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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

TPM(mg/cigarette) = [Pad Weight after(g)–Pad Weight before(g)] X 1000(mg/g)/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)*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.