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Date: December 31, 1999
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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 digestor 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 H_2SO_4.
6.3 Concentrated HNO_3 .
6.4 Type I water as per ASTM D1193.
6.5 Potassium Permanganate.
6.6 H_2O_2 (30-32 %).
6.7 Stannous Chloride.
6.8 Hydroxylamine Hydrochloride.
6.9 Atomic Absorption Reference Standards - Mercury standard solution at 1000µg/mL in 10 % HNO_3.

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 µg/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 µg/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 µg/mL.
Secondary Stock = 100 µL of Primary Stock to 100 mL = 1 µg/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 digestor, 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): Analyte [ng/cigarette] = (Analytical result [ng/mL] X 100mL X Additional Dilution factor) / 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.