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Date: December 31, 1999
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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 540n m 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 µL 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 (μg/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).

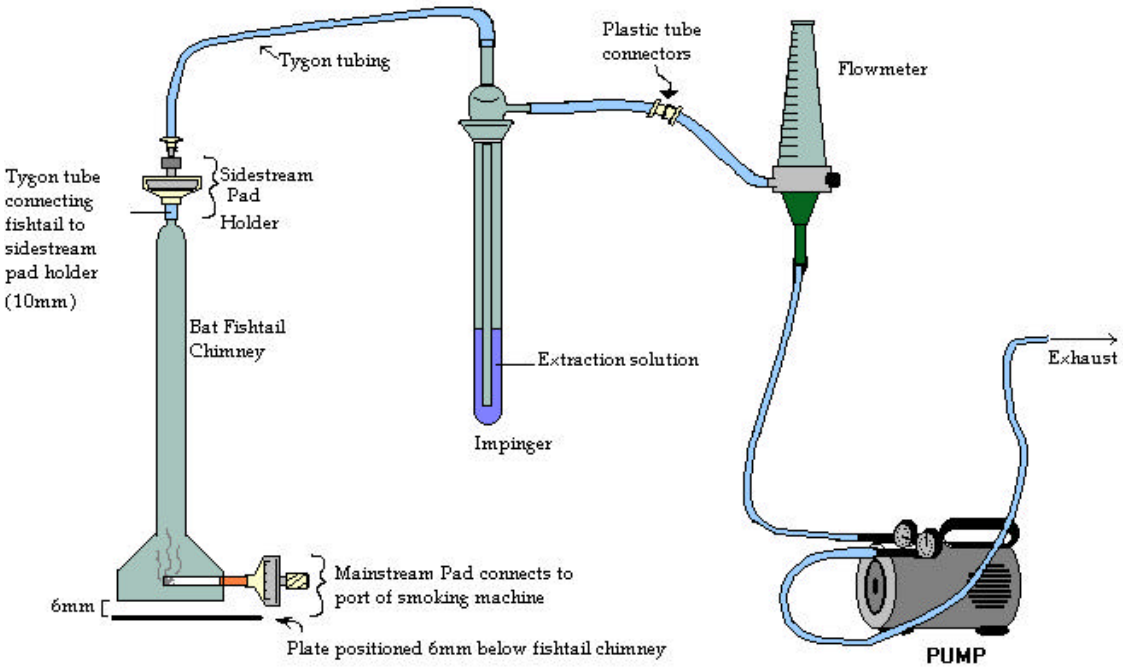

FIGURE 1a : SIDESTREAM APPARATUS

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 (µg/mL) of HCN for each extract and calculate micrograms per cigarette of HCN in sidestream smoke:

HCN (µg/cigarette) = [amount (µg/mL) X 120 (mL)] / 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 5mL 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