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Date: December 31, 1999
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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 µm) or equivalent.

5.22.6 Disposable Guard Column: Lichrocart 4 X 4 mm, Lichrosphere RP 18e (5 μm) 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 (H_2SO_4).
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:

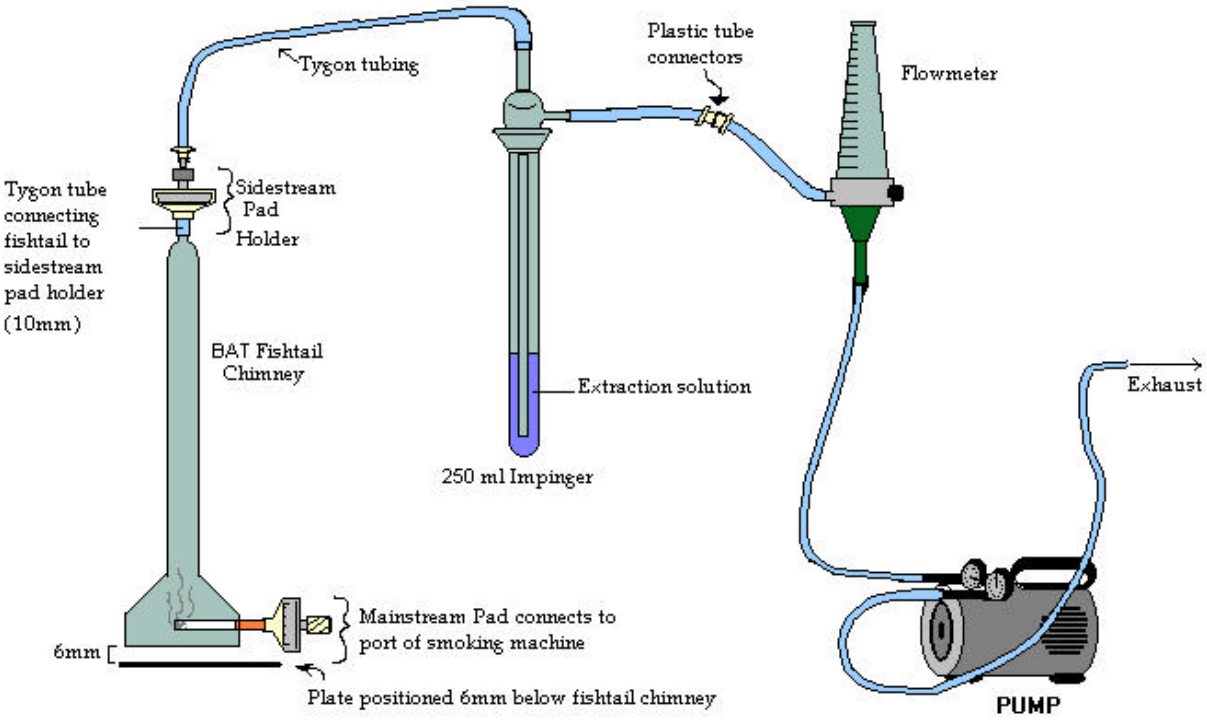

FIGURE 1a : SIDESTREAM APPARATUS

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

14.3.2 1 % Trizma base dilution solution into a 10 volumetric flask.

Add 4
the flask and mix well.

Transfer to two autosampler vials, cap and store until ready to

14.4 Laboratory Fortified Blank (LFB)

Add one blank conditioned pad to a clean 250
39 mL of fresh DNPH solution plus 1
Shake 30 minutes on wrist action shaker. Add an additional 100 mL

14.4.2 Pipette 6 mL of the Trizma base dilution solution into a 10 mL

14.4.3 Add 4 mL of the filtered, mixed DNPH/Spiking solution (section 14.4.1) mL volumetric flask.

Cap the volumetric flask and mix well.

14.4.5 analyze.

Laboratory Fortified Matrix (LFM)

After shaking the samples, prepare an LFM using a control brand with

14.5.2 Pipette 5 mL of the Trizma base dilution solution into a 10 mL

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

14.5.4 mL of filtered DNPH/smoke extract solution from a control brand to the volumetric flask.

14.5.5 analyze.

14.5.6 Compare to results of the same control sample used for preparing the LFM solution.

14.6

Chromatography

14.6.1 Column Temperature: 30 °C.

14.6.2 Injection volume: 20 µ

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	1.5mL/minute		
Time (minutes)	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]

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

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	Formula Wt	eight	Purity	Volume	Stock	Vol (ml)	Dilute to	Stock
	ıe		(mg)	(%)	(mL)	[µg/mL]	Primary Stock	Vol (mL)	[µgmL]
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	**[µgmL]**	**[µgmL]**	**[µgmL]**	**[µgmL]**	**[µgmL]**	**[µgmL]**	**[µgmL]**
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
Croton-aldehyde	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 [mgmL]	Volume (mL)	Dilute to Vol (mL)	Spike [µgmL]	as Analyzed [µgmL]
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

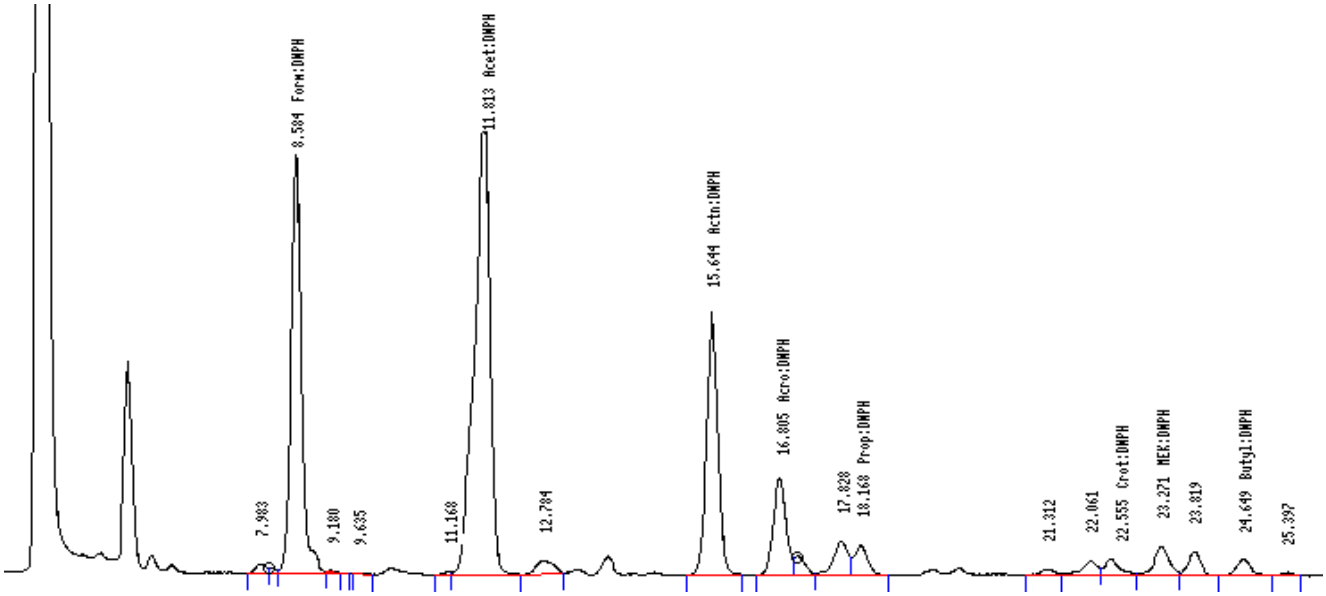

Figure 2: Analytical Chromatogram of Calibration Standard