoking or calibration.
- 12.1.3** Test T1, T2, and T3 outputs and measure on the analogue scale. This should remain constant day to day.
- 12.1.4** Switch the Range PPM to Optic and Electric test modes. Allow to stabilize at least 15-20 minutes before reading. These outputs should be consistent in the range of 40 – 80 % on a day to day basis but with some variation expected.
- 12.1.5** Switch the Range PPM back to 10 for the rest of the experiment.

12.2 Zero the NO/NO_x Analyzer with Nitrogen

Note: This is performed in vacuum mode.

- 12.2.1** Connect the nitrogen (zero gas) to FM2 to deliver 1.8 L/minute to the inlet port of the pressure equalization chamber (PEC1).
- 12.2.2** There are two outlets from PEC1, one to the exhaust and the other is connected directly to STC2.

12.2.3 Set the N₂ regulator to deliver 10 psi.

12.2.4 STC2 is directed to deliver flow to the NO/NO_x Analyzer.

12.2.5 Allow to equilibrate for 30 minutes.

12.2.6 Adjust the Zero pots on the NO/NO_x Analyzer to give an average of 0.000 over 60 seconds reading NO and NO_x on the digital multimeter.

12.3 Span the NO/NO_x Analyzer

Note: This is performed in vacuum mode.

12.3.1 Connect the 9 ppm NO primary standard to FM2 to deliver 1.8 L/minute to inlet port of PEC1.

12.3.2 Set the gas regulator to deliver 10 psi.

12.3.3 The outlet line from PEC1 is connected directly to STC2 which delivers flow to the NO/NO_x Analyzer.

12.3.4 Allow to equilibrate for 15 minutes.

12.3.5 Adjust the Span pots on the NO/NO_x Analyzer to give an average of 9.000 (see calibration gas) over 60 seconds on the digital multimeter. (Adjust the span pots to give a corresponding voltage to concentration i.e. NO=9.1 NO_x=9.3).

12.3.6 Record Span settings and adjust attenuation on plotter if necessary. **Do not adjust Span or Zero from here on.**

12.3.7 Optional: The above procedure can be repeated with a 5 ppm NO standard gas or another standard NO gas that fall in the linear range.

12.4 Confirm sidestream setup

Note: This section is confirmed in the positive displacement mode.

12.4.1 Disconnect VP1 from the NO/NO_x Analyzer and allow the NO/NO_x Analyzer to vent directly to exhaust.

12.4.2 Join the inlet ports of VP3 and VP4 using a Y connector.

12.4.3 Connect the outlet from PEC1 directly to the Y connector.

12.4.4 Connect the Nitrogen to FM2 to deliver 1.8 L/minute to the inlet port of PEC1. Set the gas regulator to deliver 10 psi.

12.4.5 Nitrogen is pumped through both VP3/FM3 and VP4/FM4 and the flow is merged and directed to the NO/NO_x Analyzer. FM3 is calibrated to 80 cc/minute and FM4 to 1420 cc/minute (total 1.5 L/minute).

12.4.6 Allow Nitrogen to equilibrate for 20-30 minutes maximum. Read voltage over 60 seconds for NO/NO_x. Do not adjust the zero pots.

12.4.7 Results should be consistent with that obtained in section 12.2.6.

12.4.8 Repeat the above procedure (12.4.1 – 12.4.6) with 9ppm standard gas and record the voltage over 60 seconds.

12.4.9 Results should be consistent with that obtained in section 12.3.5.

12.5 Calibrate Sidestream Fishtail Chimney Assembly

Note: This section is confirmed in the positive displacement mode.

12.5.1 Activate vacuum pump VP6 and adjust flow meter FM6 to deliver 2.0 L/minute. Check flow using high capacity bubble meter. STC1 is directed to room air.

12.5.2 Connect the 127 ppm NO Standard gas to FM2 to deliver 2.5 L/minute to the inlet port of PEC1.

12.5.3 Set the gas regulator to deliver 10 psi.

12.5.4 The outlet line from PEC1 is connected directly to the calibration gas port of STC1. The common port of STC1 is connected to FM6/VP6 which has been calibrated to 2 L/minute.

12.5.5 Connect Nitrogen (set to deliver 10psi) to FM5 which is set to deliver 1.5 L/minute to PEC2.

12.5.6 Connect the outlet line/port of PEC2 to VP4/FM4. Set FM4 to 1.42 L/minute. The purpose of the gas is to dilute the sidestream sub-sample.

12.5.7 Read the baseline from the V/F Integrator.

12.5.8 Take 90 seconds sidestream samples by rotating STC1 between the 127 ppm NO standard gas and room air.

12.5.9 Note the voltage on the digital multimeter and compare to the expected (as a guide):

$$\frac{127 \text{ ppm} \times 0.080 \text{ L}}{1.5 \text{ L}} = 6.7 \text{ mV.}$$

12.5.10 Add baseline to expected voltages to give actual target values.

12.5.11 Record the counts and adjust the V/F Integrator threshold pots. Repeat until total counts are reproducible and consistent between the NO and NO_x within 1-2 % from one trial to the next.

- 12.5.12** Do multiple 10 minute samplings; record count and determine average total counts per 10 minute sample. This figure is inserted into the calculation equation at a latter stage.
- 12.5.13** The NO/NO_x Analyzer is now calibrated for operation.
- 12.5.14** Repeat the above procedure (12.5.1 – 12.5.12) with the 70ppm NO Standard gas recording multiple 10 minute samplings without adjusting the voltages.
- 12.5.15** Close gas cylinders and disconnect the line from PEC1 to STC1. Be sure the room and the sidestream apparatus are clear of calibration gas before starting smoking procedure.

13 SMOKING PROCEDURE

- 13.1.1** Re-assemble configuration as per Diagram 1.
- 13.1.2** Fit a conditioned pad into the mainstream pad holder and sidestream pad holder with the rough side towards the incoming smoke.
- 13.1.3** Raise chimney level to highest position (loading position).
- 13.1.4** Attach the weighed pad holders to the smoking machine (sidestream and mainstream).
- 13.1.5** Turn the **Puff Counter** switch to **ON** and switch to automation.
- 13.1.6** Gently insert the cigarette into the cigarette holder to a depth greater than 9 mm. Withdraw the cigarette until the 9 mm mark is just visible.
- 13.1.7** Turn on the sidestream pumps (VP3, VP4 and VP6) at the beginning of the lighting procedure at t minus 30 seconds.
- 13.1.8** Light the cigarette and initiate the puff count according to the following schedule. Be sure to light the cigarette on the first puff.
- 13.1.8.1** Normal lighting procedure is 15 seconds warm-up followed by five seconds ignition. (Three seconds prior to puff plus the two seconds puff).
- 13.1.9** Lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. Do not allow the cigarette to touch the chimney. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.
- 13.1.10** Sidestream smoke pattern should be similar to **Figure 1**.
- 13.1.11** The test cigarette is smoked to the previously marked standard butt length. Remove the butt with a pair of tweezers from beneath the BAT fishtail chamber and extinguish in a beaker of water.

13.1.12 All vacuum pumps (VP3, VP4 and VP6) continue to draw any residual smoke within the sidestream chamber until the V/F Integrator counter stops. Record the time.

13.1.13 At the end of the smoking process raise the chimney and remove the pad holders.

13.1.13.1 Re-weigh the sidestream pad holder and record the "after smoking" weights of the sidestream pad holders.

13.1.13.2 Re-weigh the mainstream pad holder and record the "after smoking" weights of the mainstream pad holders.

13.1.13.3 Record the puff count.

13.1.13.4 Record the total number of counts for each channel, the NO and NO_x and the total time to the end of smoking (counting) on the run sheet.

14 CALCULATIONS

- 14.1** The total counts for each channel for each test brand are summarized and the vapour phase concentrations of NO and NO_x in [µg/cigarette] in sidestream tobacco smoke are calculated.

$$\text{NO } \mu\text{mole/cigarette} = \frac{\text{TC}_{(\text{NO})}}{\text{AC}_{(\text{NO})}} \times \frac{127}{\text{TS}} \times \frac{273}{273 + ^\circ\text{C}} \times \frac{10}{22.4} \times \frac{\text{BP}}{\text{SBP}} \times \text{DF.}$$

$$\text{NO } \mu\text{g/cigarette} = \frac{\mu\text{ mole}}{\text{cigarette}} \times \frac{\mu\text{g.}}{\mu\text{ mole}}$$

and

$$\text{NO}_x \mu\text{mole/cigarette} = \frac{\text{TC}_{(\text{NO}_x)}}{\text{AC}_{(\text{NO}_x)}} \times \frac{127}{\text{TS}} \times \frac{273}{273 + ^\circ\text{C}} \times \frac{10}{22.4} \times \frac{\text{BP}}{\text{SBP}} \times \text{DF.}$$

$$\text{NO}_x \mu\text{g/cigarette} = \frac{\mu\text{ mole}}{\text{cigarette}} \times \frac{\mu\text{g.}}{\mu\text{ mole}}$$

where:

- TC_(NO) is the total continuous counts during smoking of the NO channel.
- TC_(NO_x) is the total continuous counts during smoking of the NO_x channel.
- TS is the time to end of Smoking (minutes).
- DF is the dynamic dilution factor. Dilution factor is 1/sss/(sss+ss).
where sss = sidestream sub-sample; ss = sidestream flow (L/minute).
- 127 is the Calibration gas 127 ppm (µL/L).
- AC_(NO) is the average continuous count per NO channel for the calibration gas for 10 minutes.
- AC_(NO_x) is the average continuous count per NO_x channel for the calibration gas for 10 minutes.
- °C is the room temperature.
- 22.4 µL/µmole.
- BP is the barometric pressure (inches Hg).
- SBP is the barometric pressure at standard temperature and pressure (29.92 inches Hg).

15 QUALITY CONTROL

15.1 Typical Graph

15.1.1 See Figure 1.

15.2 Recoveries and Levels of Contamination

Each analytical run of test cigarettes should also include the following:

- 15.2.1** A Laboratory Reagent Blank (LRB) to evaluate the extent of any interferences due to glassware, trapping reagents, glass fibre filter pads, and analyzer effects. In this case it would be the Zero gas.

15.2.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss. The 9 ppm NO standard gas is the LFB.

15.2.3 Test a Reference Sample such as 1R4F to determine the inter-experimental reproducibility of the entire method of analysis.

15.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary standard gas mixtures are maintained at room temperature (22 °C).

15.4.2 All samples are analyzed on a continuous basis as smoked.

16 MODIFICATIONS FOR INTENSE SMOKING

16.1 No modifications are necessary under intense smoking conditions.

17 REFERENCES

- 17.1** Rickert, W.S., Robinson, J.C., and Collishaw, N.E., 1987. Decay of Cigarette Smoke NO_x an Ambient Air Under Controlled Conditions, *Environmental International* 13, p. 399-407.
- 17.2** Norman, V., Ibrig, A.M., Larson, T.M., and Moss, B.L., 1983. The Effect of Some Nitrogenous Blend Components on NO/NO_x and HCN Levels in Mainstream and Sidestream Smoke, *Beitrie zur Tabakforschung International* 12, No. 2, p. 55-62.
- 17.3** Neurath, G. and Dunger, M., 1972. IARC (International Agency for Research on Cancer) *Science Publication*, No.3, p.134-136.

APPENDICES

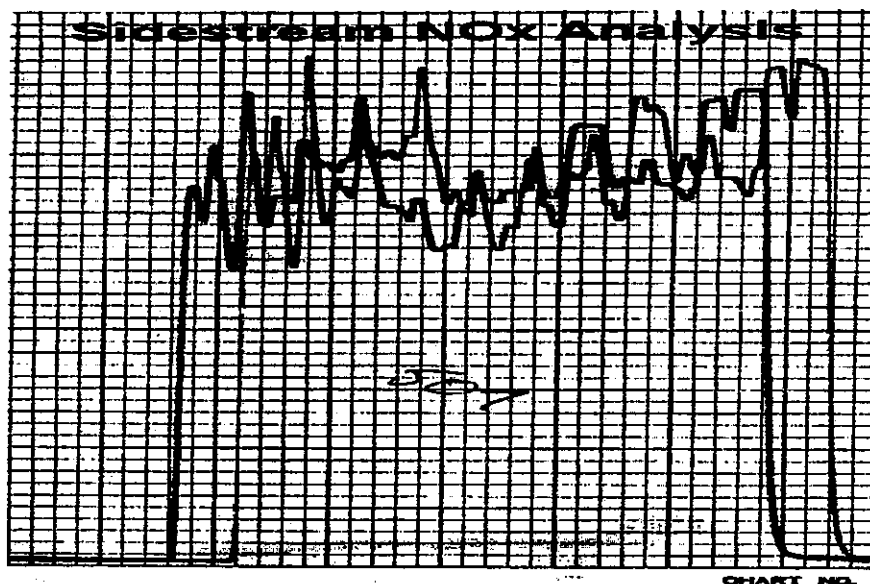
Figure 1 : Graph of Vapour Phase NO and NO_x in Sidestream Tobacco Smoke

Diagram 1: Sidestream Smoking/ Calibration Mode

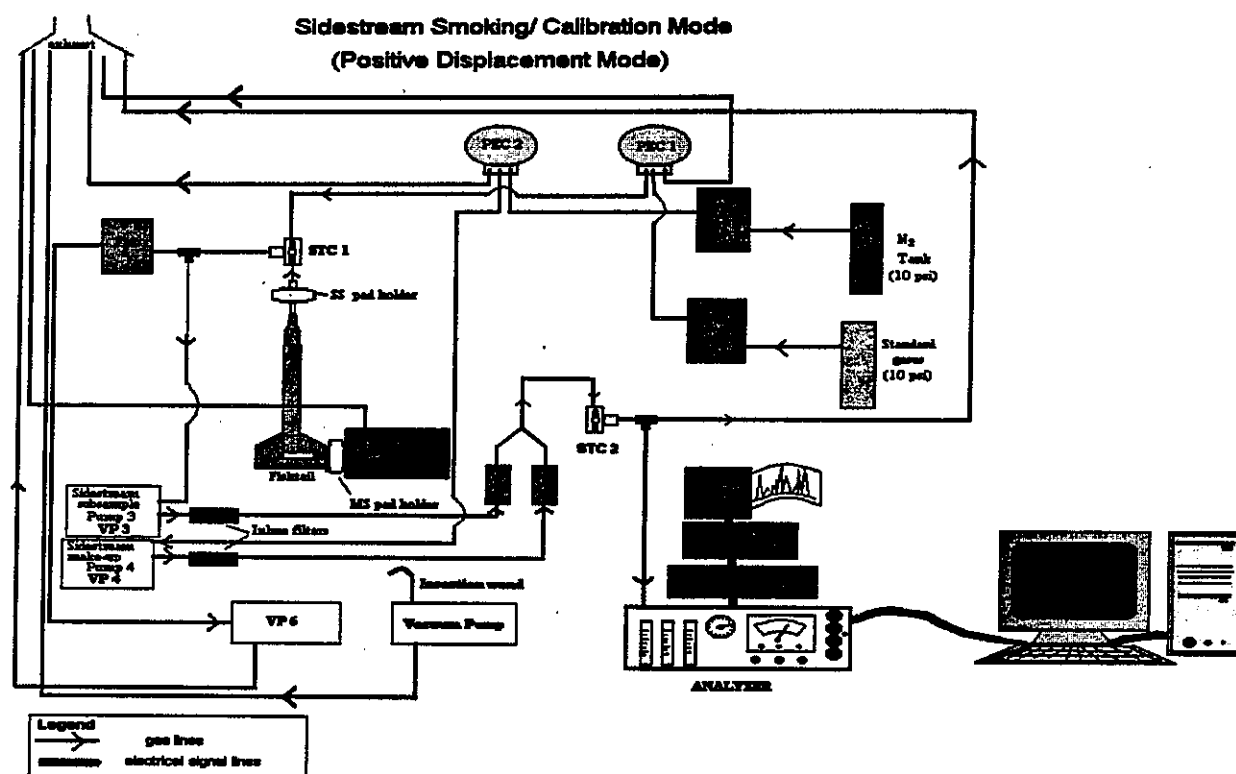
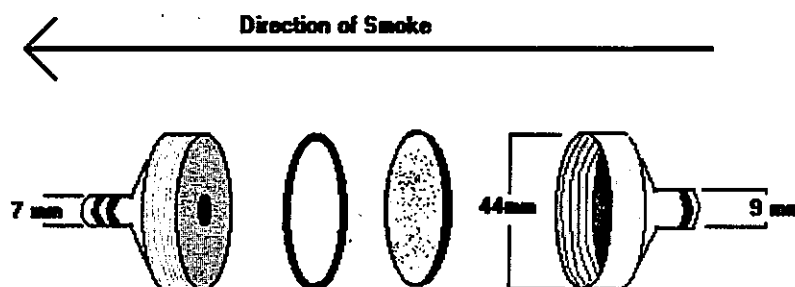


Diagram 2: Glass fibre filter holder: Threaded screw cartridge



Pad Holder Setup (Measurements are inner diameters only)

No.: T - 209
Date: December 31, 1999
Page: 1 of 12

1 SCOPE OF APPLICATIONS

- 1.1 The volatile nitrosamines (VNA) are formed during the processing of tobacco and during the smoking of tobacco products. The tobacco processing methods include air-, sun-, flue- and fire-curing, ageing and fermentation.
- 1.2 The generation of sidestream smoke is achieved under standard machine smoking conditions for cigarettes as specified in T-115. Sidestream smoke is effectively, all smoke emitted from the cigarette, other than mainstream smoke.
- 1.3 This method is suitable for the quantitative determination of four tobacco specific N-nitrosamines (TSNA) in sidestream tobacco smoke (SS): N-nitrosornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB).

2 NORMATIVE REFERENCES

- 2.1 American Society for Testing and Materials (ASTM) D1193-77 – Standard Specifications for Reagent Water, Version 1977.
- 2.2 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 Four* cigarettes are smoked individually beneath a British American Tobacco (BAT) fishtail chamber coated with ascorbic acid at a 2 L/minute flow rate in order to capture the sidestream (SS) smoke.
- 4.2 The SS smoke passes through an aqueous buffer, containing ascorbic acid, which prevents formation of TSNA by scavenging oxides of nitrogen. The SS smoke is then trapped onto a pre-treated (with ascorbic acid) 44 mm glass fibre filter disc (pad).
- 4.3 The TSNA are concentrated by combining the dichloromethane extraction of the aqueous buffer and Cambridge filter pad, followed by column chromatography onto basic alumina.
- 4.4 The fraction containing TSNA is eluted, then quantitatively analyzed by combined gas chromatography-thermal energy analysis (GC-TEA). N-nitrosoguvacoline (NG) is used as an internal standard.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

All four TSNA are carcinogenic in several species of laboratory animals. Extreme care should be taken in handling these compounds. The exhaust of the TEA detector should be vented properly in order to reduce exposure to possible excess ozone (O₃).

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.2 Equipment needed to perform conditioning as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 250 mL Round-bottom flask with ground glass joint.
- 5.5 Volumetric Flask 2, 10, 50, 100, 200 mL.
- 5.6 100 mL Beaker.
- 5.7 Wrist action shaker.
- 5.8 LC Column with Frit and Stopcock, 300 mm X 22 mm ID X 25 mm OD (Supelco 64754) or equivalent.
- 5.9 Silinized Glass Wool.
- 5.10 Short Stem Glass Funnel.
- 5.11 250 mL Separatory Funnel.
- 5.12 Glass Pasteur Pipettes.
- 5.13 Zymark TurboVap II Concentrator equipped with 200 mL tubes with graduated 1 mL stem or equivalent.
- 5.14 Thermal Energy Analyzer (Thermo-Electron Corp.) interfaced to GC or equivalent.
- 5.15 GAST Pumps or equivalent.
- 5.16 Ace flow meters or equivalent.
- 5.17 BAT Fishtail Chambers.
- 5.18 70 mL impingers with ground glass joints and Teflon sleeves.
- 5.19 Gas chromatograph, equipped with temperature programmable injector, electronic flow control and data processing software.
- 5.20 GC column, 30 m X 0.32 mm X 3.0 :m DB-1 fused silica capillary column.
- 5.21 Non-ultra violet (UV) lighting.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Dichloromethane – Distilled in Glass (DIG).
- 6.2 Acetone – DIG.
- 6.3 Sodium sulfate – Anhydrous.
- 6.4 Basic Alumina.
- 6.5 Citric Acid (Anhydrous).

- 6.6 L-Ascorbic acid.
- 6.7 Sodium Phosphate Dibasic.
- 6.8 Methano – DIG.
- 6.9 Type I water.

6.10 Aluminum foil.

6.11 Petri dish.

7 PREPARATION OF GLASSWARE

7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

8.1 1:1 Acetone:Dichloromethane solution

8.1.1 Mix Acetone and Dichloromethane in a 1:1 v/v basis.

8.2 Citrate-Phosphate Buffer containing L-Ascorbic Acid

8.2.1 Prepare a 1 L solution with Type I water containing :

8.2.1.1 55 mM Citric Acid.

8.2.1.2 90 mM Sodium Phosphate Dibasic.

8.2.1.3 20 mM L-Ascorbic Acid.

Note: pH of the solution must be between 4.3 and 4.5 or it must be remade.

8.3 Aqueous L-Ascorbic Acid Solution for SS Pad Pre-treatment

8.3.1 Weigh 33.33 g of L-ascorbic Acid into a 200 mL volumetric flask.

8.3.2 Make up to volume with Type I water.

Note: If the solution has yellowed, the solution should be re-made.

9 PREPARATION OF STANDARDS

9.1 N-nitrosoguvacoline (NG) internal standard

9.1.1 Prepare a solution at 5000 ng/mL in dichloromethane.

9.2 TSNA mixed standard solution

9.2.1 Prepare a mixed standard dilution stock solution of NNN, NAT, NAB and NNK in dichloromethane at the following range of concentrations:

9.2.1.1 NNK at 3000 ng/mL.

9.2.1.2 NNN and NAT at 1500 ng/mL.

9.2.1.3 NAB at 500 ng/mL.

Note: Concentrated solutions are stable for approximately six months if stored at -20°C in such a manner as to prevent loss of solvent from evaporation.

9.2.2 Build a calibration ranging from approximately 20 ng/mL (for NAB) to 2000 ng/mL (for NNK) containing NG as an internal standard at 500 ng/mL in each of the standards.

9.2.3 Representative calibrations are in **Appendix V**.

Note: Individual calibration stocks are stable for two months if properly stored in a freezer.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product is to be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars are to be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.1.2 Non-UV lighting shall be used in the rooms in which sample generation and sample analyses are conducted.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.2 Pre-treatment of SS Collection Pad.

12.2.2.1 Saturate the SS collection pad with the aqueous L-ascorbic acid solution by placing the pad in a Petri dish with the solution for 15 seconds, turning over and allowing to sit for an additional 15 seconds.

12.2.2.2 Place the pads in a rack in the CER for conditioning for 24-48 hours to allow the pads to dry and equilibrate.

Note: A slight yellowing of the pad will occur over the 48 hours and the pad will feel brittle.

12.2.3 Pre-treatment of BAT Fishtail

12.2.3.1 Prepare an L-Ascorbic acid slurry with methanol in a 100 mL beaker.

12.2.3.2 Pour the slurry down the fishtail from the cigarette end to the top of the fishtail to create an even coat over the entire inner surface of the fishtail.

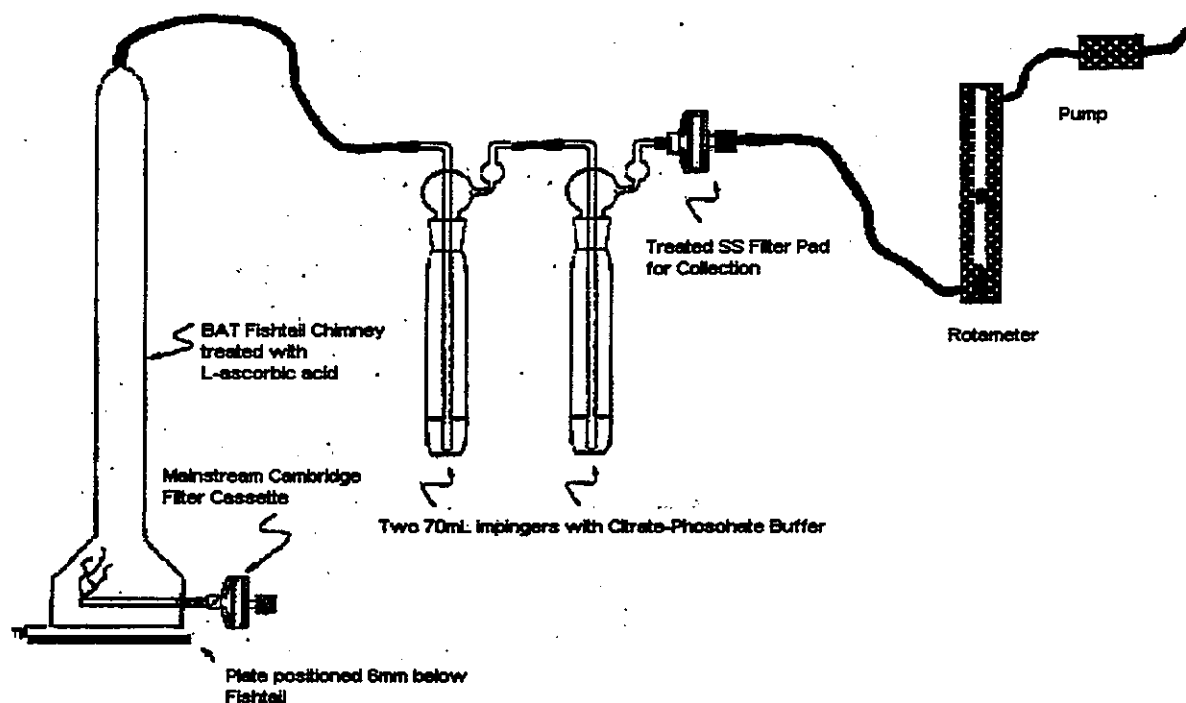
Note: The thickness of the coat should be such that one cannot look through the fishtail.

12.2.4 SS Smoke Generation and Trapping

12.2.4.1 Prepare two 70 mL impingers each containing 25 mL of the citrate-phosphate buffer containing L-ascorbic acid.

12.2.4.2 Connect these two impingers in series to the exit of the BAT fishtail and place a treated 44 mm Cambridge filter pad after the second impinger, as per the illustration.

12.2.4.3 Place a regulating flow meter between the Cambridge filter and a constant vacuum source and adjust the flow rate to 2 L/minute.

Sidestream Apparatus Set-Up Diagram**13 SAMPLE GENERATION**

- 13.1 Place a cigarette in the cigarette holder connected to the piston type smoking machine.
- 13.2 Light the cigarette and lower the sidestream apparatus over the cigarette placing a metal plate 6 mm below the edge of the fishtail. Smoke the cigarette as per T-115.
- 13.3 Smoke four cigarettes in this manner, capturing the sidestream smoke, to make one sample.

14 SAMPLE ANALYSIS**14.1 TSNA Extraction and Concentration**

- 14.1.1 Immediately after smoking, place the SS filter pad into a 250 mL round bottom flask wrapped with aluminum foil.
- 14.1.2 Add 100 μ L of internal standard solution to the flask.

- 14.1.3 Combine the buffer solutions from the two impingers into a 250 mL separatory funnel.
- 14.1.4 Wash (rinse) each of the impingers with 25 mL (total of 50 mL) of dichloromethane and add to the separatory funnel.
- 14.1.5 Shake the separatory funnel to extract the TSNA.
- 14.1.6 Drain the dichloromethane layer into the round bottom flask containing the SS filter pad.
- 14.1.7 Repeat the extraction (12.1.5 to 12.1.6) with two additional 50 mL portions of dichloromethane (total extraction volume 150 mL).
- 14.1.8 Extract the pad by placing the flask on a wrist action shake for 30 minutes until the SS pad is completely broken up.
- 14.1.9 Place a plug of silanized glass wool into the stem of a glass funnel.
- 14.1.10 Add approximately 2 g of anhydrous sodium sulphate (1 cm depth in funnel).
- 14.1.11 Pour the extract from the 250 mL round bottom flask through the funnel directly into a 200 mL TurboVap tube.
- 14.1.12 Rinse the flask with 2 X 20 mL portions of dichloromethane, passing the rinses through the funnel to aid in transfer of all of the extracted TSNA to the TurboVap tube.
- 14.1.13 After all solution has stopped dripping through the funnel, place the sample into the TurboVap II Concentrator set at 38 °C and 10 psi nitrogen.
- 14.1.14 Concentrate samples to approximately 5 mL.

14.2 Column Chromatography Clean-up Procedure

- 14.2.1 Prepare Basic Alumina Column by adding 50 mL of DCM to an empty, dry, glass LC column.
- 14.2.2 Add 10 g (+/- 0.2) of oven dried (110 °C) basic alumina to the liquid in the column. Stir the alumina slurry with a glass rod to remove any possible air pockets.
- 14.2.3 Drain the liquid from the column to waste, closing the stopcock when the solution is at the level of the alumina.
- 14.2.4 Wash the alumina by adding 50 mL of DCM to the column. Drain the liquid to waste and close the stopcock when the solution is at the level of the alumina.

- 14.2.5 Add the 5 mL sample from the TurboVap tube to the alumina column using a glass Pasteur pipette attempting not to disturb the alumina packing.
- 14.2.6 Drain the liquid from the column to waste, closing the stopcock when the solution is at the level of the alumina.
- 14.2.7 Rinse the TurboVap tube with 10 mL DCM, washing the lower portion (25 %) of the tube with repeated flushing using a Pasteur pipette.
- 14.2.8 Add the 10 mL rinse from the TurboVap tube to the alumina column using a glass Pasteur pipette attempting not to disturb the alumina packing.
- 14.2.9 Drain the liquid from the column to waste, closing the stopcock when the solution is at the level of the alumina.
- 14.2.10 Rinse the TurboVap tube and alumina column with an additional 40 mL DCM attempting not to disturb the alumina packing, draining the liquid from the column to waste.
- 14.2.11 Place a clean 200 mL TurboVap tube beneath the LC Column to collect the sample.
- 14.2.12 Elute the TSNA from the alumina column by adding 50 mL of 1:1 Acetone:DCM to the column attempting not to disturb the alumina packing.
- 14.2.13 Collect the liquid from the column into the TurboVap tube, closing the stopcock when the solution is at the level of the alumina.
- 14.2.14 Repeat steps 14.2.12 and 14.2.13 four more times, collecting the eluent in the same TurboVap tube (a total of 250 mL collected).

Note: This will require evaporating a portion of the eluent before the final 50 mL can be collected into the tube.

14.3 Sample Re-Concentration

- 14.3.1 Place the samples into the TurboVap II Concentrator set at 38 °C and 9 psi nitrogen.
- 14.3.2 After the samples have been concentrated to approximately 150 mL, increase the pressure to 10 psi.
- 14.3.3 Concentrate samples to 0.8 mL or when the sensor turns the concentration off (approximately 45 minutes).
- 14.3.4 Add dichloromethane to the tube to the 1.0 mL graduation of the tube.
- 14.3.5 Rinse the lower portion (25 %) of the tube with the final extract to dissolve any residue that may have dried to the side of the tube.

14.3.6 Transfer the sample to an amber autosampler vial with Teflon lined septa for GC analysis.

14.4 GC-TEA Analysis

14.4.1 GC-TEA Operating Conditions

14.4.1.1 Carrier flow rate (He): 2.8 mL/minute using electronic flow control (velocity = 60 cm/second).

14.4.1.2 Injector temperature: Programmable 35 to 220 °C.

14.4.1.3 Oven temperature: Programmed 50 to 170 to 212 °C.

14.4.1.4 TEA interface temperature: 240 °C.

14.4.1.5 TEA furnace temperature: 500-525 °C (dependent on analyzer sensitivity).

14.4.1.6 Analysis Run Time: 35 minutes.

14.4.2 Blank test

14.4.2.1 Blank tests using purified nitrosamine-free air should be performed periodically in order to ensure the absence of nitrosamine traces in the analytical environment, or their formation during analysis.

14.4.3 GC-TEA Calibration

14.4.3.1 Inject 1.5 µL of the TSNA mixed standard solution and determine peak areas for the four components.

14.4.4 TSNA determination

14.4.4.1 Inject 1.5 µL of the sample concentrate (14.3.6) and determine areas of the peaks having retention times corresponding to NNN, NAT, NAB and NNK.

14.5 Method of Calculation

14.5.1 The content, m_{cg} (ng/cigarette), of a given TSNA is obtained from:

$$m_{cg} = CV_s/N$$

where

C = analytical Concentration determined by ISTD calibration of given TSNA.

V_s = final volume of concentrate.

N = number of cigarettes/little cigars/cigars smoked.

15 QUALITY CONTROL

15.1 Typical Chromatogram

15.1.1 See Appendix 1.
15.2 Method Detection Limits (MDL) / Limit of Quantitation (LOQ)

- 15.2.1** The method detection limit (MDL) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The MDL is calculated as three times the standard deviation of these determinations.
- 15.2.2** The MDL (on a ng/cigarette basis) can be calculated by multiplying the determined MDL (ng/mL basis) by the final volume of the extract and dividing this by the number of cigarettes smoked.
- 15.2.3** The MDL (on a ng/cigarette basis) can be enhanced by modifying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure.
- 15.2.4** The practical limit of quantitation (LOQ) is determined by analyzing the lowest standard level a minimum of 10 times as an unknown over several days. The LOQ is calculated as 10 times the standard deviation of these determinations.
- 15.2.5** The LOQ (on a ng/cigarette basis) can be calculated by multiplying the determined LOQ (ng/mL basis) by the final volume of the extract and dividing this by the number of cigarettes smoked.
- 15.2.6** The effect of varying the number of cigarettes smoked and the volumes used for extraction and clean-up in the procedure on the LOQ is the same as for the MDL.

16 MODIFICATIONS FOR INTENSIVE SMOKING

- 16.1** None required.

17 REFERENCES

- 17.1** Adams, J.D., Brunneemann, K.D. & Hoffmann, D., 1983. Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific N-nitrosamines by gas-liquid chromatography with a thermal energy analyser. *J. Chromatogr.*, p. 256, 347-351.
- 17.2** Brunneemann, K.D. & Hoffmann, D., 1981. Assessment of the carcinogenic N-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis*, p. 2, 1123-1127.
- 17.3** Hecht, S.S., Adams, J.D. & Hoffmann, D., 1983. Tobacco-specific nitrosamines in tobacco and tobacco smoke. In: Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalter, B. & Bartsch, H., eds, *Environmental Carcinogens- Selected Methods of Analysis*, Vol. 6, N-Nitroso Compounds (IARC Scientific Publications No. 45), Lyon, International Agency for Research on Cancer, p. 93-101.

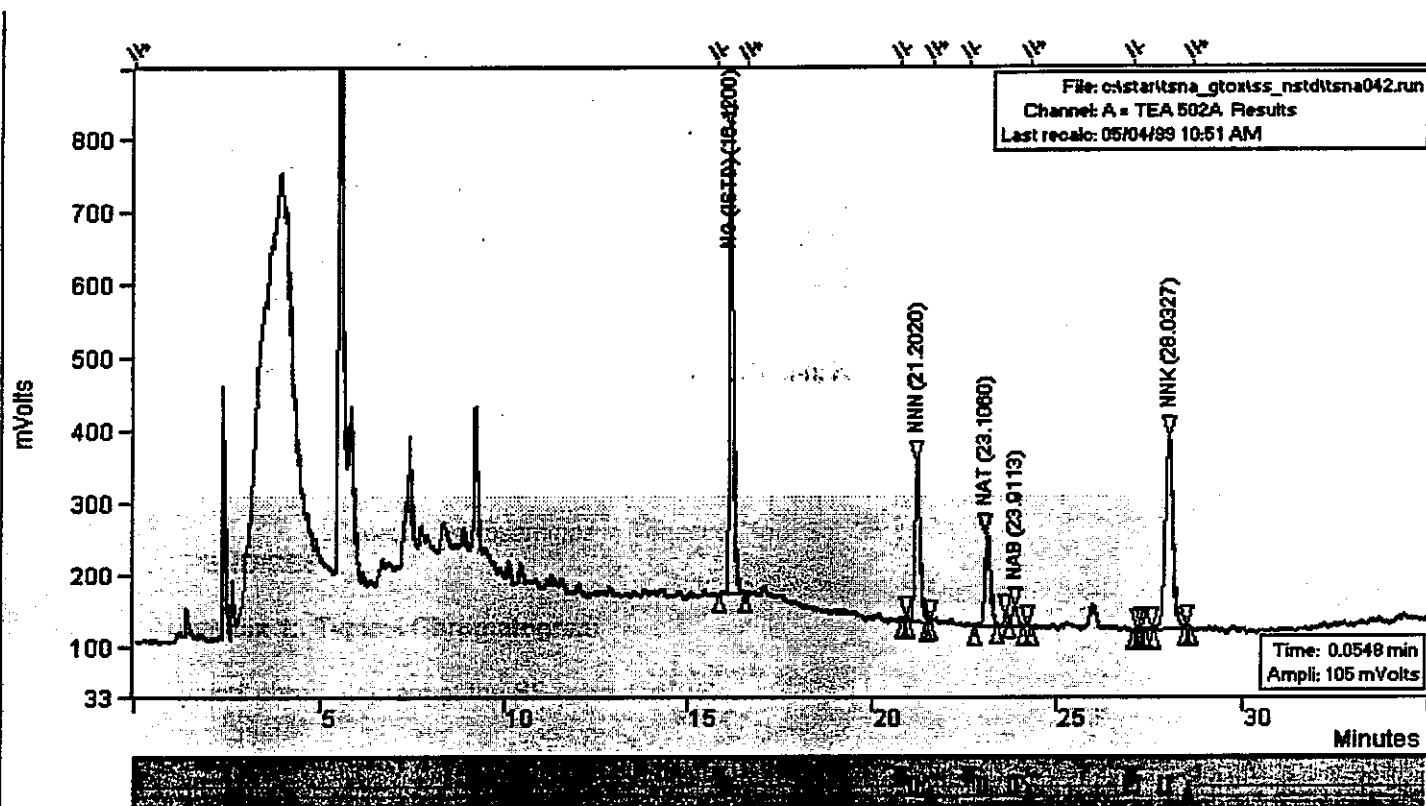
17.4 Hoffmann, D., Adams, J.D., Brunnemann, K. D. & Hecht, S. S., 1979. Assessment of tobacco-specific N-nitrosamines in tobacco products. *Cancer Res.*, p. 39, 2505-2509.

17.5 Risner, Charles H., and Wendelboe, Fred N., 1994. Quantification of Tobacco Specific Nitrosamines in Tobacco, *Tob. Sci.* 38: p.1-6.

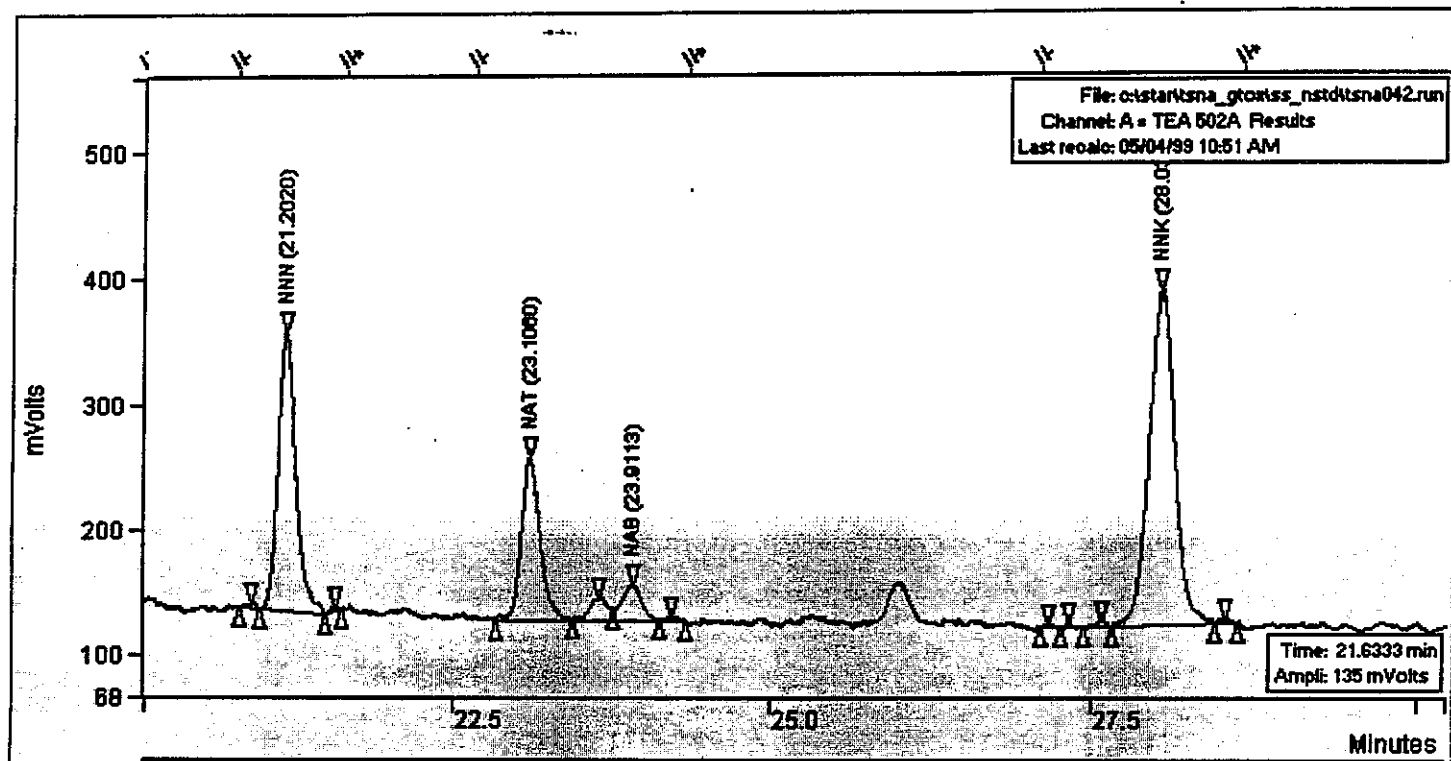
APPENDICES

Appendix 1: Typical Chromatogram

A typical chromatogram of the SS extract for 1R4F.



See the next page for an expanded region of the above chromatogram.



No.: T-210
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1 Applicable to the isolation and quantitation of the pyridine and quinoline content of sidestream tobacco smoke by gas chromatograph/mass spectrometer (GC/MS).

2 NORMATIVE REFERENCES

- 2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1 This method is used for the analysis of sidestream (SS) tobacco smoke using a British American Tobacco (BAT) fishtail chimney configuration. Sidestream smoke is all the smoke emitted from the lit end of a burning tobacco product during the smolder process. The glass fishtail chimney sits over the burning product and allows the smoke to be directed in a controlled manner for the determination of sidestream tobacco constituents.
- 4.2 Pyridine and Quinoline are collected by passing the SS smoke through a glass fibre filter disc (pad) into two impingers containing methanol. The first trap remains at room temperature while the second trap is kept at or below -70°C in a dry ice/isopropanol bath. The pad is then shaken with the internal standard (ISTD) solution (D_5 -pyridine and D_7 -quinoline) and the impinger solution and filtered. The filtrate is analyzed by GC/MS.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform smoking analyses as specified in T-115.
- 5.2 Equipment needed to perform conditioning of tobacco product as specified in T-115.
- 5.3 Equipment needed to perform marking for butt length as specified in T-115.
- 5.4 Analytical balance measuring to at least four decimal places.
- 5.5 70 mL glass impingers with extra-coarse frits.
- 5.6 Tygon tubing with connectors.
- 5.7 Fish-tail Chamber with retort stand and clamps.

-
- 5.8 Vacuum pumps (GAST or equivalent).
 - 5.9 Flow meters.
 - 5.10 125 mL polymethylpentene (PMP) Erlenmeyer flasks with screw-caps.

- 5.11 Graduated cylinder to contain 20 mL.
- 5.12 Wrist-action shaker.
- 5.13 10, 25, 50 and 100 mL volumetric flasks.
- 5.14 Volumetric pipettes or gas-tight syringes for range 100 to 1000 μ L.
- 5.15 Autosampler vials with caps and red Teflon-lined septa.
- 5.16 Varian Saturn I GC/MS system consisting of an 8100 autosampler, a 3400 GC with a 1077 split/splitless injector and an ion trap (ITD) or equivalent.
- 5.17 Supelcowax 30 m X 0.25 mm X 0.25 μ m column (or equivalent) with 1 m X 0.25 mm deactivated fused silica transfer line.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Dry ice.
- 6.2 Isopropanol (IPA).
- 6.3 Methanol (Distilled-in-Glass).
- 6.4 D₅ - Pyridine (MSD Isotopes or equivalent) – purity 98 % or greater.
- 6.5 D₇ - Quinoline (MSD Isotopes or equivalent) – purity 98 % or greater.
- 6.6 Pyridine.
- 6.7 Quinoline.
- 6.8 Disposable 5 cc syringe.
- 6.9 Syringe filters - 0.45 μ m PTFE 25 mm or equivalent.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.
- 7.2 Between samples, the impingers and fish-tail chambers are rinsed with methanol and allowed to air-dry.

8 PREPARATION OF SOLUTIONS

- 8.1 Not applicable.

9 PREPARATION OF STANDARDS

- 9.1 A primary stock solution of pyridine is prepared by accurately weighing approximately 100 mg of pyridine into a 10 mL volumetric flask. The flask is filled to the mark with methanol and mixed well. [Concentration: approximately 10 mg/mL].
- 9.2 A primary stock solution of quinoline is prepared by accurately weighing approximately 100 mg of quinoline into a 100 mL volumetric flask. The flask is filled to the mark with methanol and mixed well. [Concentration: approximately 1 mg/mL].
- 9.3 A mixed secondary stock solution is prepared by transferring 100 μ L of each stock solution into a 50 mL volumetric flask, making to the mark with methanol and mixing well. [Concentrations: approximately 20 and 2 μ g/mL, respectively].

- 9.4 A stock solution of D₅-pyridine is prepared by accurately weighing 100 mg of D₅-pyridine into a 10 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 9.5 A stock solution of D₇-quinoline is prepared by accurately weighing 25 mg of D₇-quinoline into a 25 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 9.6 An internal standard spiking solution is prepared by diluting 2 mL of each of the ISTD stock solutions to 100 mL with methanol and mixing well. Aliquots of this spiking solution are stored in 25 mL vials with Teflon-lined caps and at -20 °C. [Concentrations: approximately 200 and 20 µg/mL, respectively].
- 9.7 Five calibration standard solutions are prepared by adding 100 µL ISTD to each of five 10 mL volumetric flasks. The sides are rinsed with methanol, then appropriate aliquots (e.g. 2, 1, 0.5, 0.25 and 0.1 mL) of the secondary stock solution are added to each flask. The flasks are filled to the mark with methanol and mixed well.
- 9.8 The solutions are transferred to a series of labelled autosampler vials, capped with Teflon-lined septa and stored at -20 °C until use.

Note: Each vial is only used once.

10 SAMPLING

- 10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

- 11.1 Product shall be conditioned as specified in T-115.
- 11.2 Product shall be marked for butt length as specified in T-115.
- 11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION

12.1 Ambient Conditions

- 12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

- 12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

- 12.2.1.1 Smoking is conducted on between four and eight ports of a linear 20 port smoking machine. The sidestream smoke is collected by a fishtail chamber (see illustration below) mounted above the cigarette.

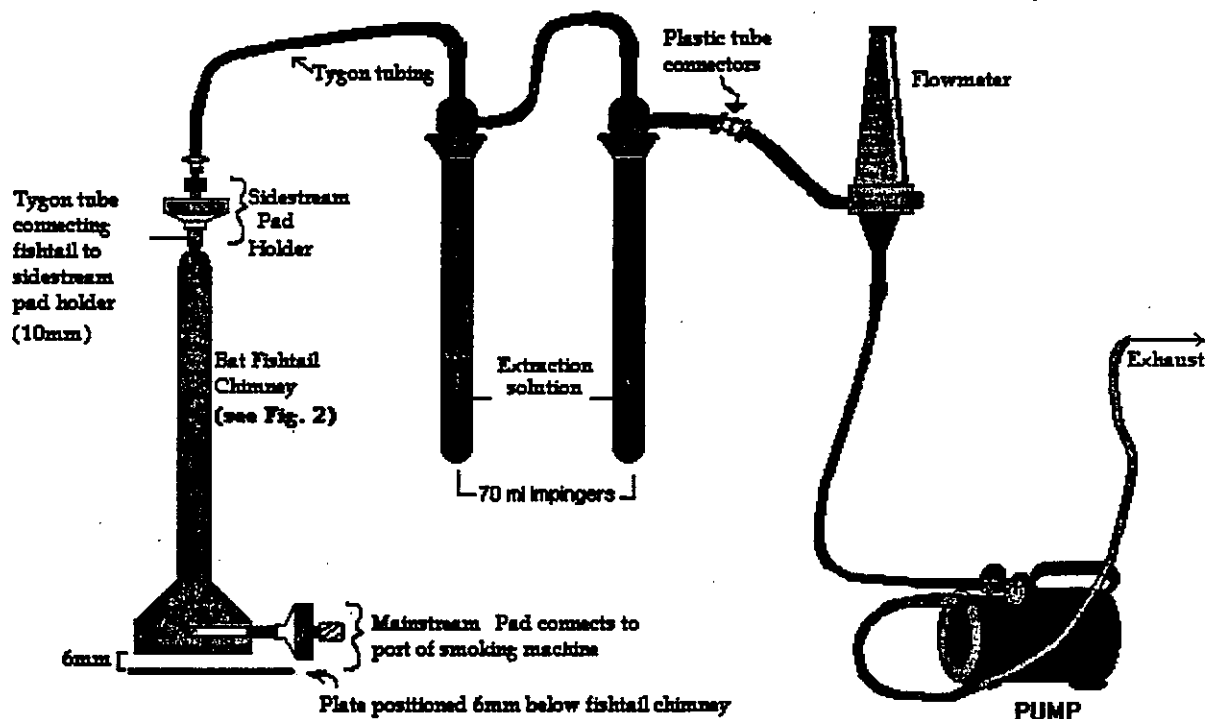


FIGURE 1b: SIDESTREAM APPARATUS USING TWO IMPINGERS

- 12.2.2 Weigh the Mainstream (MS) and SS pad holders and pads.
- 12.2.3 Insert the MS pad holder with pad into the assigned port of the smoking machine.
- 12.2.4 Prepare the impingers by adding 10 mL of methanol into the first impinger and 20 mL into the second.
- 12.2.5 Immerse the second impinger into a dry-ice/IPA bath (temperature at or below -70°C). The first impinger remains at room temperature and traps the water so that the impinger in the bath does not freeze.
- 12.2.6 Position the sidestream pad holder above the fishtail chamber and then hook up in series two impingers to the pad holder. Connect the tubing of the last impinger to the flow meter. Connect the flow meter to the vacuum pump.

13 SAMPLE GENERATION

- 13.1 Turn the pump on (flow rate of 2 L/minute) just prior to lighting the cigarette. Light the cigarette and initiate the puff count.
- 13.2 Lower the chimney to its lowest position. Do not allow the cigarette to touch the chimney. Keep the chimney approximately 6 mm from the plate insert.

- 13.3** Burn the cigarette to the previously marked standard butt length. Remove the butt. After the cigarette has been smoked to the line, leave the pump on for approximately 20 seconds to collect all of the smoke from the fishtail chimney. Raise the chimney to its highest position and turn the pump off.
- 13.4** Smoke the second cigarette in the same manner as the first.
- 13.5** After smoking two cigarettes per port, remove both mainstream and sidestream filter holders and record their final weight on the run sheet to obtain the MS and SS TPM. Total particulate matter (TPM) is determined as described in ISO 4387. Data for mainstream and sidestream TPM is used to characterize samples and to monitor the smoking process.

14 SAMPLE ANALYSIS

14.1 Extraction of Filter Pads

- 14.1.1** Place the SS pad into a clean 125 mL PMP Erlenmeyer flask. Spike the pad with 500 μ L of the ISTD solution.
- 14.1.2** Transfer the contents of both impingers into the flask. Rinse each impinger with 10 mL of methanol and pour the rinsate through the fishtail chamber into the flask (total volume 50 mL).
- 14.1.3** Close the flask with the cap and shake on the wrist-action shaker for 30 minutes.
- 14.1.4** Pour 4 mL of the solution into a 5 mL syringe fitted with a syringe filter.
- 14.1.5** Fill two labeled autosampler vials to the base of the neck and cap with an autosampler cap and Teflon-lined septum.
- 14.1.6** Store samples at -20°C for up to 48 hours prior to analysis.

14.2 Instrument Analysis

14.2.1 GC/MS Conditions

- | | |
|--|--|
| 14.2.1.1 Injector temperature: | 250 $^{\circ}\text{C}$. |
| 14.2.1.2 Column temperature: | 70 $^{\circ}\text{C}$ for two minutes.
3 $^{\circ}\text{C}$ per minute to 150 $^{\circ}\text{C}$
20 $^{\circ}\text{C}$ per minute to 250 $^{\circ}\text{C}$
hold three minutes. |
| 14.2.1.3 Column pressure: | 12 psi. |
| 14.2.1.4 Transfer line temperature: | 240 $^{\circ}\text{C}$. |
| 14.2.1.5 Manifold temperature: | 240 $^{\circ}\text{C}$. |

- 14.2.2** One μ L of the methanol solution is injected at 5 μ L per second onto the GC/MS, which is run in the splitless mode. (Split flow 20 mL/minute).
- 14.2.3** The GC/MS is operated in full-scan mode (50 to 200 amu). The following ion peak areas are used for quantitation:

D ₅ -pyridine	84
D ₇ -quinoline	136
Pyridine	79
Quinoline	129

Note: The assignment of these masses is based on selection of the best response (i.e. the base peak) and the need to avoid possible contamination from interfering peaks which may contain similar ions. The choice of quantitation ions may be different for different instrument configurations.

Note: Quantitation may be based on peak heights if interfering peaks cannot be completely resolved.

14.3 Calculations

14.3.1 Calibration Curve

14.3.1.1 A calibration curve is generated at the beginning of each sample set or "project". Each standard solution is injected once and a calibration file built using the method for internal standard quantitation available with the Saturn quantitation software.

14.3.1.2 A check standard is analyzed every 20 samples and at least once per run. This standard is treated as a sample and the observed value is compared to the expected value for that standard. A difference of more than 10 % of expected requires the following course of action.

14.3.1.3 Make fresh calibration standards and run as check standards.

14.3.1.4 If the results are within 10 % of expected, the first set of standards should be discarded and the new set used. The calibration is still valid.

14.3.1.5 If the results differ by more than 10 % of expected, the calibration is no longer valid and a new calibration curve must be generated.

14.3.2 Sample Calculation

14.3.2.1 The software on the GC/MS is used to generate results for each analyte based on the concentrations of the standard solutions. The results are reported in µg/mL. To calculate the final results, the following calculation is used:

$$\text{Analyte (ug/cigarette)} = \frac{\text{Conc. of Analyte in Sample (ug/mL)} \times \text{Volume (mL)}}{\text{\# of cigarettes}}$$

Note: Typically, the volume is 50 mL and the number of cigarettes is two.

15 QUALITY CONTROL

15.1 Recoveries and Levels of Contamination

15.1.1 This involves the use of laboratory reagent blanks (LRB) to evaluate potential interference of the reagents. One LRB should be analyzed every 20 samples. A CFP is placed in a PMP Erlenmeyer with 500 μL of the ISTD solution and 50 mL of methanol. The LRB is then treated as a sample through the rest of the procedure.

Note: In lieu of an LRB, a smoking blank can be used to monitor contamination of reagents and the air in the smoking room. This involves conducting a smoking run with the same number of puffs as a control cigarette but with no cigarette in place.

15.1.2 This also involves the use of laboratory fortified blanks (LFB) to evaluate the extent of potential analyte loss. One LFB may be analyzed every 20 samples. A CFP is placed in a flask with 500 μL of the ISTD solution, an appropriate aliquot of the secondary mixed stock solution, and 50 mL of methanol. The LFB is then treated as a sample through the rest of the procedure. Recoveries should be very close to 100 % due to the simplicity of the method and the use of deuterated internal standards.

15.1.3 A laboratory fortified matrix (LFM) may be analyzed to assess potential matrix interference. A sample of a control brand is smoked and the pad transferred to the serum bottle. The pad is spiked with the ISTD solution and an aliquot of the mixed secondary stock solution. The impinger solutions are added to the pad and the sample is taken through the remainder of the procedure. The recoveries should be close to 100 %.

15.2 Method Detection Limit (MDL)/Limit of Quantitation (LOQ)

15.2.1 The MDL can be defined as the level which gives a signal to noise ratio of three to one. The LOQ can be defined as the level which gives a signal to noise ratio of 10 to one.

Note: Because this method involves the analysis of the methanol soluble components of whole tobacco smoke, with no sample clean-up, the chromatography must be very carefully monitored so that the peaks are sharp and the analytes of interest are well resolved from other components.

15.3 Stability of Reagents and Samples

15.3.1 Stock solutions are stable for at least one month if kept at $-20\text{ }^{\circ}\text{C}$.

15.3.2 Calibration standards are stable for at least one week if kept at $-20\text{ }^{\circ}\text{C}$. Once punctured, the more volatile pyridine may be lost so vials are typically used once and discarded.

15.3.3 Samples are stable at $-20\text{ }^{\circ}\text{C}$ for at least one week if the septum has not been punctured. It is essential that at least two vials be prepared for each sample as the vial is discarded once punctured.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

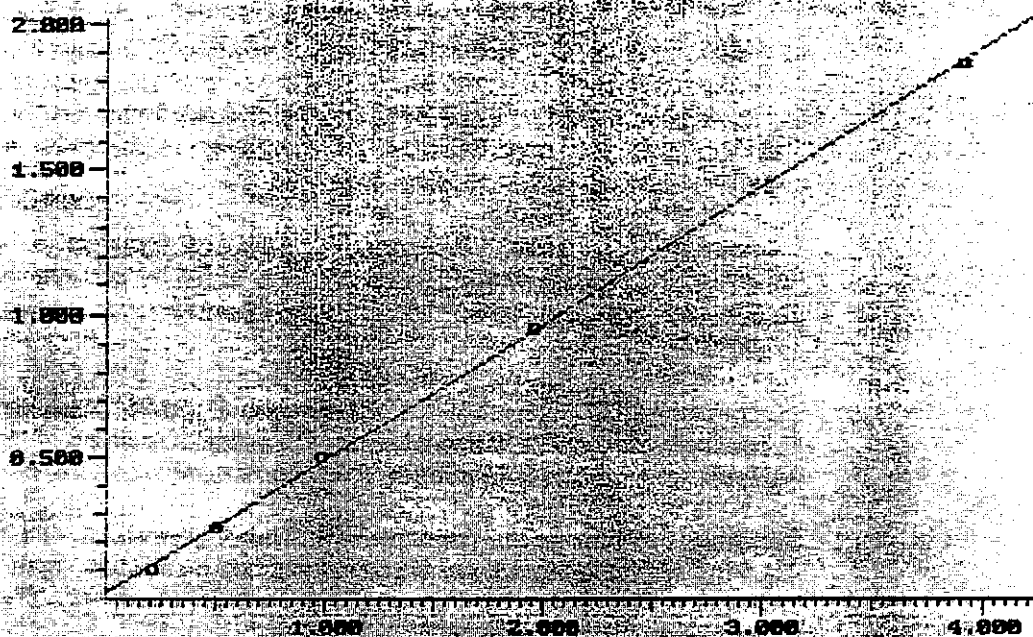
- 16.1** No changes for smoking under intense smoking conditions.

17 REFERENCES

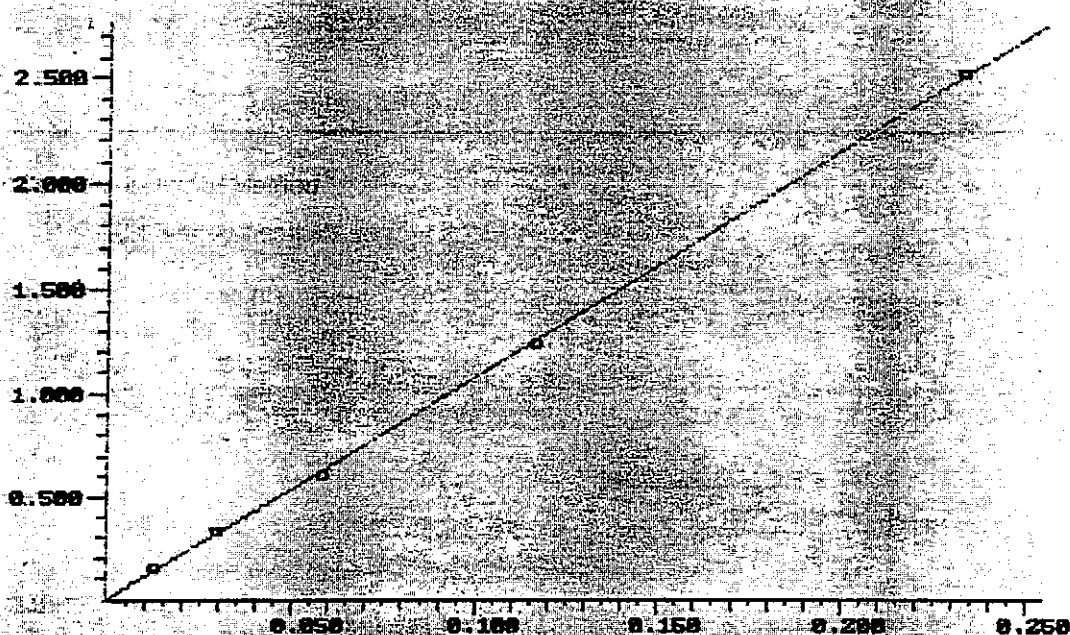
- 17.1** White, E., Uhrig, M., Johnson, T., Gordon, B., Hicks, R., Borgerding, M., Coleman, W., and Elder, J., 1990. Quantitative Determination of Selected Compounds in a Kentucky 1R4F Reference Cigarette Smoke by Multidimensional Gas Chromatography and Selected Ion Monitoring - Mass Spectrometry. *Journal of Chromatographic Science* 26, p. 393-399.
- 17.2** Sakuma, H., Kusama, M., Yamaguchi, K., Matsuki, T., and Sugawara, S., 1984. The Distribution of Cigarette Smoke Components between Mainstream and Sidestream Smoke. II. Bases, *Beiträge zur Takakforschung International* 12, p. 199-209.
- 17.3** Brunnemann, K.D., Stahnke, G., and Hoffmann, D., 1978. Chemical Studies on Tobacco Smoke. LXI. Volatile Pyridines: Quantitative Analysis in Mainstream and Sidestream Smoke of Cigarettes and Cigars, *Analytical Letters* A11, p. 545-560.
- 17.4** Sakuma, H., Kissama, M., Yamaguchi, K., and Sugawara, S., 1984. The Distribution of Cigarette Smoke Components between Mainstream and Sidestream Smoke. III. Middle and Higher Boiling Components, *Beiträge zur Takakforschung International* 12, p. 251-258.
- 17.5** Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

APPENDIX

Calibration Plot (Int Std) Filename: PYR8317 Correlation Coeff: 1.000
Pyridine Compound: 3 of 5 Standard Deviation: 0.013
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin(Lin))



Calibration Plot (Int Std) Filename: PYR8317 Correlation Coeff: 1.000
Quinoline Compound: 5 of 5 Standard Deviation: 0.015
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin(Lin))



No: T - 211
Date: December 31, 1999
Page: 1 of 21

1 SCOPE OF APPLICATIONS

- 1.1** This method describes the extraction and determination of phenolic compounds in the sidestream (SS) tobacco smoke by reversed phase high performance liquid chromatography (HPLC).
- 1.2** Applicable to the trapping and quantitation of phenolic compounds in sidestream tobacco smoke on the sidestream glass fibre filter disc (pad) and impinger only.

2 NORMATIVE REFERENCES

- 2.1** American Society for Testing and Materials (ASTM) D 1193-77 – Standard Specification for Reagent Water, Version 1977.
- 2.2** Health Canada Test Method: T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** 10 equidistant ports of a standard 20 port linear smoking machine are reconfigured with the BAT (British American Tobacco) fishtail chambers and flow-controlled vacuum pumps.
- 4.2** Cigarettes or other tobacco products are smoked beneath the fishtail chambers and the smoke is swept up the chimney at the rate of 3 L/minute.
- 4.3** The total particulate matter (TPM) from the sidestream smoke is collected on a pad at the top of the chimney. The filtered puff is then bubbled through an impinger containing 100 mL of 1 % acetic acid.
- 4.4** After smoking two cigarettes*, the sidestream pad is placed in a glass-stoppered Erlenmeyer flask that contains the impinger solution and 2 X 20 mL rinses of the BAT fishtail chamber and is extracted by wrist-action shaking.
- *For other tobacco products, select a number such that breakthrough does not occur.
- 4.5** An aliquot of the TPM extract is then syringe filtered and subjected to reversed-phase gradient high performance liquid chromatography (HPLC).
- 4.6** Phenols are monitored using selective fluorescence detection and quantified by comparison to an external standard calibration.

- 4.7 The sample generation and analysis should be completed in one day.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1 Equipment needed to perform conditioning as specified in T-115.
- 5.2 Equipment needed to perform marking for butt length as specified in T-115.
- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Glass fibre filter holders.
- 5.5 Glass fibre filter discs (pads), 44 mm in diameter, with no more than 5 % acrylic type binder.
- 5.6 Analytical Balance capable of measuring to at least four decimal places.
- 5.7 Wrist Action Shaker.
- 5.8 Vacuum Pumps.
- 5.9 Flow meters.
- 5.10 Fishtail Chambers - BAT (10).
- 5.11 Glass Impingers with frits and cooling jackets - 10 X 10", capacity 250 mL.
- 5.12 Cooling Bath.
- 5.13 250 mL Erlenmeyer flasks with ground glass stoppers.
- 5.14 Volumetric flasks 10 mL, 25 mL and 50 mL, Actinic Red.
- 5.15 Glass Micropipettes - assorted volumes (100, 150, 300, 400, 500, 800, 1000, and 2000 μ L).
- 5.16 Glass Transfer Pipettes - 1, 2, 5, 6, 7, 8, and 20 mL.
- 5.17 Glass Graduated Measuring Cylinders 25 mL and 50 mL.
- 5.18 Erlenmeyer flasks with ground glass joints 50 mL, Actinic Red.
- 5.19 High Pressure Liquid Chromatography System consisting of:
 - 5.19.1 Solvent Delivery System - tertiary gradient pump.
 - 5.19.2 Refrigerated Autosampler with 20 μ L sampling loop.
 - 5.19.3 Programmable Wavelength Spectrofluorometer.
 - 5.19.4 Column Temperature Modifier.
 - 5.19.5 Cooling Bath.
 - 5.19.6 Data collection system.
 - 5.19.7 RP18e 250 mm X 4 mm and 5 μ m Column with 10 mm X 4 mm guard column.

6 REAGENT AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

- 6.1 Syringe Filters 0.45 μ m PVDF.
- 6.2 Disposable syringes.
- 6.3 Disposable Glass Pasteur Pipettes.
- 6.4 Rubber Bulbs.
- 6.5 Autosampler vials, screw caps and septa.
- 6.6 Masking Tape.

- 6.7 Aluminum Foil.
- 6.8 Methanol - Distilled in Glass (DIG).
- 6.9 Acetonitrile - DIG.
- 6.10 Isopropanol (IPA) - DIG.
- 6.11 Ethanol - DIG.
- 6.12 Acetic Acid - HPLC grade.
- 6.13 Octanol > 99 % purity.
- 6.14 Type I water (meets ASTM D 1193 specification).
- 6.15 Hydroquinone > 99 % purity.
- 6.16 Resorcinol > 99 % purity.
- 6.17 Catechol > 99 % purity.
- 6.18 Phenol > 99 % purity.
- 6.19 m-Cresol > 99 % purity.
- 6.20 p-Cresol > 99 % purity.
- 6.21 o-Cresol > 99 % purity.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS

- 8.1 Prepare 4 L fresh 1 % acetic acid solution in Type I water (40 mL diluted up to 4 L) and test by HPLC for contamination.

9 PREPARATION OF STANDARDS

9.1 Primary (1°) Phenol Standards (See Appendix 1)

- 9.1.1 Weigh 25 mg of the following phenols (Hydroquinone, Resorcinol, Catechol, Phenol, m-Cresol, p-Cresol and o-Cresol) into individual 25 mL volumetric flasks and make up to the mark with fresh 1 % acetic acid solution.
- 9.1.2 Concentrations will be in the range of 1.0 mg/mL. Prepare fresh primary phenol stock standards every 10 working days.

9.2 Secondary (2°) Phenol Standard Solutions (See Appendix 1)

- 9.2.1 Take appropriate volumes of the 1° Phenol Standards and dilute to 10 mL with 1 % acetic acid.
- 9.2.2 Prepare 2° phenol stock standards fresh with each new primary stock standards.

9.3 Tertiary (3°) Phenol Solution (See Appendix 1)

- 9.3.1 Take corresponding volumes of each phenol solution and add to a single 50 mL volumetric flask. Dilute up to the mark with 1 % acetic acid.
- 9.3.2 Prepare phenol working stock solution fresh every five working days.

9.4 Phenol Working Standards

9.4.1 Take appropriate volumes (0.100 to 7.5 mL) of the Tertiary (3°) Stock Phenol solution and dilute to 10 mL with 1 % acetic acid to give calibration standards with appropriate phenol concentrations.

9.4.2 Transfer to autosampler vials.

9.4.3 Phenol calibration standards are prepared fresh every five working days.

9.5 Phenol Spiking Solution for laboratory fortified blanks (LFB)

9.5.1 Add selected volumes of the phenol stock standards together in a 50 mL volumetric flask and make up to the mark with 1 % acetic acid.

9.5.2 Prepare phenol spiking solution fresh every five working days.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 with the following modifications as detailed below:

12.2.2 Assemble the Phenol sidestream apparatus as per the diagram.

12.2.3 Raise chimney level to highest position (loading position).

12.2.4 The sidestream impinger is loaded with 100 mL of 1 % acetic acid plus two drops of octanol and the impinger jackets are connected to a cooling bath at 10 °C.

- 12.2.5 Install the weighed sidestream filter pad assembly at the top of the fishtail chamber and place impingers with tops connected to coarse frits onto the rear section of smoke machine.
- 12.2.6 Tubing from impinger front (internal stem connection) attaches to SS filter pad holder and from impinger rear (bulb) to vacuum pump.
- 12.2.7 Calibrate the vacuum pumps to draw at the rate of 3 L/minute. Record the flow meter settings.
- 12.2.8 Attach the mainstream filter pad holders to the corresponding port on the smoking machine.

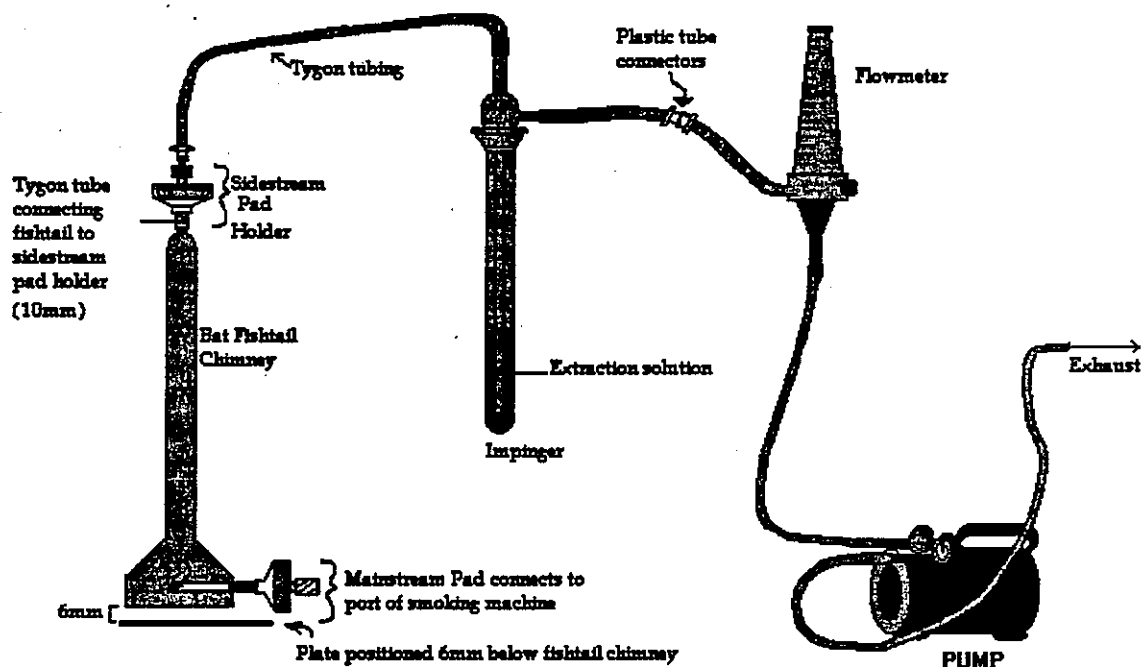


FIGURE 1a: SIDESTREAM APPARATUS

13 SAMPLE GENERATION

Note: It is important to ensure that at least 50 to 100 mg of TPM has been deposited on the sidestream filter pad before proceeding with the analysis.

- 13.1 Using the vacuum bar, install the first test cigarette to be smoked in position below the fishtail in the 10 calibrated ports. Gently insert the cigarette into the cigarette holder to the marked butt length.
- 13.2 Turn on the sidestream pumps (3 L/minute) at the beginning of the lighting procedure at t minus 30 seconds.
- 13.3 Light the cigarette (on the first puff) and initiate the puff count according to the following schedule:

- 13.3.1** Normal lighting procedure is 15 second warm-up beginning at t-18 seconds followed by five seconds ignition. (Three seconds prior to puff plus the two seconds puff).
- 13.4** Lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. Do not allow the cigarette to touch the chimney. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.
- 13.5** The test cigarettes are smoked to the previously marked standard butt length. Extinguish and remove from beneath the BAT fishtail chamber.
- 13.6** The pump continues for an additional 30 seconds to sweep any residual smoke up to the sidestream pad.
- 13.6.1** The smoking process is repeated for the second cigarette.
- 13.6.2** Smoking is terminated when the final test cigarette has been consumed to the predetermined end mark.
- 13.6.3** At the end of the smoking process raise the chimney and disassemble the sidestream apparatus.
- 13.6.4** Re-weigh and record the "after smoking" weights of the sidestream filter holders.

14 SAMPLE ANALYSIS

14.1 Extraction of filter pads

- 14.1.1** One run consists of 10 samples (pads). Process 10 samples at a time but not more than two runs or 20 samples per day. Do not smoke more than can be analyzed in a 24 hours period. Hydroquinone is especially temperature and time sensitive.
- 14.1.2** Remove the sidestream pad, fold in half and in half again with the "clean" side facing out. Grasp with a pair of clean tweezers, and wipe the holder. Place the pad into a 250 mL Erlenmeyer flask.
- 14.1.3** Add the 100 mL of impinger solution to the Erlenmeyer flask.
- 14.1.4** Rinse the fishtail chimney with 2 X 20 mL of fresh 1 % Acetic Acid. Use a glass rod to free up any debris on the chimney. Add the chimney washings to the Erlenmeyer flask for a total volume of 140 mL.
- 14.1.5** Place a piece of 1" masking tape over the ground glass stopper to hold it in place.
- 14.1.6** Prepare an Laboratory Reagent Blank (LRB) with each day's smoking as follows to demonstrate that interference from the analytical system, glassware, and reagents are not present.

- 14.1.6.1** LRB: Add one blank filter pad from the smoking room to a clean 250 mL Erlenmeyer flask, add 140 mL of 1 % Acetic Acid solution and stopper.
- 14.1.7** Prepare an Laboratory Fortified Blank (LFB) with each day's smoking as follows to determine whether there is any loss of analyte as a result of the analytical process.
- 14.1.7.1** LFB: Add one blank filter pad from the smoking room to a clean 250 mL Erlenmeyer flask, add 139 mL of 1 % acetic acid plus 1 mL of phenol spiking solution and stopper.
- 14.1.8** Wrap flasks with tin foil completely. Clamp flasks onto armature of wrist action shaker and agitate 30 minutes. The pad should be disintegrated once agitation is complete.
- 14.1.9** After shaking, syringe-filter the smoke extract directly into autosampler vials in duplicate. Rinse the vial first, discard the rinse; and then fill to minimize headspace.
- 14.1.10** After shaking, prepare an Laboratory Fortified Matrix (LFM) using a standard control brand with each day of smoked samples:
- 14.1.10.1** Attach a 0.45 µm syringe filter to a disposable syringe and filter the smoke extract directly into 10 mL volumetric flasks that have been preloaded with 1 % acetic acid and the phenol spike as necessary to dilute the smoke extract to 10 mL. Mix the volumetric flask well and then using a Pasteur pipette, fill autosampler vials in duplicate. Rinse vial first and then fill to minimize head space.
- 14.1.11** The LRB and LFB are syringe-filtered directly into autosampler vials.
- 14.1.12** Place the vials in a vial file and store at 4 °C, protected from light, until instrument analysis takes place.
- 14.1.13** A run log is then generated to record the total time samples are at room temperature from smoking to the end of analysis.

Note: It is very critical that analysis be completed in minimal time without interruption as the samples will decompose with prolonged exposure at room temperature.

14.2 Instrument Analysis: HPLC Equipment

- 14.2.1** High Pressure Liquid Chromatography System consisting of:
- 14.2.2** Solvent Delivery System - ternary gradient pump.
- 14.2.3** Refrigerated Autosampler with 20 µL sampling loop.
- 14.2.4** Programmable Wavelength Spectrofluorometer at Gain 100, ATTN 8.
- 14.2.5** Slit Width: Ex. 18 nm, Em 18 nm.
- 14.2.6** Wavelength Profile:

Time	Excitation (nm)	Emission (nm)
------	-----------------	---------------

Initial

0.0	304	338
5.5	274	298
32.0	274	298
33.5	304	338

14.2.7 Cooling Bath with column temperature modifier attachment.

14.3 Chromatographic Conditions (Reversed Phase Analysis)

14.3.1 Column Temperature: 20 °C.

14.3.2 Mobile Phase: Reagents.

14.3.2.1 Solvent A: Prepare 2 L of 1 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

14.3.2.2 Solvent B: Prepare 2 L of 28 % Acetonitrile, 1 % Acetic Acid, 1 % IPA filter and degas. (UHP Helium sparged).

14.3.2.3 Solvent C: Acetonitrile (UHP Helium sparged).

14.3.3 Sample Wash: Solvent A.

14.3.4 Mobile Phase: Gradient.

Flowrate: 1.5 mL/minute.

Time (minutes)	Composition		
0.0	100 % A	0 % B	0 % C
5.0	100 % A	0 % B	0 % C
15.0	75 % A	25 % B	0 % C
20.0	25 % A	75 % B	0 % C
28.0	0 % A	100 % B	0 % C
30.0	0 % A	0 % B	100 % C
32.0	0 % A	0 % B	100 % C
34.0	95 % A	0 % B	5 % C

Method End Action 100 % A 0 % B 0 % C
(Equilibrate 10 minutes).

14.3.5 Sample vials are loaded onto the autosampler such that every 10th vial is a standard solution and in such quantities that the total analysis time does not exceed 24 hours.

14.3.6 Twenty µL of each sample vial is injected onto the HPLC. Elution pattern should be similar to Figure 1.

14.4 Calculations

14.4.1 Construct a Calibration Curve

14.4.2 Twenty μL of each calibration standard is injected onto the HPLC column and analyzed as per the chromatographic conditions. Do in duplicate. Elution pattern should be similar to **Figure 2**.

14.4.3 Determination of Response Factor

14.4.3.1 A calibration curve of the various hydroxybenzene compounds is prepared by plotting the concentration of the standards versus their respective peak areas.

14.4.3.2 Determine the Response Factor from the calibration curve.

14.5 Sample Quantification

14.5.1 The amount of the various phenolic compounds in smoke samples is quantified by the external standard method.

14.5.2 The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.

14.6 Determination of Phenol Deliveries in µg/cigarette

$$14.6.1 \text{ Hydroxybenzene } [\mu\text{g/cigarette}] = \frac{\text{Peak Area}}{\text{Resp Factor}} \times \frac{\text{DF.}}{\text{No. of Cigarettes}}$$

where DF is the dilution factor.

15 QUALITY CONTROL**15.1 Typical Chromatogram**

15.1.1 See Figure 1, 2.

15.2 Recoveries and Levels of Contamination

15.2.1 Each analytical run of test cigarettes should also include:

A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to glassware, trapping reagents, filter pads, and analyzer effects.

A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss.

15.2.2 A standard run as a sample to verify the calculation process and validate the calibration.

15.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)**15.3.1 Method Detection Limit (MDL)**

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary stock Phenol standards are prepared fresh weekly.

15.4.2 All work standards, and reagents are prepared fresh weekly.

15.4.3 All samples are analyzed as soon as they are smoked and within 24 hours.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 The number of cigarettes does not have to be altered for intense smoking.

17 REFERENCES

17.1 Risner, C.H. and Cash, S.L. "A High Performance Liquid Chromatographic Determination of Major Phenolic Compounds in Tobacco Smoke", *Journal of Chromatographic Science*, p. 28, 1990.

17.2 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

APPENDICES

Appendix 1: Calibration Standards

(a): Phenol Standards										
Phenol	Primary Standard *				Secondary Standard *			Tertiary Solution **		
	Weight (g)	Purity (%)	Volume (mL)	Stock [mg/mL]	Vol (mL) Prim Stock	Dilute to Vol (mL)	Stock [mg/mL]	Vol (mL) Stock	Dilute to Vol (mL)	Stock [ug/mL]
Hydroquinone	0.0243	99.0	25.0	0.96228				0.50	50.0	9.62280
Resorcinol	0.0294	99.0	25.0	1.16424	2.0	10.0	0.23285	0.20	50.0	0.93139
Catechol	0.0223	99.0	25.0	0.88308				0.25	50.0	4.41540
Phenol	0.0272	99.0	25.0	1.07712				0.50	50.0	10.77120
m-Cresol	0.0335	99.0	25.0	1.3266	1.0	10.0	0.13266	1.00	50.0	2.65320
p-Cresol	0.0351	99.0	25.0	1.38996	0.5	10.0	0.06950	1.00	50.0	1.38996
o-cresol	0.0303	99.0	25.0	1.19988	0.4	10.0	0.04800	2.00	50.0	1.91981
m+p-Cresol		99.0	25.0							4.04316
* In 1% (v/v) Acetic Acid										
** In 1% (v/v) Acetic Acid in a single 50mL volumetric flask										
(b): Phenol Working Standards +										
Label	5	10	100	200	350	500	750	1000		
ol (mL) Tertiary	0.050	0.100	1.000	2.000	3.500	5.000	7.500	10.000		
Phenol	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]	[ug/mL]		
Hydroquinone	0.04811	0.09623	0.96228	1.92456	3.36798	4.81140	7.21710	9.62280		
Resorcinol	0.00466	0.00931	0.09314	0.18628	0.32599	0.46570	0.69854	0.93139		
Catechol	0.02208	0.04415	0.44154	0.88308	1.54539	2.20770	3.31155	4.41540		
Phenol	0.05386	0.10771	1.07712	2.15424	3.76992	5.38560	8.07840	10.77120		
m-Cresol	0.01327	0.02653	0.26532	0.53064	0.92862	1.32660	1.98990	2.65320		
p-Cresol	0.00695	0.01390	0.13900	0.27799	0.48649	0.69498	1.04247	1.38996		
o-cresol	0.00960	0.01920	0.19198	0.38396	0.67193	0.95990	1.43986	1.91981		
m+p-Cresol	0.02022	0.04043	0.40432	0.80863	1.41511	2.02158	3.03237	4.04316		
+ In 1% (v/v) Acetic Acid in single 10mL volumetric flasks										
(c): Spiking Solution										
Phenol	LFB Spiking Solution ***						LFM Spike ++			
	Stock Level	Stock [mg/mL]	Volume (mL)	Dilute to Vol (mL)	Spike [ug/mL]	Analyzed [ug/mL]	Volume Spike (mL)	Dilute to Vol (mL)	Spike [ug/mL]	Analyzed [ug/mL]
Hydroquinone	Primary	0.96228	1.0		38.4912	0.76982			19.24560	1.92456
Phenol	Primary	1.07712	0.6	25.0	25.85088	0.51702	5.0	10.0	12.92544	1.29254
o-cresol	Secondary	0.04800	1.4		2.68773	0.05375			1.34387	0.13439
*** In 1% (v/v) Acetic Acid in a single 25mL volumetric flask										
++ In 1% (v/v) Acetic Acid in a single 10mL volumetric flask										

Figure 1: Chromatogram of a Typical Phenol Calibration Standard

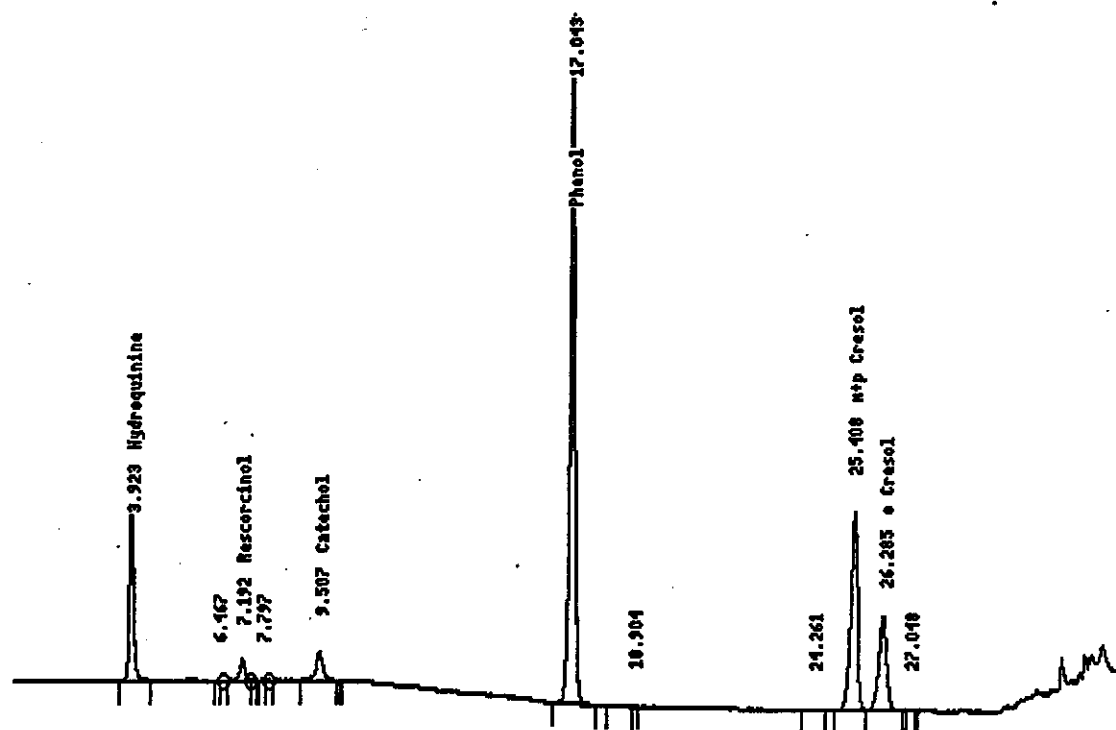


Figure 2: Chromatogram of The Analysis of Sidestream TPM for Hydroxybenzenes.

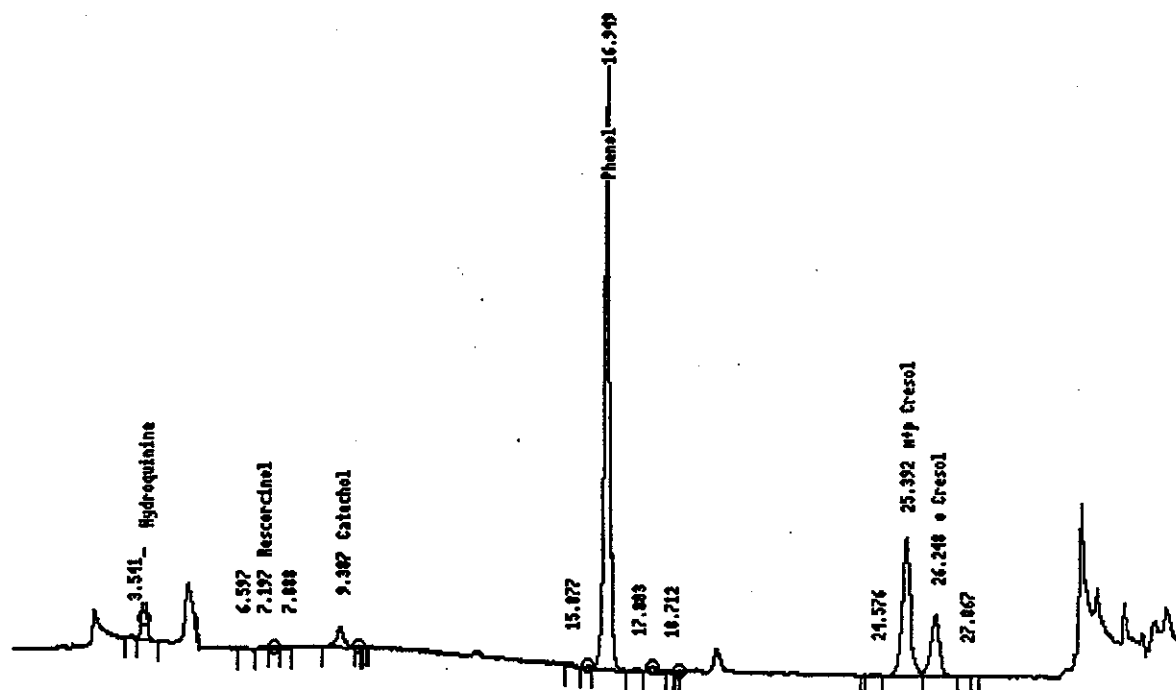
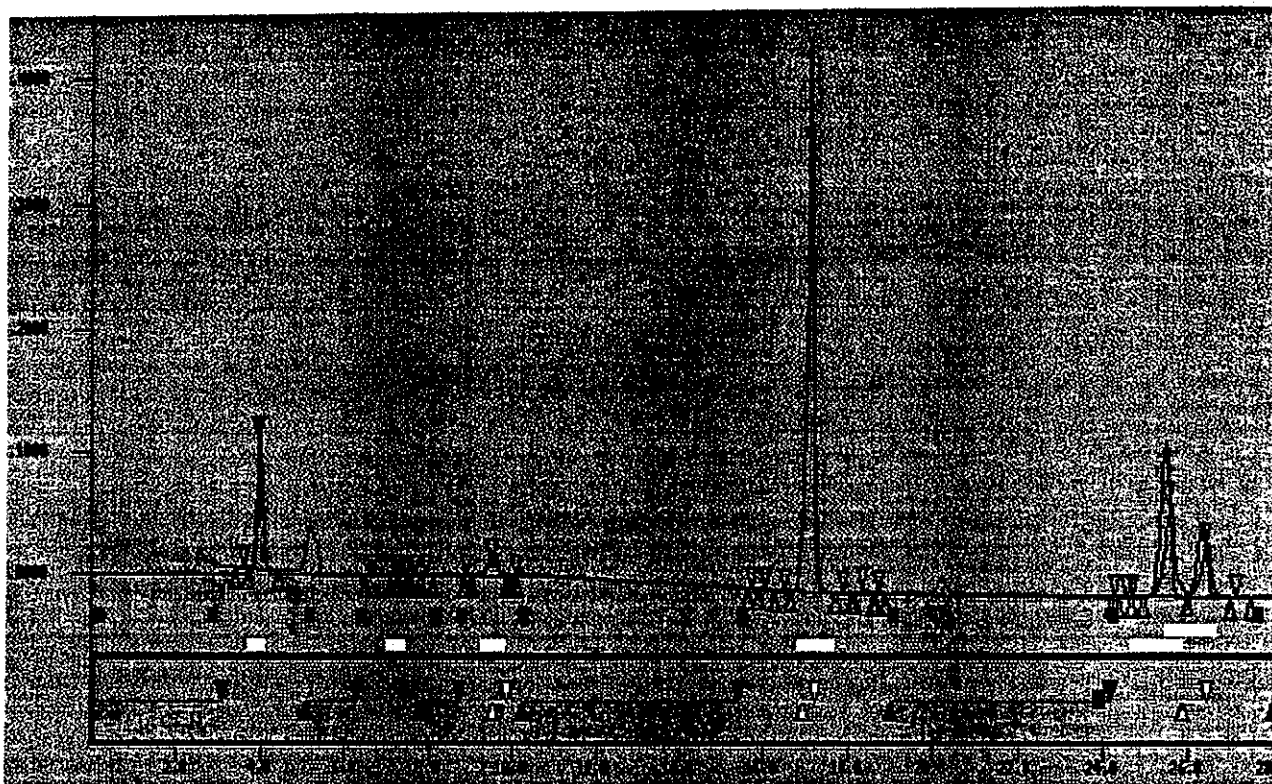


Figure 3: Overlay of Control Cigarette Phenols and Calibration Standard

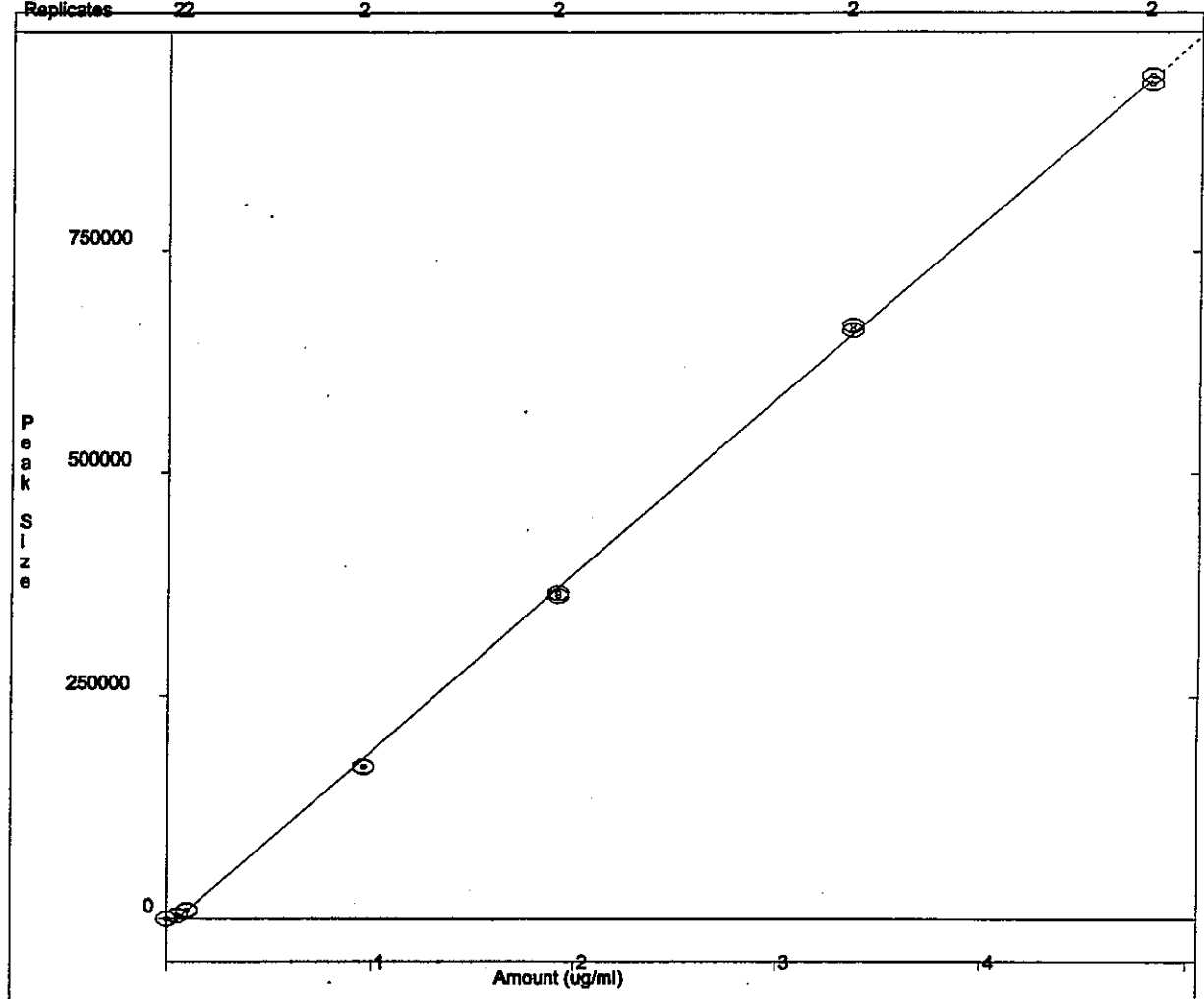


Appendix 4a: Hydroquinone Calibration Curve

Calibration Curve Report
File: f:\home_dir\zavitsk\m24ssphe\phen475.mth
Detector: ADC Board, Address: 16, Channel ID: A

External Standard Analysis
Curve Type: Linear
Origin: Include
 $y = +1.980434e+005x - 9.836358e+003$
Replicates: 22 2 2 2 2

Hydroquinone
Resp. Fact. RSD: 31.64%
Corr. Coef. (R^2): 0.999628



Appendix 4b: Resorcinol Calibration Curve

Calibration Curve Report

File: f:\home_dir\zavitsk\m24ssphe\phen475.mth

Detector: ADC Board, Address: 16, Channel ID: A

External Standard Analysis

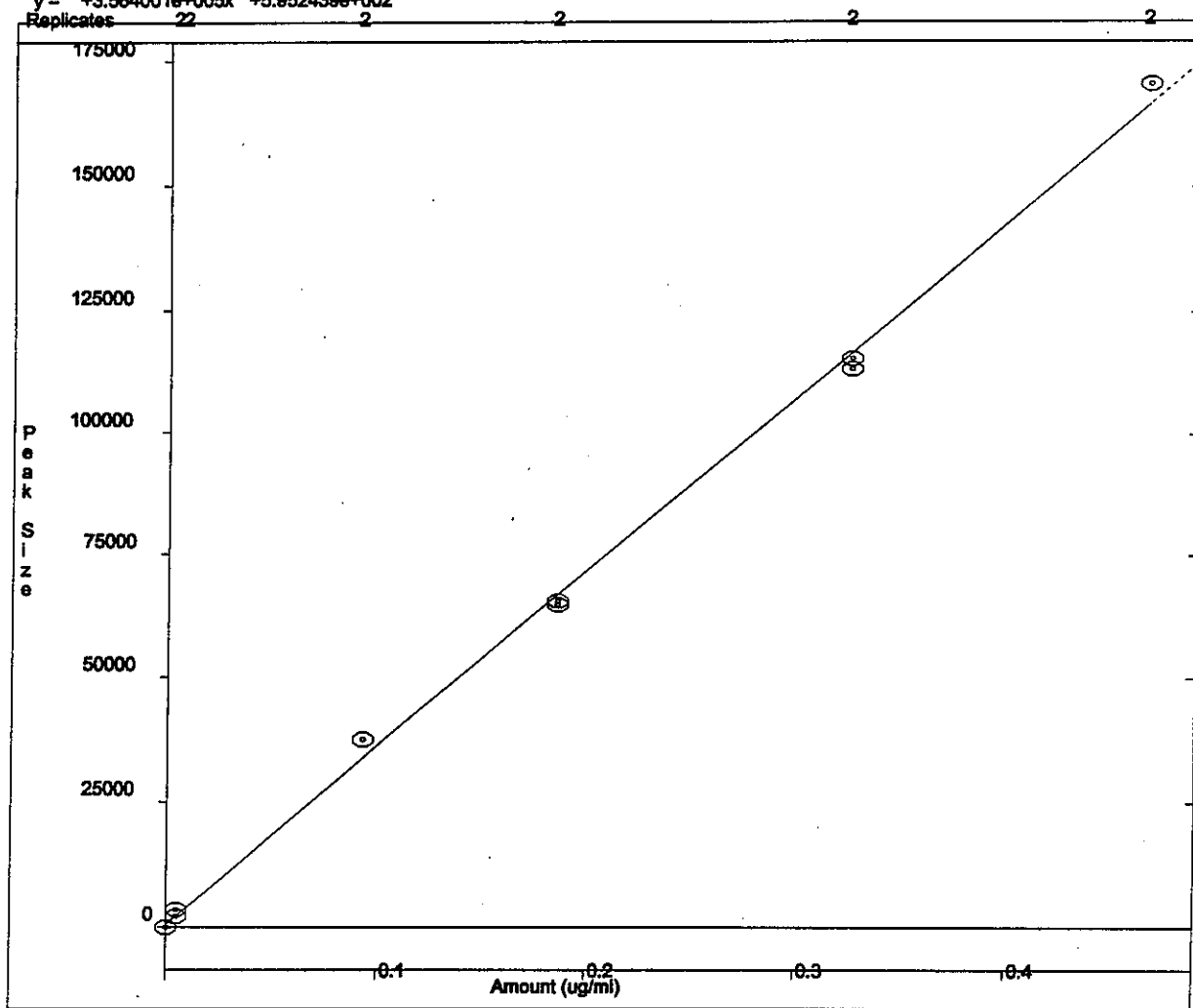
Curve Type: Linear

Origin: Include

 $y = +3.564001e+005x + 5.952439e+002$

Resorcinol

Resp. Fact. RSD: 33.80%

Corr. Coef.(R²): 0.998308

Appendix 4c: Catechol Calibration Curve

Calibration Curve Report

File: f:\home_dir\zavitsk\m24ssphe\phen475.mth

Detector: ADC Board, Address: 16, Channel ID: A

External Standard Analysis

Curve Type: Linear

Origin: Include

 $y = +1.091808e+005x + 2.252483e+003$

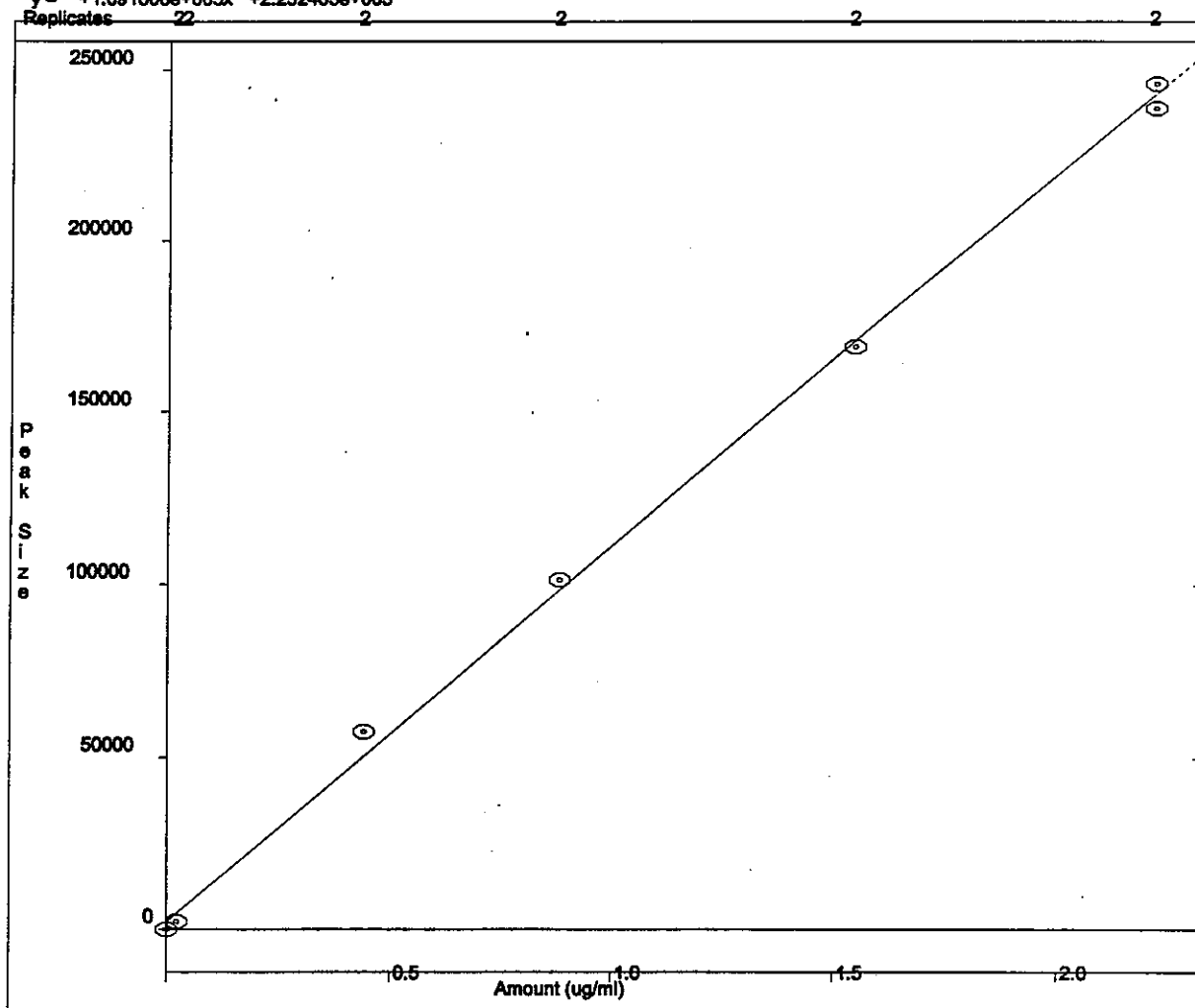
Replicates

22

2

Catechol

Resp. Fact. RSD: 8.328%

Corr. Coef.(R²): 0.998611

Appendix 4d: Phenol Calibration Curve

Calibration Curve Report

File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth

Detector: ADC Board, Address: 16, Channel ID: A

External Standard Analysis

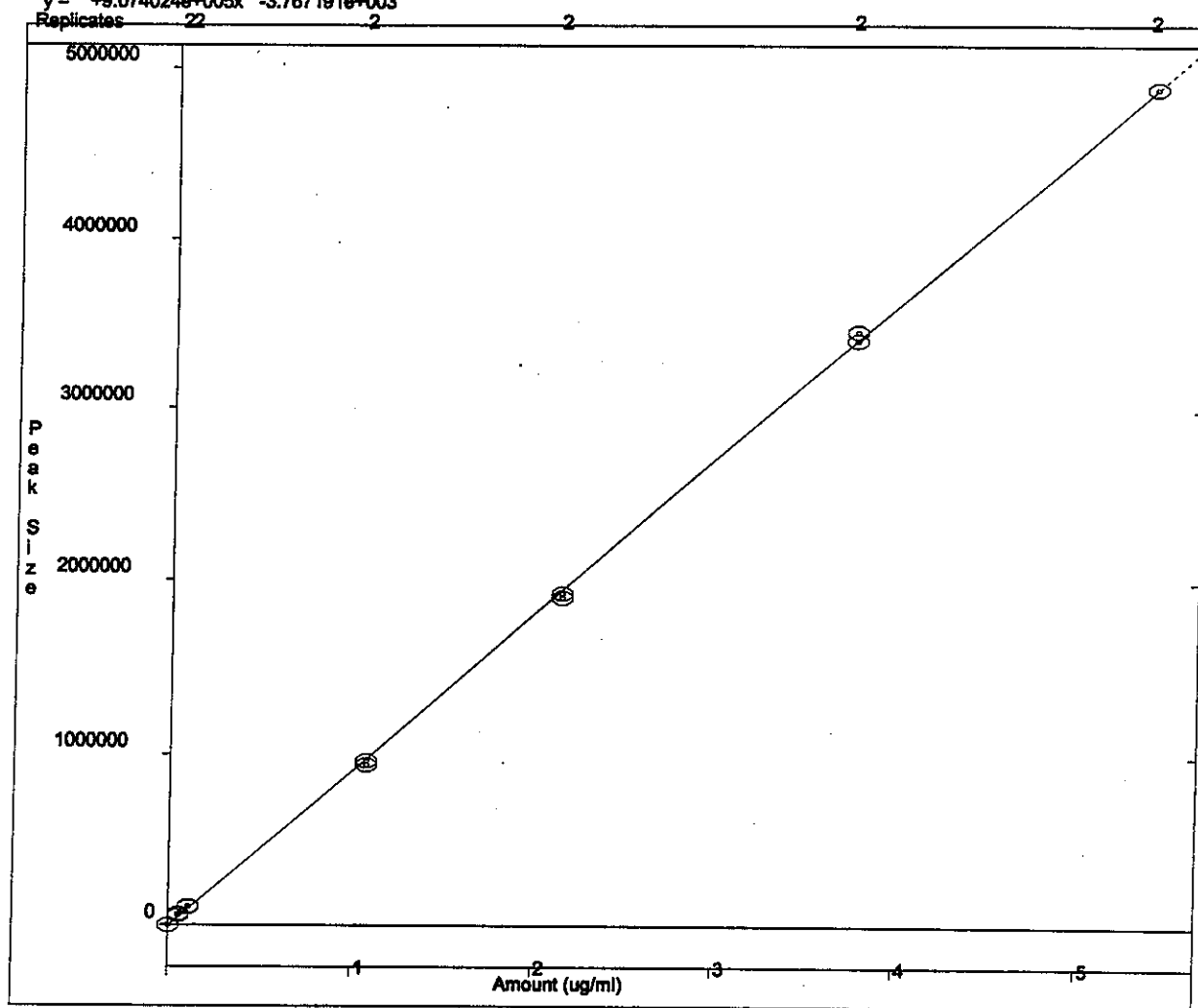
Curve Type: Linear

Origin: Include

$$y = +9.074024e+005x - 3.767191e+003$$

Phenol

Resp. Fact. RSD: 10.50%

Corr. Coef.(R²): 0.999767

Appendix 4e: m+p Cresol Calibration Curve

Calibration Curve Report

File: f:\home_dir\jzavitsk\m24ssphe\phen475.mth

Detector: ADC Board, Address: 16, Channel ID: A

External Standard Analysis

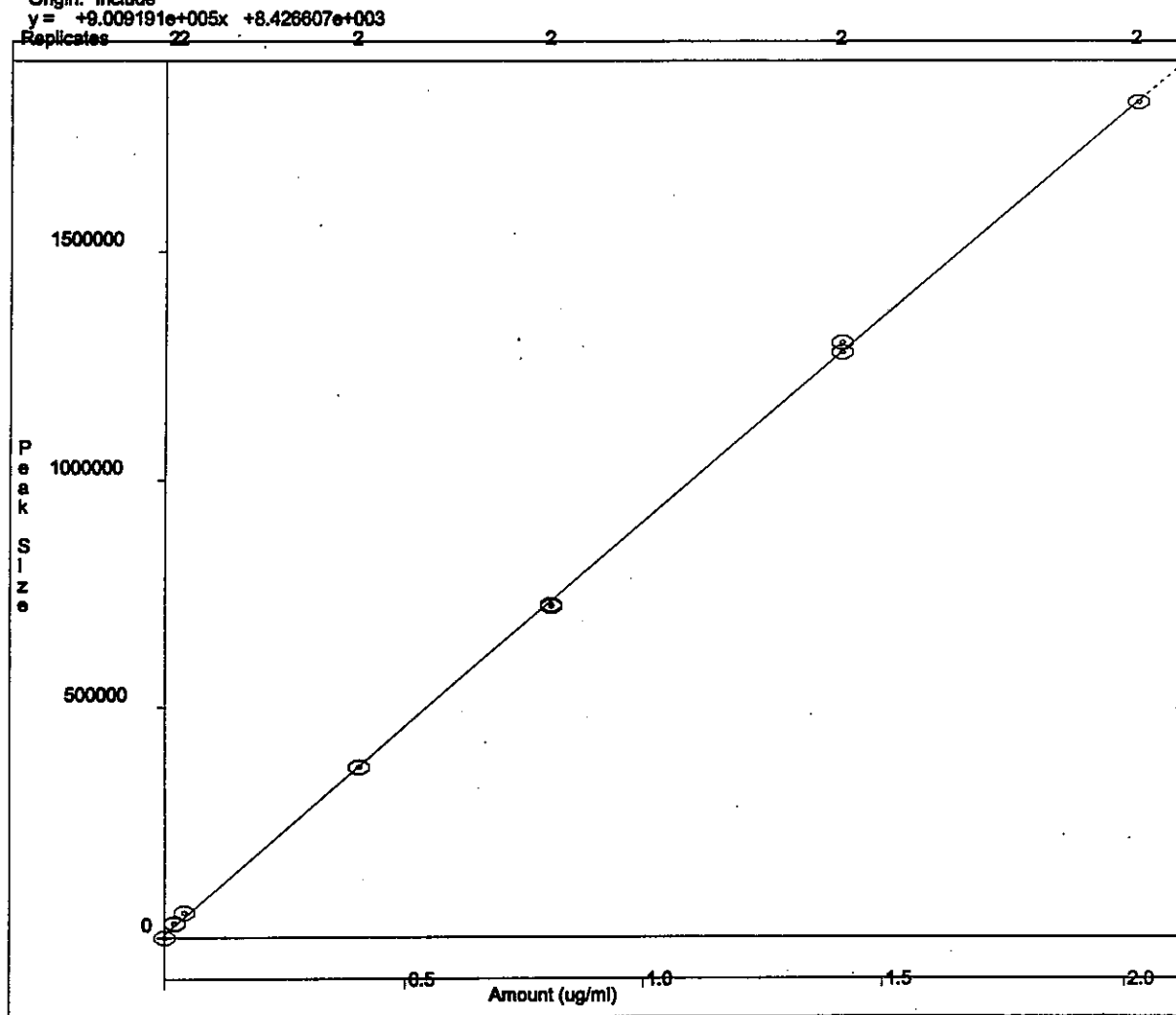
Curve Type: Linear

Origin: Include

 $y = +9.009191e+005x + 8.426607e+003$

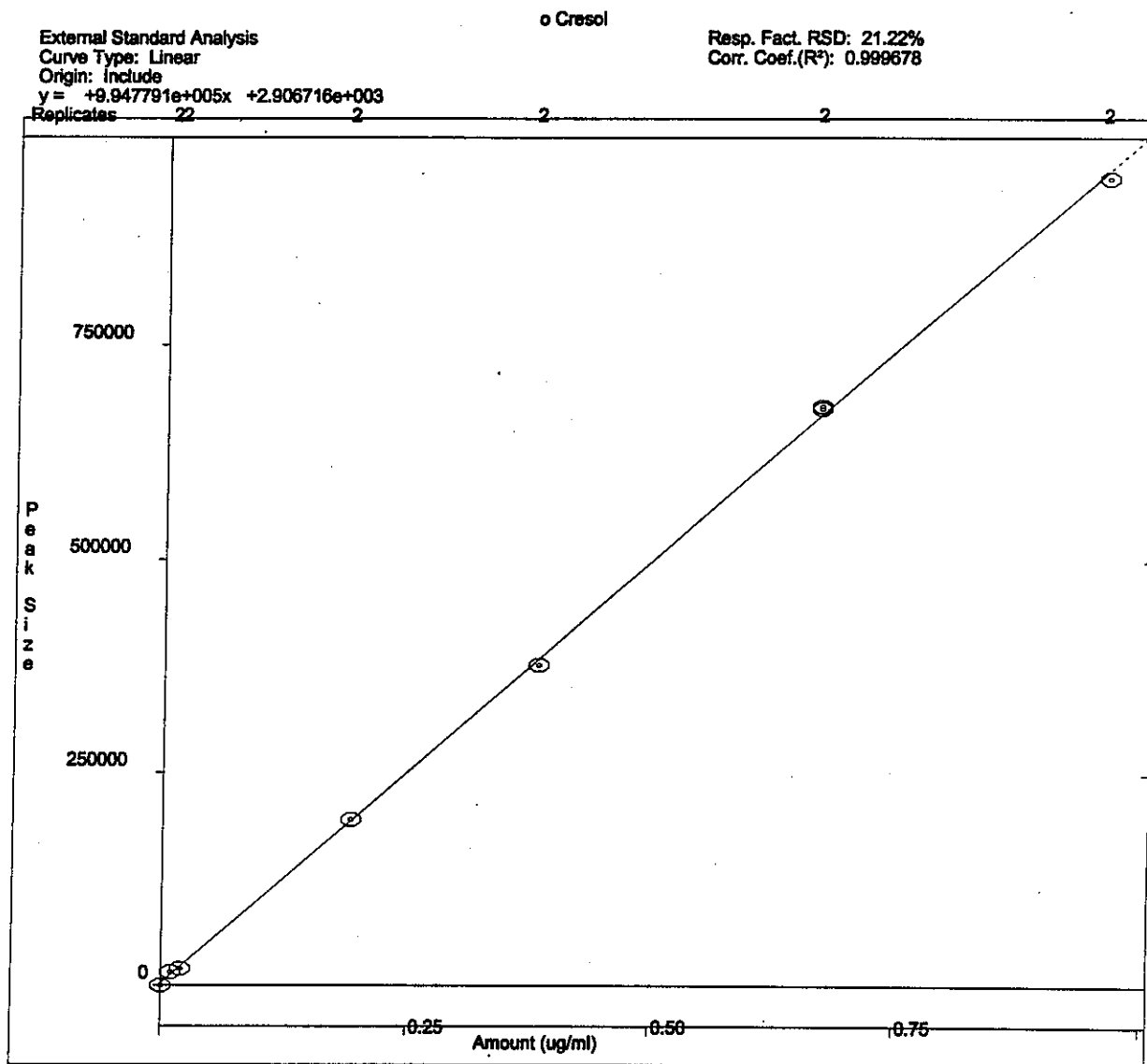
m+p Cresol

Resp. Fact. RSD: 26.32%

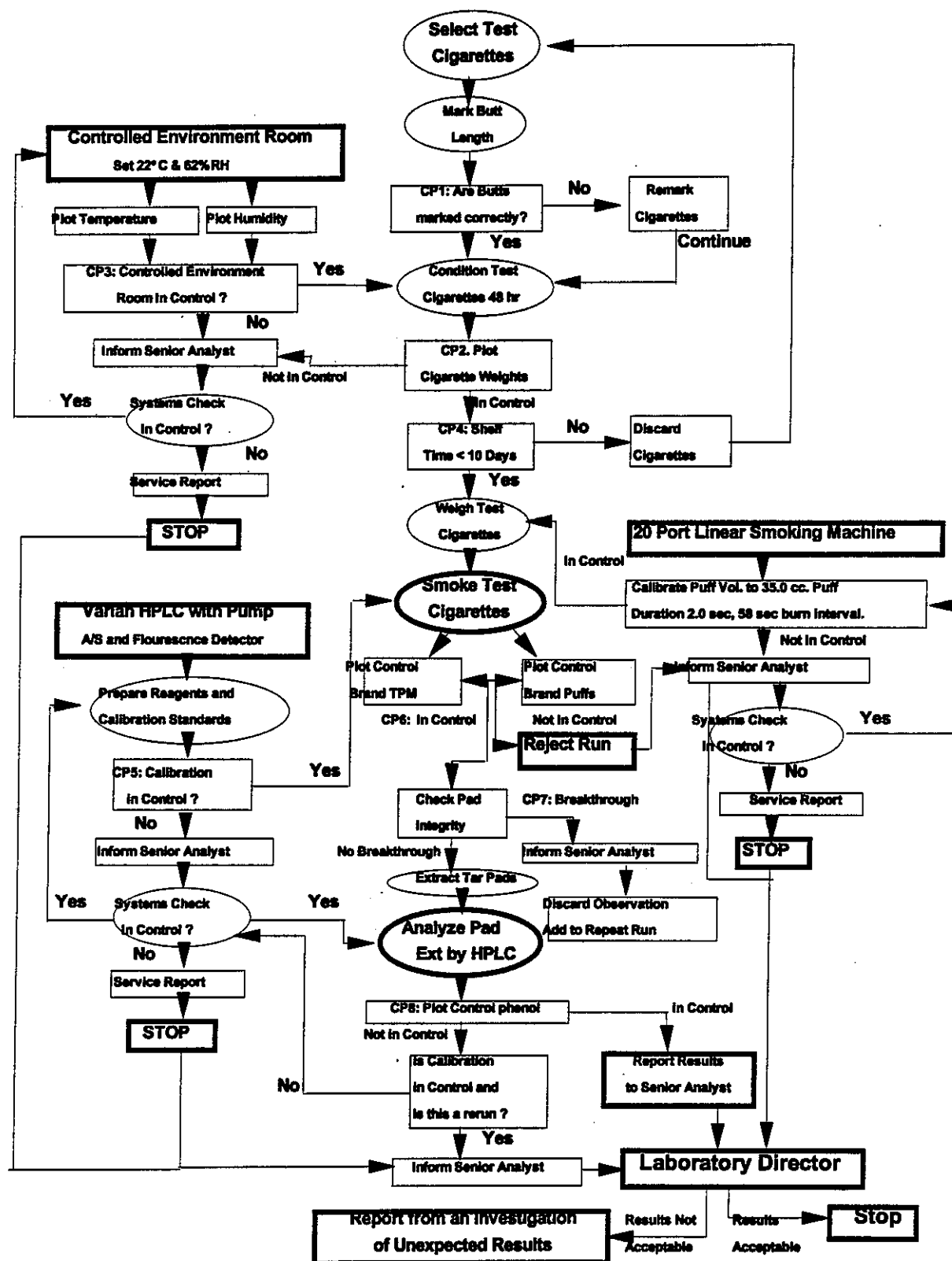
Corr. Coef.(R²): 0.999778

Appendix 4f: o-Cresol Calibration Curve

Calibration Curve Report
File: f:\home_dir\zavitsk\m24ssphen475.mth
Detector: ADC Board, Address: 16, Channel ID: A



Appendix 5: Phenols Process Control Flow Diagram



Appendix 6a: Laboratory Reagent Blanks (LRB) from a Recent Sidestream Study

Sample Description	Hydroquinone (ug/ml)	Resorcinol (ug/ml)	Catechol (ug/ml)	Phenol (ug/ml)	m+p Cresol (ug/ml)	o-Cresol (ug/ml)
Day1_LRBa	Not Det	0.0112	0.0260	0.0113	0.0218	0.0003
Day2_LRBa	Not Det	0.0107	0.0235	0.0113	Not Det	0.0076
Day3_LRBa	Not Det	0.0139	0.0468	0.0136	Not Det	0.0044

Sample Description	Hydroquinone (ug/cig)	Resorcinol (ug/cig)	Catechol (ug/cig)	Phenol (ug/cig)	m+p Cresol (ug/cig)	o-Cresol (ug/cig)
Day1_LRBa	Not Det	0.112	0.260	0.113	0.218	0.003
Day2_LRBa	Not Det	0.107	0.235	0.113	Not Det	0.076
Day3_LRBa	Not Det	0.139	0.468	0.136	Not Det	0.044
Average	Not Det	0.119	0.321	0.121	0.218	0.041

Appendix 6b: Laboratory Fortified Blanks (LFB) from a Recent Sidestream Study

Results are reported on a Per cent Recovered Basis

Sample Description	Hydroquinone (ug/ml)	Phenol (ug/ml)	o-Cresol (ug/ml)
Day1_LFBa	73.40	94.00	114.23
Day2_LFBa	83.10	96.75	74.23
Day3_LFBa	90.23	97.25	125.21
Average	82.24	96.00	104.56

Appendix 6c: Laboratory Fortified Blanks (LFM) from a Recent Sidestream Study

Results are reported on a Per Cent Recovered Basis

Sample Description	Hydroquinone (ug/ml)	Phenol (ug/ml)	o-Cresol (ug/ml)
R04_P12_LFMa	98.76	93.75	95.84
R08_P09_LFMa	99.02	102.82	102.76
R12_P17_LFMa	100.78	109.32	124.93
Average % LFM Recovered	99.52	101.96	107.85

Appendix 7: Minimum Detection Limit (MDL) and Limit of Quantitation (LOQ) for Sidestream Phenols.

Phenols	Standard 1 Hydroquinone (ug/ml)	Standard 2 Resorcinol (ug/ml)	Standard 2 Catechol (ug/ml)	Standard 1 Phenol (ug/ml)	Standard 2 m+p Cresol (ug/ml)	Standard 2 o-Cresol (ug/ml)
	0.0512	0.0092	0.0543	0.0427	0.0355	0.0215
	0.0510	0.0069	0.0598	0.0474	0.0342	0.0193
	0.0493	0.0052	0.0557	0.0485	0.0356	0.0229
	0.0514	0.0071	0.0619	0.0474	0.0354	0.0211
	0.0490	0.0088	0.0570	0.0484	0.0351	0.0179
	0.0543	0.0054	0.0506	0.0489	0.0368	0.0182
	0.0544	0.0056	0.0439	0.0424	0.0366	0.0202
	0.0497	0.0059	0.0472	0.0424	0.0357	0.0205
	0.0506	0.0071	0.0485	0.0451	0.0353	0.0221
	0.0538	0.0073	0.0628	0.0452	0.0347	0.0189
Average	0.0515	0.0067	0.0542	0.0456	0.0355	0.0203
Std Dev	0.0020	0.0012	0.0065	0.0025	0.0008	0.0017
Coeff of Var	3.9	17.9	11.9	5.4	2.2	8.3
MDL (ug/ml)	0.0061	0.0036	0.0194	0.0074	0.0023	0.0050
MDL (ug/cig)	0.122	0.071	0.387	0.149	0.047	0.100
LOQ (ug/ml)	0.0203	0.0119	0.0646	0.0248	0.0078	0.0167
LOQ (ug/cig)	0.406	0.238	1.291	0.496	0.156	0.335

Appendix 8: Kentucky Reference cigarette (1R4F) Yields
**Sidestream Yield Summary for Kentucky Reference
1R4F (Brand 507)**

Analyte	Mean	Units	Std. Dev.	Coeff. Var.
Phenolic grp				
Hydroquinone	116	ug/cig	17.3	14.9%
Resorcinol	0.806	ug/cig	0.568	70.5%
Catechol	93.4	ug/cig	13.9	14.9%
Phenol	247	ug/cig	25.7	10.4%
mp_cresol	72.9	ug/cig	10.1	13.8%
o_cresol	34.7	ug/cig	5.82	16.8%

No.: T - 212
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the collection and quantitation of the tar and nicotine content of sidestream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Smoke, 1999-12-31.
- 2.2** American Society for Testing and Materials (ASTM) D1193-77 – Standard Specifications for Reagent Water, Version 1977.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** This method describes the routine analysis of sidestream (SS) tobacco smoke using a British American Tobacco (BAT) fishtail chamber configuration. Sidestream smoke is all the smoke emitted from the lit end of a burning cigarette during the smoulder process. A glass fishtail chamber sits over a burning cigarette and allows the smoke to be directed in a controlled manner for the determination of sidestream particulate matter, water, nicotine and tar.
- 4.2** Four conditioned cigarettes* are smoked per port, using a constant volume smoking machine. The SS smoke is collected on a glass fibre filter disc (pad). Sidestream total particulate matter (TPM), water, nicotine and tar are determined as specified in T-115.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and/or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
- 5.2** Equipment needed to perform marking for butt length as specified in T-115.
- 5.3** Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4** Sidestream pad holders.

- 5.5 Analytical balance measuring to at least four decimal places.
- 5.6 Anti-static wipes.
- 5.7 Tweezers and gloves for transferring pads.
- 5.8 50 mL amber serum bottles with stoppers.
- 5.9 Desiccator.
- 5.10 Constant rate platform shaker.
- 5.11 Volumetric Flasks - 10, 25 and 50 mL.
- 5.12 Volumetric pipettes or gas-tight syringe for range 100 to 1000 μ L.
- 5.13 Hewlett-Packard (HP) 5890 GC with FID and TCD and 6890 autosampler (or equivalent).
- 5.14 Data collection system.
- 5.15 Water Column - 6' X 1/8" o.d. (two metres X 3.2 mm o.d.) stainless steel - Poropak Type Q: 80 - 100 mesh.
- 5.16 Nicotine Column - 6' X 1/8" o.d. (two metres X 3.2 mm o.d.) stainless steel - 16 % Apiezon L, 2 % KOH, 2 % Carbowax 20M on Chromosorb W: 80-100 mesh.
- 5.17 UV Spectrophotometer - Spectronic Genesys 5 (or equivalent) with quartz cuvettes (1 cm path length).
- 5.18 Vacuum pumps (GAST or equivalent).
- 5.19 Flow Meter (15 mL capacity).
- 5.20 Retort stand and clamps (one set per "fishtail").
- 5.21 Impingers - 70 mL without frits.
- 5.22 Tygon tubing.
- 5.23 Electric lighter.
- 5.24 Rubber bulb.
- 5.25 Fishtail Chamber.
- 5.26 Scintillation vials (10 mL) with foil-lined plastic caps.
- 5.27 Screw-cap culture tubes (15 mL) with plastic caps.
- 5.28 Glass funnels - 75 mm i.d., short stem.
- 5.29 Rinsing rack.
- 5.30 Pipette (200 μ L-1000 μ L), Pipette (1-5 mL).

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

- 6.1 Isopropanol.
- 6.2 Methanol.
- 6.3 Anethole (at least 99 % purity).
- 6.4 Nicotine (at least 98 % purity).
- 6.5 Type I Water - as specified in ASTM D1193.
- 6.6 Glass fibre filter discs, 44 mm in diameter, with no more than 5 % acrylic type binder.
- 6.7 Amber Autosampler vials with rubber septa lined caps.
- 6.8 Disposable syringes - 5 mL.
- 6.9 Syringe filters 0.45 μ m, 25 mm diameter
- 6.10 Parafilm® or equivalent.
- 6.11 Disposable pipette tips.
- 6.12 Wash bottles.
- 6.13 Argon gas.

7 PREPARATION OF GLASSWARE

-
- 7.1** Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF SOLUTIONS**8.1 Preparation of Extraction Solution**

8.1.1 Prepare extraction solution as specified in T-115.

9 PREPARATION OF STANDARDS

9.1 Prepare standards as specified in T-115.

10 SAMPLING

10.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

11 TOBACCO PRODUCT PREPARATION

11.1 Product shall be conditioned as specified in T-115.

11.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.

11.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

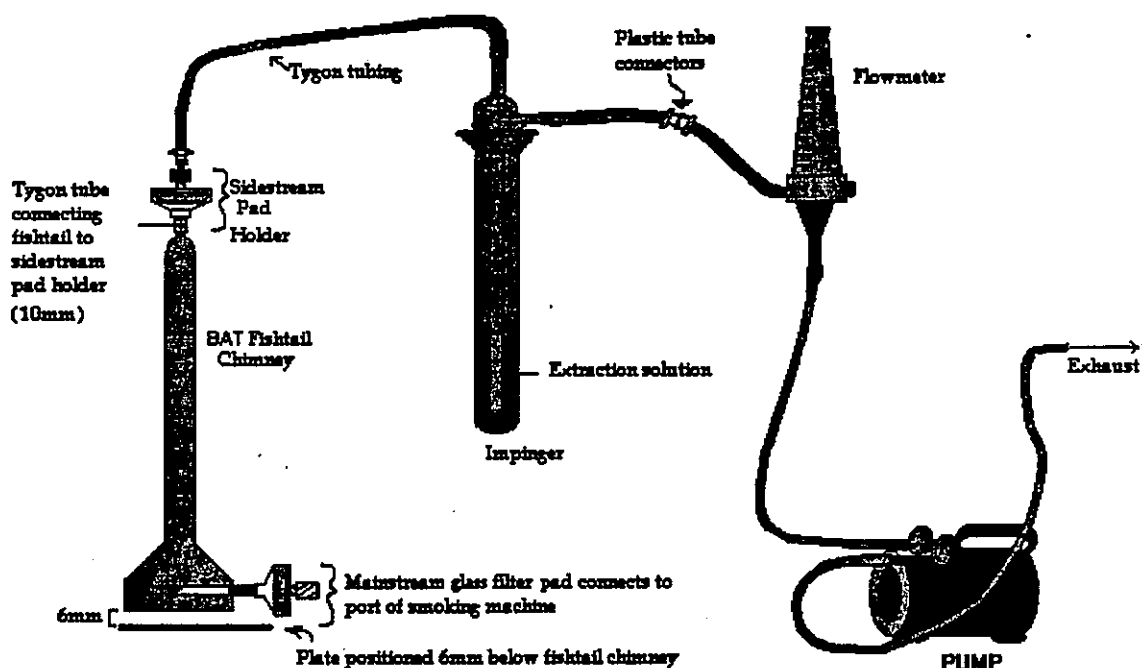
12 SMOKING MACHINE PREPARATION**12.1 Ambient Conditions**

12.1.1 The ambient conditions for smoking shall be as those specified in T-115.

12.2 Machine Conditions

12.2.1 The machine conditions shall be as those specified in T-115 (with the following modifications as detailed below).

12.2.2 Set up the Sidestream Apparatus as shown.



12.2.3 Accurately transfer 30 mL of extraction solution into each impinger.

12.2.4 Attach the SS holders to the top of the fishtail.

12.2.5 Calibrate the flow meter to 3 L/minute.

12.2.6 Insert cigarette into MS holder.

Note: Before lighting the cigarette, lower the fishtail to the smoking position. Adjust the alignment of the fishtail and cigarette so that they are not touching. Raise the fishtail and prepare to start run.

12.2.7 Start the run initiating the puff interval.

12.2.8 At 30 seconds, turn on vacuum pump.

12.2.9 At 51 seconds, light the cigarette using the lighter. Remove lighter immediately after puff has been taken.

12.2.10 Position the bottom plate beneath the cigarette.

12.2.11 Lower the fishtail over the cigarette to approximately 6 mm from the plate.

12.2.12 Smoke the cigarette to the butt mark.

12.2.13 Raise the fishtail and extinguish the cigarette using tweezers.

12.2.14 Allow the pump to run for 30 seconds after the cigarette is extinguished to ensure all SS smoke is collected.

12.2.15 Remove the cigarette butt.

12.2.16 Repeat the smoking procedure for the remaining cigarettes.

Note: The number of cigarettes smoked for this analysis is four. Because of the large amount of TPM in SS smoke, only the smoke of the first two cigarettes is collected on the SS pad. After the second cigarette, the SS pad holder is removed, weighed for TPM and the pad is transferred to a 50 mL serum bottle. Another pad is placed into the SS pad holder and the run is continued. The second pad is disposed of at the end of the run. If the SS TPM levels are known to be very low, all four cigarettes may be smoked onto one pad.

13 SAMPLE ANALYSIS

13.1 Extraction of pads (SS)

13.1.1 After two cigarettes are smoked, remove the SS pad holder from the smoking machine and weigh to determine TPM.

13.1.2 Open the filter holder and, with gloves on and using clean tweezers, fold the pad into quarters, TPM inside.

13.1.3 Wipe the internal surface of the holder with the clean surface of the pad and transfer the pad into a desiccated, labelled 50 mL amber serum bottle, TPM side up.

13.1.4 Three blanks must be prepared with each smoking run. Place one conditioned pad into each of three desiccated 50 mL serum bottles and treat as samples.

13.1.5 Add 20 mL of the extraction solvent to each serum bottle and seal with a stopper.

13.1.6 Shake the bottles for 45 minutes on a platform shaker.

13.1.7 Rinse two autosampler vials with the contents of each bottle, then fill each vial, cap and label with run #, port #, and A or B and place A samples on GC autosampler tray for analysis.

13.1.8 Store B samples in the dark to be used if necessary.

13.1.9 Filter a portion of the SS pad extract into a vial using a syringe and filter. Store until ready to do Tar analysis on the Spectrophotometer.

13.2 Rinsing of Fishtail Chamber

13.2.1 At the end of the run, remove the fishtail from the clamps and place upside down into a rack for rinsing.

- 13.2.2 Place a 50 mL volumetric flask beneath the fishtail.
- 13.2.3 Place a glass funnel into the 50 mL volumetric.
- 13.2.4 Rinse the fishtail down into the volumetric flask using a squirt bottle containing extraction solution.
- 13.2.5 Make the flask to volume with extraction solution, stopper and mix well.
- 13.2.6 Decant the solution into two GC vials (A and B) for nicotine analysis.
- 13.2.7 Pour the remaining solution into scintillation vials. Store until ready to do Tar analysis on the Spectrophotometer.

13.3 Impinger Solution

- 13.3.1 Rinse the impinger solution through the Tygon tubing and pour into a 50 mL volumetric flask.
- 13.3.2 Rinse the impinger and tubing twice with 5 mL aliquots of extraction solution and transfer to the flask.
- 13.3.3 Make the flask to volume with extraction solution, stopper and mix well.
- 13.3.4 Decant the solution into two GC vials (A and B) for nicotine analysis.

14 SAMPLE ANALYSIS

14.1 GC Analysis

14.1.1 Typical GC Conditions:

Oven Temperature:	190 °C.
Injector Temperature:	230 °C.
Detector Temperature:	230 °C.
Carrier Gas:	Purified Helium @ pressure 60 psi.

Flow Rates

FID: Column flow:	20 mL/minute.
Column + Hydrogen:	60 mL/minute.
Column + Air + Hydrogen:	350 mL/minute.

TCD: Column flow:	20 mL/minute.
Column + Reference:	30 mL/minute.

- 14.1.2 Two μ L of each standard and sample are injected onto the GC for nicotine analysis. Only the sidestream pad extracts are analyzed for water.

14.2 Spectrophotometric Analysis

14.2.1 Turn on the spectrophotometer and set the wavelength to 310 nm.

14.2.2 Zero the spectrophotometer with a "blank" cuvette containing extraction solution.

14.2.3 Measure and record the absorbance of the SS pad extract and the Fishtail rinse. Make dilutions as necessary with extraction solution so that the absorbance of all solutions falls between 0.2 and 0.8. Record any dilutions to be used in the final calculation.

Note: Typical dilutions are 0.25-0.50 mL to 10 mL for the SS pad extract and 2.5-5.0 mL to 10 mL for the fishtail solution.

14.3 Calculations

14.3.1 Calibration Curve

14.3.2 With each new batch of extraction solution prepared, the GC must be recalibrated to determine new slopes and intercepts for water and nicotine calculations as well as to monitor any changes in GC performance. Each recalibration involves preparing new stock and standard solutions.

14.4 Sample Calculations

14.4.1 TPM (Sidestream Pad)

$$\text{TPM (mg/cig)} = [\text{CFH after(g)} - \text{CFH before(g)}] \times 1000(\text{mg/g}) / 2 (\text{cigarettes}).$$

Note: the number of cigarettes may be four for very low SS TPM brands.

14.4.2 Water (SS Pads)

Water results are calculated from the calibration curve and are reported in mg/cigarette. The water content of the three blanks is determined from the calibration curve for water. The average of the three blanks is subtracted from the water results for that run.

14.4.3 Nicotine (SS Pads, Fishtail and Impinger Solutions)

Nicotine results are calculated from the calibration curve and are reported in mg/cigarette.

14.4.4 Tar (SS Pads)

The Tar value is determined for each observation by the following:

$$\text{Tar (mg/cigarette)} = \text{TPM} - \text{Nicotine} - \text{Water}.$$

14.4.5 Tar (Fishtail)

The Fishtail Tar value is calculated from the following equation:

$$\text{FT Tar (mg/cigarette)} = (\text{SS}_{\text{Tar}} \times \text{FT}_{\text{Abs}} \times \text{FT}_{\text{DF}}) / (\text{SS}_{\text{Abs}} \times \text{SS}_{\text{DF}}).$$

where: SS_{Tar} is the tar value obtained from the SS pad.
 FT_{Abs} is the absorbance of the fishtail solution.
 FT_{DF} is the dilution factor of the fishtail solution.
 SS_{Abs} is the absorbance of the SS pad extract.
 SS_{DF} is the dilution factor of the SS pad extract.

14.4.6 SS Tar and Nicotine

The total SS tar and nicotine are calculated as follows:

$$\text{SS Tar (mg/cigarette)} = \text{SS Pad}_{\text{Tar}} + \text{FT}_{\text{Tar}}.$$

$$\text{SS Nicotine (mg/cigarette)} = \text{SS Pad Nicotine} + \text{Fishtail Nicotine} + \text{Impinger Nicotine}.$$

15 QUALITY CONTROL

15.1 Recoveries and Levels of Contamination

15.1.1 Laboratory Reagent Blanks (LRB) are used to monitor the level of water and nicotine contamination in the reagents (including glassware and pads). Although nicotine is typically ND in these blanks, there is always some water due to the presence of the water in the extraction solution and the conditioned pad.

15.1.2 Laboratory Fortified Blanks (LFB) are used to evaluate the extent of potential analyte loss during the extraction process. LFBs should be run whenever there is a question about the validity of results.

15.2 Method detection limit (MDL)/Limit of Quantitation (LOQ)

This involves the use of either a test material with a low level of the analyte or the lowest standard. The standard deviation is then determined and the MDL is determined to be three times the standard deviation. LOQ is taken as 10 times the standard deviation.

15.3 Stability of Reagents and Samples

15.3.1 Extraction solution is stable but can become contaminated with water over time. For this reason, and to ensure nicotine calibration remains constant, fresh standards for nicotine and water should be made weekly.

16 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

16.1 Under intense smoking conditions, the number of cigarettes smoked per port is two.

17 REFERENCES

17.1 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113:

p. 1509-1513.

- 17.2** Cigarettes - Sampling, *International Reference Number ISO 8243:1991.*
- 17.3** Tobacco and tobacco products - Atmosphere for conditioning and testing, *International Standard Reference Number ISO 3402:1991 (E).*
- 17.4** Routine analytical cigarette-smoking machine - Definitions and standard conditions, *International Standard Reference Number ISO 3308:1991 (E).*
- 17.5** Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical cigarette smoking machine, *International Standard Reference Number ISO 4387:1991 (E).*
- 17.6** Cigarettes - Determination of nicotine in smoke condensates - Gas-chromatographic method, *International Standard Reference Number ISO 10315:1991 (E).*
- 17.7** Cigarettes - Determination of water in smoke condensates - Part 1: Gas-chromatographic method, *International Standard Reference Number ISO 10362-1:1991 (E).*
- 17.8** Cigarettes - Determination of carbon monoxide in the vapour phase of cigarette smoke - NDIR method, *International Standard Reference Number ISO 8454:1995 (E).*

No.: T-213
Date: December 31, 1999
Page: 1 of 9

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the isolation and quantitation of the 1,3-Butadiene, Isoprene, Acrylonitrile, Benzene, Toluene and Styrene ("Volatiles") content of sidestream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide (CO) in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** This method is used for the analysis of sidestream (SS) tobacco smoke using a fishtail chimney configuration. Sidestream smoke is all the smoke emitted from the lit end of a burning cigarette during the smolder process. The glass fishtail chimney sits over a burning cigarette and allows the smoke to be directed in a controlled manner for the determination of sidestream tobacco constituents.
- 4.2** The sidestream smoke of two cigarettes* is drawn through a fishtail chamber and passed through a glass fibre filter pad which is subsequently used for sidestream (SS) Total Particulate Matter (TPM) determination. The gas phase is led through three 70 mL impingers, each containing methanol. The first trap remains at room temperature while the second and third traps are kept at or below -70 °C in a dry ice/isopropanol bath. The impinger solutions are pooled, spiked with D₆-benzene and injected onto a GC/MS for quantitation.

*For other tobacco products, select a number such that breakthrough does not occur.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
5.2 Equipment needed to perform marking for butt length as specified in T-115.

- 5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4 Analytical balance measuring to at least four decimal places.
- 5.5 Vacuum pumps (GAST or equivalent).
- 5.6 Victor Flow meter or equivalent.
- 5.7 BAT (British American Tobacco) Fishtail Chimney mounted on a retort stand.
- 5.8 Tygon tubing.
- 5.9 70 mL glass impingers with extra-coarse frits.
- 5.10 10, 25, 50 and 100 mL volumetric flasks.
- 5.11 Volumetric pipettes or gas-tight syringes for range 100 to 1000 μ L.
- 5.12 Screw-cap autosampler vials with caps and Teflon-lined septa.
- 5.13 Varian Saturn I GC/MS system consisting of an 8100 autosampler, a 3400 GC with a 1077 split/splitless injector and an ion trap detector (or equivalent).
- 5.14 J&W Scientific 60 m X 0.32 mm X 1 μ m DB-5MS column (or equivalent) with 1 m X 0.25 mm deactivated fused silica transfer line.
- 5.15 Dewar flasks.
- 5.16 Pasteur pipettes.
- 5.17 Vortex.
- 5.18 Thermometer (-100 to 40 °C).
- 5.19 Spectrophotometer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical reagent grade quality.

- 6.1 Dry ice.
- 6.2 Propan-2-ol (IPA).
- 6.3 Methanol (Distilled-in-Glass).
- 6.4 Reagent Alcohol (Distilled-in-Glass).
- 6.5 D₆ – Benzene - Purity of D₆ > 99 %.
- 6.6 1,3-butadiene.
- 6.7 Isoprene.
- 6.8 Acrylonitrile.
- 6.9 Benzene.
- 6.10 Toluene.
- 6.11 Styrene.

7 PREPARATION OF GLASSWARE

- 7.1 Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

8 PREPARATION OF STANDARDS

8.1 Preparation of Standards (except 1,3-butadiene)

- 8.1.1 Five primary stock solutions are prepared by accurately weighing 100 μ L each of isoprene, acrylonitrile, benzene, toluene and styrene into five 10 mL volumetric flasks, filling each flask to the mark with methanol and mixing well.

- 8.1.2 A combined secondary stock solution is prepared by transferring appropriate aliquots of each of the primary stock solutions into a 25 mL volumetric flask, filling it to the mark with methanol and mixing well.
- 8.1.3 A stock solution of D₆-benzene is prepared by transferring the contents of a 1 g ampoule to a 10 mL volumetric flask, filling the flask to the mark with methanol and mixing well.
- 8.1.4 An internal standard spiking solution is prepared by diluting 4 mL of the stock to 100 mL with methanol and mixing well. Aliquots of this spiking solution are stored in 25 mL vials with Teflon-lined caps and at minus 20 °C.
- 8.1.5 Five calibration standard solutions are prepared by adding 100 µL ISTD to each of five 10 mL volumetric flasks. The sides are rinsed with methanol, then appropriate aliquots of the secondary stock solution are added to each flask. The flasks are filled to the mark with methanol and mixed well.
- 8.1.6 The solutions are transferred to a series of labeled autosampler vials, capped with Teflon-lined septa and stored at minus 20 °C until use.

Note: Each vial is only used once.

8.2 Preparation of 1,3-Butadiene Standards

- 8.2.1 Attach a piece of Tygon tubing to the valve of a 1,3-butadiene cylinder. Place a Pasteur pipette on the other end and immerse the tip of the pipette into a 100 mL volumetric flask containing methanol up to the base of the neck of the flask. Open the valve and gently bubble the 1,3-butadiene into the methanol for about five minutes. Make the volume to the mark with methanol and mix well.
- 8.2.2 Pipette 1 mL of the stock solution into a clean 100 mL volumetric flask and make to the mark with methanol and mix well. This is the secondary stock solution.
- 8.2.3 Determination of Secondary Stock Concentration
- 8.2.3.1 Pipette 1 mL of the secondary stock solution into a 100 mL volumetric flask and make to the mark with reagent alcohol and mix well.
- 8.2.3.2 Measure the absorbance of the solution against a reagent alcohol blank on the spectrophotometer at 217 nm. Make dilutions as necessary so that the absorbance (A) falls between 0.2 and 0.6.
- 8.2.3.3 Calculate the concentration of the secondary stock solution according to the following:

$$\text{Conc. (}\mu\text{g/mL)} = \text{A} \times 54 \text{ g/mole} \times 1000 \text{ mg/g} \times 100 \text{ mL} \times 1000 \text{ }\mu\text{g/mg.}$$

20 893 L/mole

1000 mL/L 1 mL

- 8.2.4** Once the concentration of the secondary stock solution is known, make a minimum of four calibration standard solutions in the range appropriate for the expected delivery levels (typically five to 50 µg/mL).
- 8.2.5** Add internal standard solution (100 µL) to each 10 mL volumetric. Add an appropriate aliquot of secondary stock solution, and make up to the mark with methanol.

- 8.2.6 Transfer the solutions to a series of labeled autosampler vials. Cap with Teflon-lined septa and store at minus 20 °C until use.

Note: Each vial is used only once.

9 SAMPLING

- 9.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

10 TOBACCO PRODUCT PREPARATION

- 10.1 Product shall be conditioned as specified in T-115.
- 10.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 10.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

11 SMOKING MACHINE PREPARATION

11.1 Ambient Conditions

- 11.1.1 The ambient conditions for smoking shall be as those specified in T-115.

11.2 Machine Conditions

- 11.2.1 The machine conditions shall be as those specified in T-115 (with the following modifications as detailed below:)

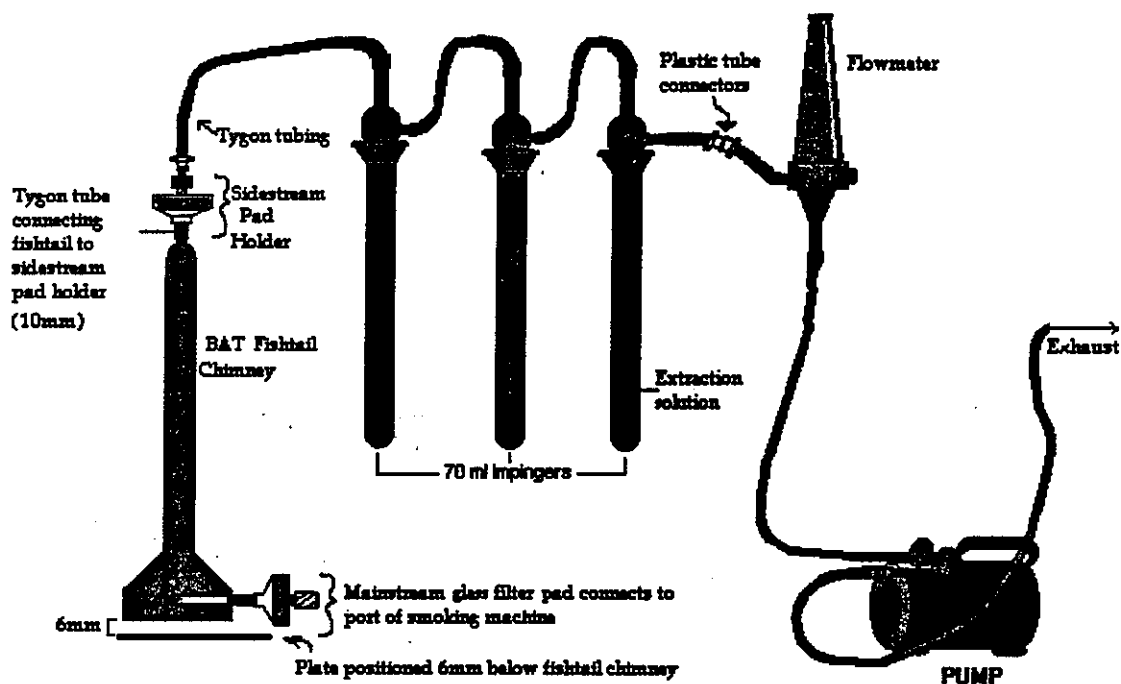


FIGURE 1b: SIDESTREAM APPARATUS USING THREE IMPINGERS

12 SAMPLE GENERATION

- 12.1 Smoking is conducted using between four and eight ports of a linear 20 port smoking machine. The sidestream smoke is collected by a fish-tail chamber mounted above the cigarette.
- 12.2 Insert the mainstream (MS) pad holder with pad into the assigned port of the smoking machine.
- 12.3 Prepare the impingers by adding 10 mL of methanol to the first impinger and 20 mL of methanol into the second and third impingers.
- 12.4 Immerse the second and third impingers into a dry-ice/IPA bath (temperature at or below -70°C) leaving the first at room temperature.

Note: The first impinger is left at room temperature due to the large amount of water in the sidestream smoke which causes the impingers to freeze in the dry ice/IPA bath.
- 12.5 Position the SS filter pad holder above the fish-tail chamber and then hook up in series three impingers to the filter pad holder. Connect the tubing of the last impinger to the flow meter. Connect the flow meter to the vacuum pump.
- 12.6 Turn the pump on (flow rate of 2 L/minute) just prior to lighting the cigarette. Light the cigarette and initiate the puff count.

- 12.7 Lower the chimney to its lowest position. Do not allow the cigarette to touch the chimney. Keep the chimney approximately 6 mm from the plate insert.
- 12.8 Burn the cigarette to the previously marked standard butt length. Remove the butt and save if required. After the cigarette has been smoked to the line, leave the pump on for approximately 20 seconds to collect all of the smoke from the fishtail chimney. Raise the chimney to its highest position and turn the pump off.
- 12.9 Smoke the second cigarette in the same manner as the first.
- 12.10 After smoking two cigarettes per port, remove both mainstream and sidestream filter holders and record their final weight on the run sheet to obtain the MS and SS TPM. Total particulate matter (TPM) is determined as described in T-115. Data for mainstream and sidestream TPM is used to characterize samples and to monitor the smoking process.

13 SAMPLE ANALYSIS

- 13.1 Immediately after smoking is completed, pool the contents of the three impingers and spike with 500 µL ISTD solution. Vortex for approximately 10 seconds.
- 13.2 Decant aliquots of each impinger solution into two labeled autosampler vials. Fill each vial to the base of the neck and cap with an autosampler cap and Teflon-lined septum. DO NOT OVERFILL VIALS.
- 13.3 Store samples at minus 20 °C for up to 48 hours prior to analysis.
- 13.4 Instrument Analysis: GC/MS Conditions

Injector temperature:	20 °C.
Column temperature:	35 °C for five minutes. 20 °C/minute to 225 °C, hold six minutes.
Column pressure:	13 psi.
Transfer line temperature:	240 °C.
Manifold temperature:	240 °C.

- 13.5 One µL of the methanol solution is injected onto the GC/MS, which is run in the split mode (split flow 30 mL/minute).
- 13.6 The GC/MS is operated in full-scan mode (50 to 200 amu). The following ion peak areas are used for quantitation:

1,3-butadiene	51+52
Isoprene	67
Acrylonitrile	52
Benzene	78
D ₆ Benzene	84
Toluene	91
Styrene	104

Note: The assignment of these masses is based on selection of the best response (i.e. the base peak) and the need to avoid possible contamination from interfering peaks which may contain similar ions. The choice of quantitation ions may be different for different instrument configurations.

14 CALCULATIONS

14.1 Calibration Curve

14.1.1 A calibration curve is generated at the beginning of each sample set or "project". Each standard solution is injected once and a calibration file built using the method for internal standard quantitation available with the Saturn quantitation software.

14.1.2 A check standard is analyzed every 20 samples and at least once per run. This standard is treated as a sample and the observed value is compared to the expected value for that standard. A difference of more than 10 % of expected requires the following course of action.

14.1.2.1 Make fresh calibration standards and run as check standards.

14.1.2.2 If the results are within 10 % of expected, the first set of standards should be discarded and the new set used. The calibration is still valid.

14.1.2.3 If the results differ by more than 10 % of expected, the calibration is no longer valid and a new calibration curve must be generated.

14.2 Sample Calculation

14.2.1 The software on the GC/MS is used to generate results for each analyte based on the concentrations of the standard solutions. The results are reported in µg/mL. To calculate the final results, the following calculation is used:

$$\text{Analyte } (\mu\text{g/cigarette}) = \frac{\text{Conc. of Analyte in Sample } (\mu\text{g/mL}) \times \text{Volume (mL)}}{\text{No. of cigarettes}}$$

15 QUALITY CONTROL

15.1 Recoveries and Levels of Contamination

15.1.1 To determine trapping efficiency, the three impingers may be analyzed separately. The amount of each analyte is determined in each impinger and is reported as a % of the total.

15.1.2 Laboratory reagent blanks (LRB) should be analyzed every 20 samples. There is occasionally a small amount of toluene present in methanol and this should be monitored closely. A laboratory fortified blank (LFB) is not necessary for this analysis as there is no sample work-up.

- 15.1.3** In lieu of an LRB, a smoking blank can be used to monitor contamination of reagents and the air in the smoking room. This involves conducting a smoking run with the same number of puffs as a control cigarette but with no cigarette in place.

15.2 Method Detection Limit (MDL)/Limit of Quantitation (LOQ)

15.2.1 The MDL can be defined as the level that gives a signal to noise ratio of three to one. The LOQ can be defined as the level that gives a signal to noise ratio of 10 to one. Because of chromatographic differences and the effect of solvent on the 1,3-butadiene peak, each analyte has different MDL and LOQ. They are estimated as follows (units $\mu\text{g/mL}$):

	MDL	LOQ
1,3-butadiene	0.3	1
Isoprene	0.05	0.2
Acrylonitrile	0.3	1
Benzene	0.05	0.2
Toluene	0.05	0.2
Styrene	0.05	0.2

Note: The DB-5 column used in this method would not be the best choice for analyzing acrylonitrile alone. It gives a poor peak shape and this is reflected in the much higher MDL and LOQ for this analyte. A more polar column would be more suitable for acrylonitrile, but for most purposes, the detection limits are acceptable for the matrices covered by this test method.

15.3 Stability of Reagents and Samples

15.3.1 Volatile mix standards are stable for at least one week if kept at minus 20 °C. Once punctured, the Isoprene is lost rapidly so each vial is used only once.

15.3.2 1,3-butadiene standards are stable for approximately one week if kept at minus 20 °C. Once punctured, the vial is discarded.

15.3.3 Volatile stock solutions should be made fresh at the beginning of every project and can be stored in the freezer for at least two weeks to be used for working standards.

15.3.4 Acrylonitrile, benzene, toluene and styrene are significantly less volatile than 1,3-butadiene and isoprene, and stock solutions may be stable for up to a month if kept at minus 20 °C.

15.3.5 The secondary stock solution for 1,3-butadiene can be re-used almost indefinitely as the actual concentration of this solution is determined every time working standards are prepared from it.

15.3.6 Samples are stable at minus 20 °C for up to 48 hours if the septum has not been punctured. It is essential that at least two vials be prepared for each sample as the vial is discarded once punctured.

16 REFERENCES

- 16.1** Byrd, G.D., K.W. Fowler, R.D. Hicks, M.E. Lovette and M.F. Borgerding, 1990. Isotope dilution gas chromatography-mass spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke. *J. Chromat.* 503, p. 359-368.
- 16.2** Brunneemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann, 1990. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. *Carcinogenesis* 11, p. 1863-1868.
- 16.3** Brunneemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann, 1989. Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MSD. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. *Exp. Pathol.* 11, p. 108-113.

No.: T - 214
Date: December 31, 1999
Page: 1 of 5

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the collection and quantitation of the carbon monoxide content of sidestream tobacco smoke.

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** One pre-conditioned cigarette or other tobacco product is smoked per port, using a constant volume smoking machine.
- 4.2** This method describes the routine analysis of sidestream (SS) tobacco smoke using a BAT (British American Tobacco) fishtail chamber configuration. Sidestream smoke is all the smoke emitted from the lit end of a burning cigarette during the smoulder process. A glass fishtail chamber sits over a burning cigarette and allows the smoke to be directed in a controlled manner through a glass fibre filter disc (pad) into a gas sampling bag for the determination of sidestream Carbon Monoxide (CO).

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Equipment needed to perform conditioning as specified in T-115.
- 5.2** Equipment needed to perform marking for butt length as specified in T-115.
- 5.3** Equipment needed to perform smoking of tobacco products as specified in T-115.
- 5.4** 50 litre gas sampling bags (or equivalent).
- 5.5** Sidestream glass fibre filter disc holders.
- 5.6** Analytical balance measuring to at least four decimal places.
- 5.7** Anti-static wipes.
- 5.8** Diaphragm vacuum pumps (GAST or equivalent).
- 5.9** Flow Meter (15 mL capacity).
- 5.10** Retort stand and clamps (one set per fishtail).
- 5.11** Tygon tubing.

- 5.12 Electric lighter.
- 5.13 BAT Fishtail Chambers.

6 REAGENTS AND SUPPLIES

- 6.1 Glass fibre filter discs (pads), 44 mm in diameter, with no more than 5 % acrylic type binder.
- 6.2 Four Primary Standard Grade CO gas standards (approx. 0.1 %, 0.3 %, 0.5 % and 1.0 % balanced with nitrogen, and with exact analysis, accurate to \pm 0.005 %, accompanying each tank).

7 PREPARATION OF CO BAGS

- 7.1 CO bags should be cleaned in such a manner to ensure that contamination from CO bags does not occur.

8 SAMPLING

- 8.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

9 TOBACCO PRODUCT PREPARATION

- 9.1 Product shall be conditioned as specified in T-115.
- 9.2 Cigarettes, cigarette equivalents, bidis, kreteks and cigars shall be marked for butt length as specified in T-115.
- 9.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

10 SMOKING MACHINE PREPARATION

10.1 Ambient Conditions

- 10.1.1 The ambient conditions for smoking shall be as those specified in T-115.

10.2 Machine Conditions

- 10.2.1 The machine conditions shall be as those specified in T-115 (with the following modifications as detailed below:)
- 10.2.2 Set up the Sidestream Apparatus as shown in the **Appendix**.

Note: For CO analysis, there is no impinger after the sidestream pad. This analysis cannot be done in conjunction with the tar and nicotine analysis but requires a separate smoking.
- 10.2.3 Calibrate the CO analyzer with the four primary grade standards (at least once per day).
- 10.2.4 Weigh pad holders and record weights on the run sheet.

- 10.2.5 Insert the mainstream (MS) holders into the specified ports on the smoking machine.
- 10.2.6 Clamp the fishtail chamber in position in front of the MS pad holder.
- 10.2.7 Attach the SS pad holder to the top of the fishtail.
- 10.2.8 Connect the SS pad holder to the flowmeter with Tygon tubing.
- 10.2.9 Connect the flowmeter to the diaphragm vacuum pump inlet with Tygon tubing.
- 10.2.10 Connect the outlet of the pump to a 50 L gas sampling bag.
- 10.2.11 Calibrate the flowmeter to 3 L/minute.
- 10.2.12 Insert cigarette into MS pad holder.

Note: Before lighting the cigarette, lower the fishtail to the smoking position. Adjust the alignment of the fishtail and cigarette so that they are not touching. Raise the fishtail and prepare to start run.
- 10.2.13 At 40 seconds, turn on diaphragm vacuum pump. Once the pump is turned on, immediately start the stopwatch.
- 10.2.14 At 51 seconds, light the cigarette using the electric lighter. Remove lighter immediately after puff has been taken.
- 10.2.15 Position the bottom plate beneath the cigarette.
- 10.2.16 Lower the fishtail over the cigarette to approximately 6 mm from the plate.
- 10.2.17 Smoke the cigarette to the butt mark.
- 10.2.18 Raise the fishtail and extinguish the cigarette using tweezers.
- 10.2.19 Allow the pump to run for 30 seconds after the cigarette is extinguished to ensure all SS smoke is collected. Turn off the stopwatch exactly when the pump is turned off. Clamp the gas sampling bag.
- 10.2.20 Remove the cigarette butt.

11 SAMPLE ANALYSIS

11.1 CO Analysis

- 11.1.1 Perform CO analysis as specified in T-115 with the following modifications:
 - 11.1.1.1 Once the cigarette has been extinguished, allow the pump attached to the sidestream pad holder to run for 30 seconds

longer. Turn off the pump and the stopwatch at the same time and clamp the gas sampling bag and record the time.

- 11.1.1.2** Remove the collection bag, and insert into the CO meter's inlet port. Turn the sampling pump on. Allow the display to stabilize and record the %CO.

11.2 Calculations

11.2.1 Calculation of the average carbon monoxide volume per cigarette

The average volume of carbon monoxide per cigarette is given by the following equation:

$$V_{as} = (CxVxp_xT_o)/Sx100xp_o x(t+T_o).$$

Where

- V_{as} is the average volume of carbon monoxide per cigarette in millilitres.
- C is the percentage by volume of carbon monoxide observed;
- V is the total volume collected in millilitres. $V = \text{total pump time (minutes)} \times 3000 \text{ (mL/minute)}$.
- p is the ambient pressure, in kilopascals.
- p_o is the standard atmospheric pressure in kilopascals.
- S is the number of cigarettes smoked.
- T_o is the temperature for the triple point of water, in degrees Kelvin.
- t is the ambient temperature, in degrees Celsius.

11.2.2 Calculation of the average mass of carbon monoxide per cigarette

The average mass of carbon monoxide per cigarette is given by the following equation:

$$m_{dg} = Cxp_xT_o xM_{co} xV_m / Sx100xp_o x(t+273) xV_m.$$

Where

- m_{dg} is the average mass of carbon monoxide per cigarette in milligrams.
- M_{co} is the molar mass of carbon monoxide in grams per mole.
- V_m is the molar volume of an ideal gas, in litres per mole.

12 QUALITY CONTROL

12.1 Recoveries and Levels of Contamination

- 12.1.1** Each analytical run of test cigarettes should include:

- 12.1.1.1** A Laboratory Reagent Blank (LRB) to evaluate the extent of any interference due to CO bags, pads, and analyser effects.

12.2 Method Detection Limit (MDL) and Limit of Quantitation

12.2.1 Method Detection Limit (MDL)

- 12.2.1.1** The method detection limit is determined by analysing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

12.2.2 Limit of Quantitation (LOQ)

- 12.2.2.1** The limit of quantification is determined by analysing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

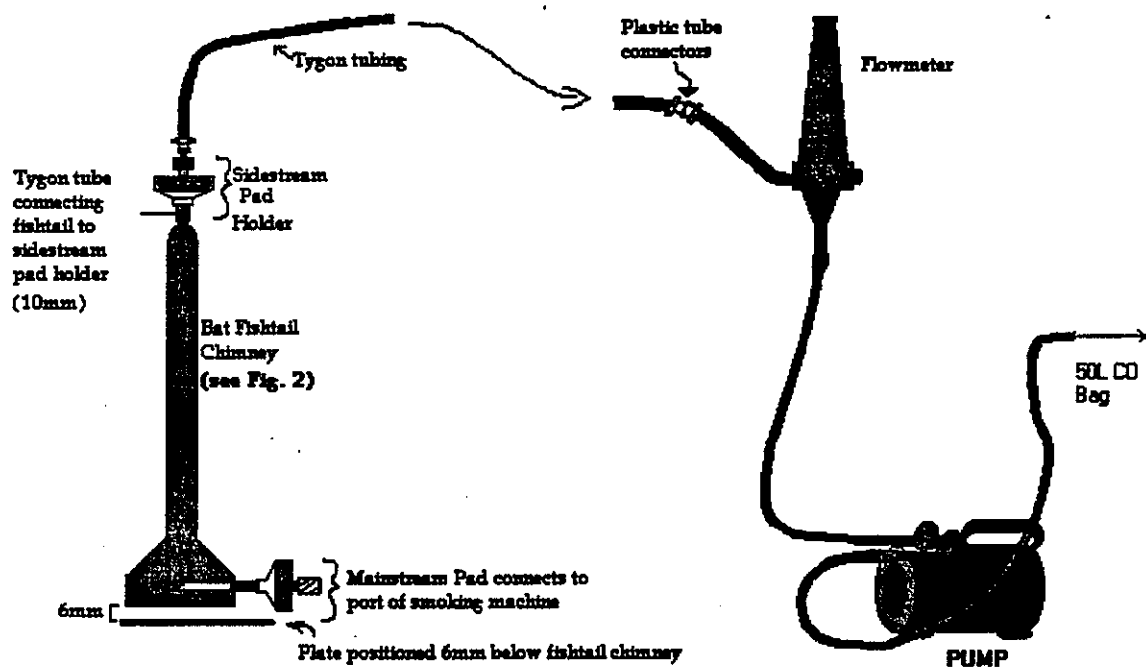
13 MODIFICATIONS FOR INTENSE SMOKING CONDITIONS

- 13.1** No modifications required for intense smoking conditions.

14 REFERENCE

Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst* 113: p. 1509-1513.

APPENDIX

**FIGURE 1a: SIDESTREAM APPARATUS**

No: T - 401
Date: December 31, 1999
Page: 1 of 5

1 SCOPE OF APPLICATIONS

- 1.1** Applicable to the preparation of processed, and cured commercially available whole tobacco leaf for incorporation into cigarettes.

2 NORMATIVE REFERENCES

- 2.1** Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.
- 2.2** CAN/CGSB-176.1-92 – Preparation of Cigarettes from Cigarette Tobacco for Testing. National Standard of Canada. Canadian General Standards Board. Appendix A, December 1992.
- 2.3** International Organization for Standardization (ISO) Method
- 2.3.1** ISO 4387:1991 Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine, 1991-10-15.

3 DEFINITIONS

- 3.1** Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

- 4.1** Whole tobacco leaf is dissected using a utility knife in order to separate the midrib from the lamina. The midrib is discarded while the top third of the leaf (tip) and the lamina are added to a Robot Coupe batch processor or equivalent.
- 4.2** 75 g of the dissected tobacco (lamina + tip) are subject to maceration at 3000 rpm for six minutes. The resulting chopped tobacco is then sieved through a 4 mm, and 850 µm sieve for 30 seconds. Tobacco retained by the 4 mm sieve, and tobacco that has passed through the 850 µm sieve, is discarded.
- 4.3** The tobacco retained by the 850 m sieve is then spread in a monolayer (2 – 4 mm thick), onto aluminum sheets, and conditioned as specified in T-115.
- 4.4** The cigarettes are made using a hand-operated cigarette-making device (e.g. Matinee Filter Master or equivalent) to inject 900 ± 10 mg of the conditioned samples of tobacco into the cigarette tube to a uniform packing density. The number of cigarettes to be prepared shall be determined using ISO 4387:1991 as a guide.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated

with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

- 5.1** Utility knife.
- 5.2** Hard Surfaced Cutting Board.
- 5.3** Robot Coupe RSI 2V Batch Processor (or equivalent).
- 5.4** USA Standard Testing Sieve (A.S.T.M.E.-11) – 4.00 mm (0.157") No. 5.
- 5.5** USA Standard Testing Sieve (A.S.T.M.E.-11) – 850 µm (0.0331") No. 20.
- 5.6** Aluminum pan or cookie sheet.
- 5.7** Weighing paper.
- 5.8** Analytical Balance capable of measuring to four decimal places.
- 5.9** Matinee Filter Master (or equivalent).
- 5.10** Cigarette tubes.
- 5.11** Equipment needed to perform conditioning as specified in T-115.

6 SAMPLING

- 6.1** The sampling of tobacco for this procedure shall be according to CAN/CGSB-176.1-92, Appendix A.

7 SAMPLE PROCESSING

7.1 Dissection of Whole Leaf Tobacco

- 7.1.1** Place a single whole leaf of tobacco onto a cutting board.
- 7.1.2** Dissect the leaf by first cutting the top 1/3 of the leaf (tip), and then remove the lamina from the midrib by cutting along both sides of the midrib from the remaining 2/3 of the leaf (see illustration below).



7.1.3 Discard the midrib portion of the dissected leaf.

7.1.4 Place a total of 75 ± 5 g of the dissected lamina (tip + lamina) into the Robot Coupe batch processor.

7.2 Maceration of Tobacco

7.2.1 Secure lid of Robot Coupe batch processor.

7.2.2 Set the processor to 3000 rpm.

7.2.3 Turn the unit on and process the sample for a total of six minutes at 3000 rpm.

7.3 Sieving of Processed Tobacco

7.3.1 Assemble the sieving apparatus from bottom to top as follows: Collection pan, 850 μ m sieve, 4 mm sieve.

7.3.2 Pour the processed tobacco sample from the batch processor container into the sieve.

7.3.3 Place the lid onto the sieve and shake in a back and forth motion for 30 seconds.

7.3.4 Remove lid from the sieve and discard any tobacco retained by the 4 mm sieve.

- 7.3.5 Pour the tobacco sample retained by the 850 μ m sieve onto an aluminum sheet.
- 7.3.6 Discard remaining tobacco from the sieve.
- 7.3.7 The processed tobacco should appear similar to the following:



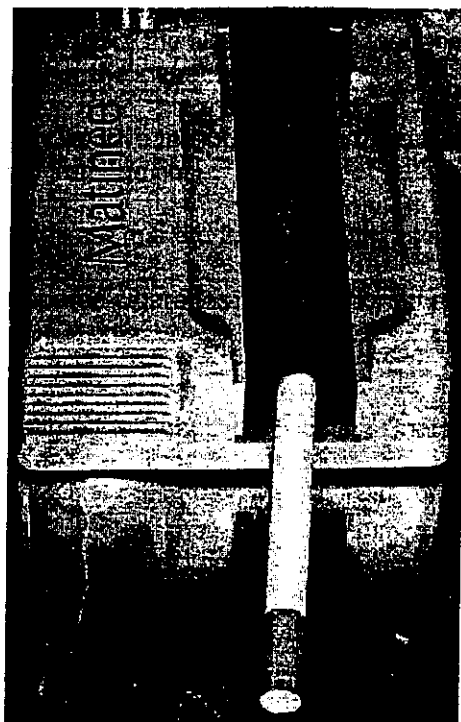
- 7.3.8 The above shows a comparison of processed leaf tobacco (bottom) to normal fine cut tobacco (top).

7.4 Conditioning of Tobacco

- 7.4.1 The tobacco retained by the 850 μ m sieve is spread in a monolayer (2 – 4 mm thick), onto aluminum sheets.
- 7.4.2 The tray is placed into a controlled environment room (CER) at $22 \pm 1^\circ\text{C}$ and $60 \pm 2\%$ relative humidity (RH) for 48 hours to condition as specified in T-115.

7.5 Preparation of Cigarettes for Testing

- 7.5.1 Weigh 0.900 ± 0.01 g of conditioned tobacco onto weigh paper using a four decimal place balance.
- 7.5.2 Transfer the tobacco to the Matinee Filter Master such that there is an even distribution of tobacco in the trough.
- 7.5.3 Place the cigarette tube onto the Filter Master (see illustration below).



- 7.5.4 Close the top of the filter master to load the tobacco into the cigarette tube as per the instructions.
- 7.5.5 Carefully remove the cigarette from the filter master as to not lose tobacco from the tip.
- 7.5.6 Cigarettes are marked for butt length as specified in T-115.
- 7.5.7 The prepared cigarettes are conditioned as specified in T-115.

8 REFERENCES

- 8.1 Protocol to Measure the Quantity of Nicotine Contained in Smokeless Tobacco Products Manufactured, Imported, or Packaged in the United States, Federal Registrar, Vol. 62, No. 85, Friday, May 2, 1997. p. 24115 – 24117.

SCHEDULE D

CIGARETTE BRANDS

Company	Brand			
Imperial Tobacco Limited	Players	Light	Regular Size	Filter Tip
Imperial Tobacco Limited	DuMaurier		King Size	Filter Tip
Imperial Tobacco Limited	Players		Regular Size	Filter Tip
Imperial Tobacco Limited	Players	Light	King Size	Filter Tip
Imperial Tobacco Limited	Players	Extra Light	Regular Size	Filter Tip
JTI Macdonald Corp.	Export A	Light	Regular Size	Filter Tip
Imperial Tobacco Limited	DuMaurier	Light	King Size	Filter Tip
Imperial Tobacco Limited	DuMaurier		Regular Size	Filter Tip
Imperial Tobacco Limited	Matinee	Extra Mild	King Size	Filter Tip
Rothmans, Benson & Hedges Inc.	Rothmans		King Size	Filter Tip
Imperial Tobacco Limited	Players	Extra Light	King Size	Filter Tip
JTI - Macdonald Corp.	Export A	(Full Flavour)	Regular Size	Filter Tip
Imperial Tobacco Limited	DuMaurier	Light	Regular Size	Filter Tip
Rothmans, Benson & Hedges Inc.	Craven	Menthol	King Size	Filter Tip
Rothmans, Benson & Hedges Inc.	Rothmans	Special Mild	King Size	Filter Tip
Imperial Tobacco Limited	DuMaurier	Extra Light	King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Number 7		King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Craven A		King Size	Filter Tip
JTI - Macdonald Corp.	Export A	Extra Light	Regular Size	Filter Tip
JTI - Macdonald Corp	Export A	Medium	Regular Size	Filter Tip
Imperial Tobacco Limited	DuMaurier	Ultra Light	King Size	Filter Tip
JTI - Macdonald Corp	Export A	Ultra Light	Regular Size	Filter Tip
Rothmans, Benson & Hedges Inc	Benson & Hedges 100's	Deluxe Ultra Light	(Premium Size)	Filter Tip
Imperial Tobacco Limited	Matinee	Slims 100		Filter Tip
Imperial Tobacco Limited	DuMaurier	Extra Light	Regular Size	Filter Tip
Imperial Tobacco Limited	Players	Light Smooth	Regular Size	Filter Tip
Imperial Tobacco Limited	Matinee	Extra Mild	Regular Size	Filter Tip
Imperial Tobacco Limited	DuMaurier	Ultra Light	Regular Size	Filter Tip
Rothmans, Benson & Hedges Inc	Benson & Hedges 100's	Light	(Premium Size)	Filter Tip
Rothmans, Benson & Hedges Inc	Viscount	Extra Mild 100's		Filter Tip
JTI - Macdonald Corp	Export A	Light	King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Viscount 1	Ultra Mild	King Size	Filter Tip
Imperial Tobacco Limited	Players	Medium	Regular Size	Filter Tip
JTI - Macdonald Corp	Export A	Ultra Light	King Size	Filter Tip
Imperial Tobacco Limited	Medallion		King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Craven A	Ultra Mild	King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Rothmans	Ultra Light	King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Craven A		Regular Size	Filter Tip
Imperial Tobacco Limited	Players	Premier	King Size	Filter Tip
Rothmans, Benson & Hedges Inc	Craven A	Ultra Light	Regular Size	Filter Tip
JTI - Macdonald Corp	Export A	Ultra Light	King Size	Filter Tip

