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 µL.
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 µm 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 D_5 - Pyridine (MSD Isotopes or equivalent) – purity 98 % or greater.
6.5 D_7 - 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 µm PTFE 25 mm or equivalent.

7 PREPARATION OFGLASSWARE

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 µL of each stock solution into a 50 mL volumetric flask, making to the mark with methanol and mixing well. [Concentrations: approximately 20 and 2 µg/mL, respectively].

9.4 A stock solution of D_5-pyridine is prepared by accurately weighing 100 mg of D_5-pyridine into a 10 mL volumetric flask, filling the flask to the mark with methanol and mixing well.

9.5 A stock solution of D_7-quinoline is prepared by accurately weighing 25 mg of D_7-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.

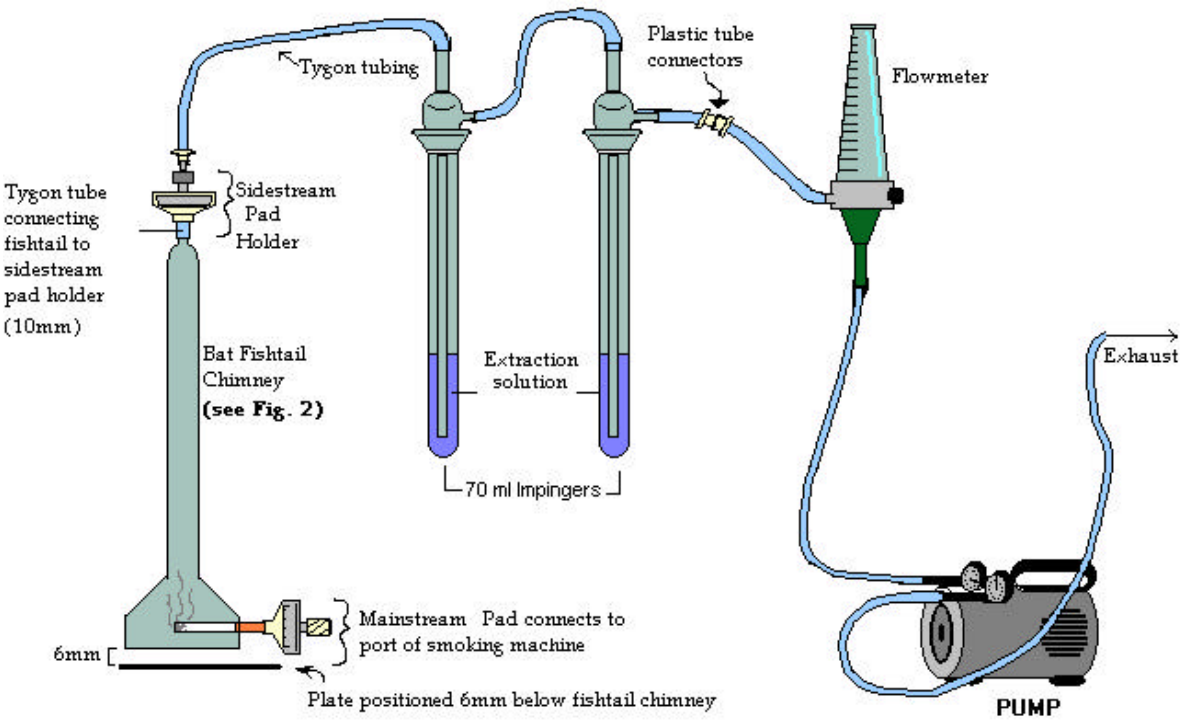

FIGURE 1b: SIDESTREAM APPARATUS USING TWO IMPINGERS

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 µL 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 °C.
14.2.1.2	Column temperature:	70 °C for two minutes. 3 °C per minute to 150 °C 20 °C per minute to 250 °C hold three minutes.
14.2.1.3	Column pressure:	12 psi.
14.2.1.4	Transfer line temperature:	240 °C.
14.2.1.5	Manifold temperature:	240 °C.

14.2.2 One 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).

14.2.3 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:

$$\text{Analyte (ug/cigarette)} = \frac{\text{Conc. of Analyte in Sample (μg/mL)} \times \text{Volume (mL)}}{\text{\# of cigarettes}}$$

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 50mL 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 °C.

15.3.2 Calibration standards are stable for at least one week if kept at –20 °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 °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.

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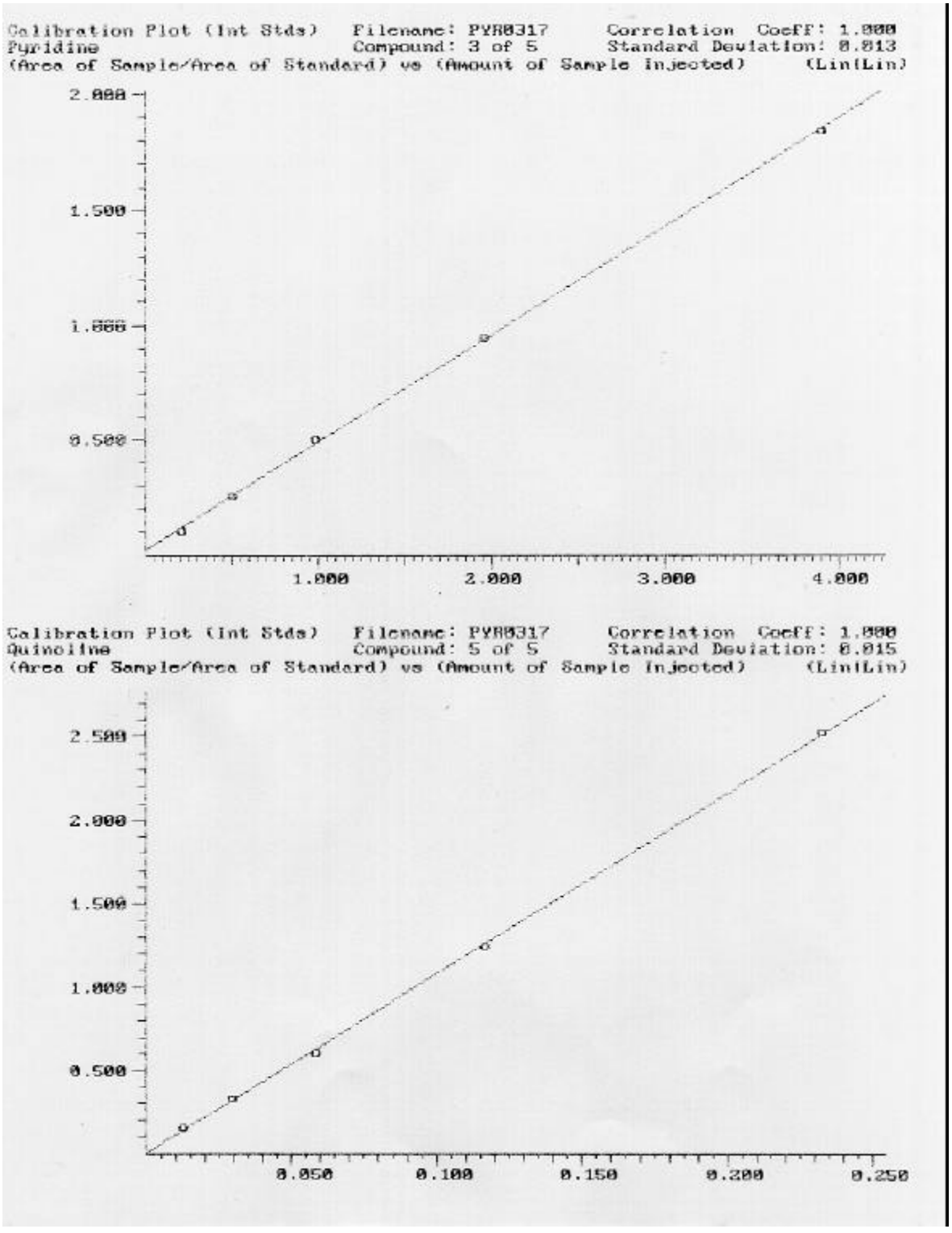
17.5 Proctor, C.J., Martin, C., Beven, J.L., and Dymond H.F., 1988. Evaluation of an Apparatus Designed for the Collection of Sidestream Tobacco Smoke, *Analyst 113:* p. 1509-1513.

APPENDIX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(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin|Lin)
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1.500
1.000
0.500
1.000
2.000
3.000
4.000
Calibration Plot (Int Stds) Filename: PYR0317 Correlation CoeFF: 1.000
Quinoline Compound: 5 of 5 Standard Deviation: 0.015
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0.200
0.250