

CORESTA RECOMMENDED METHOD N° 74

DETERMINATION OF SELECTED CARBONYLS IN MAINSTREAM CIGARETTE SMOKE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

(August 2011)

0. INTRODUCTION

At the outset of this work, discussions in the CORESTA Special Analytes Sub-Group determined that most laboratories used a method involving derivatisation of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) because they considered it most suitable and so this was chosen as the basis of the recommended method. The method involved smoke collection in impinger traps, derivatisation of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) using reversed phase high performance liquid chromatography for separation and detection by Ultra Violet or Diode Array.

Initial joint experiments and on-going discussions addressed some methodological aspects that needed to be considered before moving to a recommended method. The recommended method was produced through a final collaborative experiment involving 15 laboratories from 11 countries. The method includes notes to inform other laboratories that might wish to adopt it about some of the main features that need to be well controlled to provide data as robust and consistent as the repeatability and reproducibility data provided. Statistical evaluations were made according to ISO recommendations and are included.

1. FIELD OF APPLICATION

This method is applicable to the quantification of carbonyls (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and n-butyraldehyde) as their 2,4-dinitrophenylhydrazones in mainstream cigarette smoke by using reversed phase high performance liquid chromatography with UV or DAD detection (HPLC-UV/DAD).

2. REFERENCES

- 2.1.** *ISO 3308: 2009* - Routine analytical cigarette-smoking machine – Definitions and standard conditions.
- 2.2.** *ISO 8243: 2006* - Cigarettes – Sampling
- 2.3.** *ISO 3402: 1999* - Tobacco and tobacco products – Atmosphere for conditioning and testing
- 2.4.** *ISO 4387: 2008* - Cigarettes – Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine

3. METHOD SUMMARY

- 3.1.** Cigarettes are smoked on a standard smoking machine that has been fitted with impingers.

- 3.2. The unfiltered mainstream tobacco smoke is scrubbed of volatile carbonyls by passing each puff through an impinger device containing an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile.
- 3.3. An aliquot of the reacted DNPH-smoke extract is then syringe-filtered and diluted with 1% Trizma™ base in aqueous acetonitrile.
- 3.4. The samples are subjected to reverse phase high performance liquid chromatography (HPLC) and quantified via ultra violet detection.

4. APPARATUS AND EQUIPMENT

Normal laboratory apparatus and equipment is needed and in particular following items:

- Equipment needed to perform conditioning of tobacco products
- Equipment needed to perform marking for butt length
- Equipment needed to perform smoking of tobacco products complying with ISO 3308: 2009
- Impingers for trapping mainstream smoke
- Erlenmeyer flasks (150 mL) with ground glass stoppers (or equivalent for combining impinger solutions)
- Tubing (e.g. Nalgene) 1/4" ID X 3/8" OD

The necessary general laboratory equipment for the preparation of samples, standards, and reagents. Examples:

- Analytical balance, capable of measuring to four decimal places
- Volumetric flasks 10 mL, 25 mL, 200 mL, 1 L, and 2 L(amber)
- Glass micropipettes - assorted volumes (50, 100, 150, 300, 400, 500, 800, 1000, and 2000 µL)
- Glass transfer pipettes - 1, 2, 5, 6, 7, 8, and 20 mL
- Glass graduated measuring cylinders 25 mL, 50 mL, and 100 mL
- Dispenser (35 mL for trapping solutions)
- Hot Plate/Stirrer

High Performance Liquid Chromatography System consisting of:

- Tertiary gradient pump
- Auto-sampler with appropriate sampling loop
- UV Detector (or equivalent)
- Data Collection System
- Column: 250 X 4 mm, 100 Å, Reversed Phase (RP) 18e (5 µm) or equivalent
- Disposable Guard Column: 4 X 4 mm RP 18e (5 µm) or equivalent
- Vacuum filter
- Amber bottles 1 L and 4 L
- Dessicator
- Rotovap

5. REAGENTS AND SUPPLIES

- Acetonitrile (MeCN) – HPLC Grade
- Isopropanol (IPA) – HPLC Grade
- Ethyl Acetate – HPLC Grade
- Tetrahydrofuran (THF) – HPLC Grade
- Ethanol – HPLC Grade
- Phosphoric acid (85 %)
- Deionised water
- Formaldehyde-DNPH (min. 99 %)
- Acetaldehyde-DNPH (min. 99 %)
- Acetone-DNPH (min. 99 %)
- Acrolein-DNPH (min. 99 %)
- Propionaldehyde-DNPH (min. 98 %)
- Crotonaldehyde-DNPH (min. 99 %)
- 2-Butanone-DNPH (min. 98 %); methyl ethyl ketone-DNPH derivative
- n-Butyraldehyde-DNPH (min. 99 %)
- Trizma Base (Tris-(hydroxymethyl)-aminomethane; ACS Reag. Grade)
- 2,4-dinitrophenylhydrazine (2,4 DNPH)
- Syringe filter - 0.45 µm PVDF or equivalent
- Disposable syringes – 5 mL
- Disposable glass Pasteur pipettes
- Rubber bulbs
- Autosampler Vials, caps and Teflon faced septa
- Helium (UHP) – if necessary for sparging of HPLC system mobile phase or equivalent degassing system

Note: All reagents shall be, at the least, recognized as analytical reagent grade in quality.

Warning notice: The solvents and chemicals to be used for this method are classified as toxic, highly toxic, harmful, carcinogenic, mutagenic, sensitising, teratogenic, irritant, corrosive, easily flammable and dangerous for the environment. The instructions specified in the individual material safety data sheets concerning safe handling; storage and waste disposal as well as protective equipment must be followed.

6. PREPARATION OF GLASSWARE

Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

Note: It is extremely important that all possible sources of contamination are removed from the work area e.g. acetone solvent wash bottles.

7. PREPARATION OF SOLUTIONS

7.1. Preparation of DNPH Solution (*using Phosphoric Acid*)

- About 150 mL of water is added to a 200 mL volumetric flask and then 28 mL of 85 % phosphoric acid is also carefully added. The solution is made up to volume with deionised water.

- Weigh 6.792 g (24.0 mmol) of commercially available 2,4 dinitrophenylhydrazine (ca. 30% water) into a 2 L amber volumetric flask and add 1 L of fresh acetonitrile. Dissolve DNPH by alternating: gently swirling and warming the flask. Make sure there are no crystals remaining before proceeding.

Warning! Do not sonicate - precipitation of DNPH may occur.

Note: If using re-crystallized 2,4 DNPH, weigh 4.755 g to achieve the same molarity. (see Appendix 2 for preparation)

- After the DNPH is dissolved, add 58 mL of the diluted phosphoric acid solution with gentle mixing. The solution will turn yellow at this point. Dilute to volume with deionised water. The solution will turn to a bright orange upon addition of the water.

Note: The addition of water will cool the solution and may promote the precipitation of the DNPH. Add the water slowly. Gentle heating and stirring may be required to maintain the solution at room temperature and prevent the precipitation of DNPH. If crystals appear -
Do not sonicate.

- 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.

Note: There might also other mineral acids being suitable for this purpose. However, only perchloric acid was investigated in a joint experiment and no differences were detected in comparison to phosphoric acid.

7.2. Preparation of TrizmaTM Base Dilution Solution (80:20, MeCN:1 % aqueous TrizmaTM)

- Dissolve 2.00 g of Trizma Base in 200 mL of distilled deionized water (Type I water) in a 1 L volumetric flask. Dilute to volume with acetonitrile.
- Store in a 1 L amber bottle with Teflon-lined cap or equivalent at room temperature. This solution should remain stable for several weeks under these conditions.

8. PREPARATION OF STANDARDS

8.1. HPLC Calibration Standards and Working Solutions

- The calibration should cover the concentrations of interest. The following calibration steps were found to be suitable for this purpose.

8.1.1. Primary (1°) Carbonyl Standards

- Weigh the hydrazones in the amounts described in example found in **Appendix 1a**. Put into individual 25 mL volumetric flasks and dissolve in acetonitrile. The concentrations of the free aldehydes are recorded in µg/mL.

Note: When properly stored (sealed and refrigerated at 4 °C), solutions are stable for six months up to one year.

8.1.2. Secondary (2°) Carbonyl Standards

- Pipette predetermined volumes of each primary hydrazone standard into a single 25 mL volumetric flask and dilute up to the mark with acetonitrile.

Note: Seal and refrigerate at 4 °C. Prepare new working standards every 20 days. See Appendix 1a.

8.2. Carbonyl Working Standards

- Take appropriate volumes (0.050 to 10 mL) of the 2° carbonyl standard and dilute to 10 mL with acetonitrile to provide calibration standards with approximate carbonyl concentrations in the ranges noted in **Appendix 1b**.
- Transfer to auto-sampler vials
- New carbonyl calibration standards should be prepared every 20 days.

9. SAMPLING

Sampling is done in accordance with ISO 8243: 2006.

10. TOBACCO PRODUCT PREPARATION

Conditioning of the cigarettes is done in accordance with ISO 3402: 1999.

11. SMOKING MACHINE PREPARATION

11.1. Smoking Machine Setup

- A routine analytical cigarette-smoking machine complying with the requirements of 3308: 2009 is required with the following modifications as detailed below:

Note: It is important to ensure that the mainstream tobacco smoke is characteristic of the test sample before proceeding with the analysis. Since the mainstream total particulate matter (TPM) is not filtered, no filter pad is present and therefore puff count information must be used to characterize the smoke extract samples and monitor the smoking process.

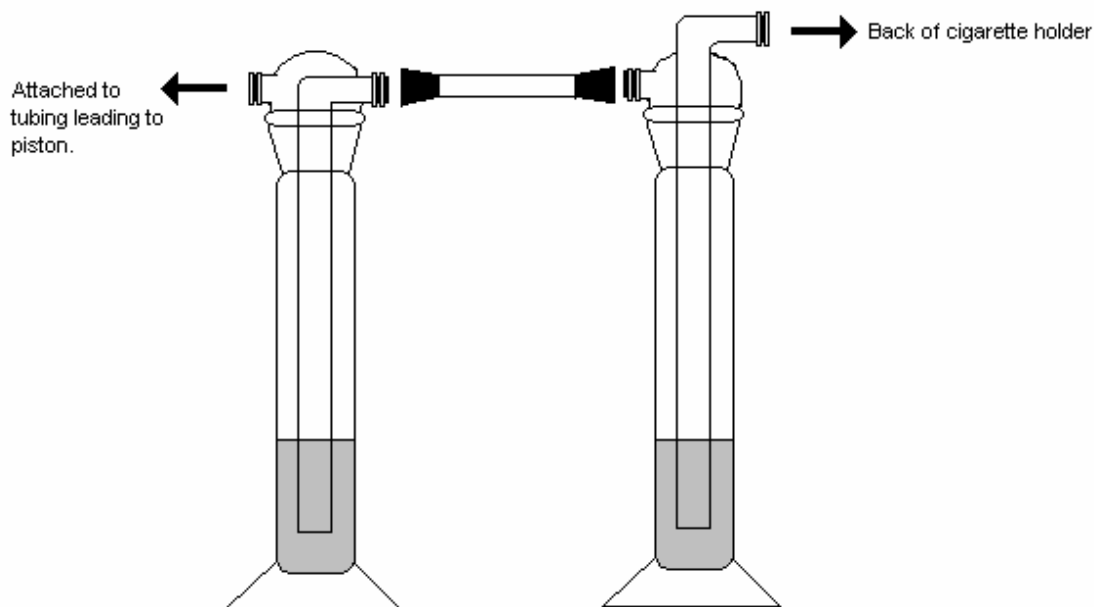
- Assemble the carbonyl mainstream apparatus on the smoking machine without using the filter pads and filter holders.
- Check and adjust the puff volume drawn by the smoking machine at all ports at the cigarette end of the port with the impingers and DNPH in line as described in ISO 4387: 2008.

Note: The same impinger can be used to adjust each port before smoking begins. Discard the DNPH solution after the puff volumes have been measured and adjusted.

- Add 35 mL of freshly prepared 2,4-DNPH solution to each impinger.

Figure 1 - Example of a suitable Trapping System

Two impingers containing 35 mL of DNPH solution in each



Note: It is recommended that the trapping efficiency be checked when validating this method. To check the trapping efficiency of the method, add an additional impinger and follow the method accordingly. Analyze each impinger individually for the compounds of interest. If no compounds are detected in the additional impinger then only the prescribed number of impingers is required to trap all the carbonyls effectively.

12. SAMPLE GENERATION – SMOKING OF CIGARETTES

The cigarettes are smoked according to ISO 4387: 2008 with the following modifications:

12.1. Linear Smoking

- Check the puff volume of each port and adjust accordingly.
- Typically two cigarettes are smoked per trap.

12.2. Rotary Smoking

- Check the puff volume of the rotary smoking machine and adjust accordingly.
- Typically five or ten cigarettes are smoked.

13. SAMPLE ANALYSIS

13.1. Preparation of Mainstream Smoke Extract Solution

- Rinse the tubing with the impinger solution by forcing the solution back up the impinger e.g. by using positive air pressure, then with negative air pressure until air is drawn back through the solution.
- Repeat this rinsing procedure at least three times for each impinger to dissolve any smoke condensate in the gas transfer lines.
- Allow the DNPH smoke extract solution to sit for five to thirty minutes before continuing with sample preparation.
- Pipette 6 mL of 1 % Trizma™ base solution into a 10 mL volumetric flask.
- Add 4 mL of syringe-filtered DNPH smoke extract to the volumetric flask.
- Mix the volumetric flask well. Transfer a portion of this solution to an autosampler vial.
- Cap the vials with Teflon faced septa and store at room temperature until analyzed.
- Repeat above steps for each smoke extract sample.

13.2. Reversed phase high performance liquid chromatography

13.2.1. Chromatographic Conditions (Example)

- Column Temperature: 30 °C.
- Auto-sampler Tray Temperature: room temperature.
- Injection volume: 20 µL.
- UV or DAD detection at 365 nm.

Note: These settings are detector-dependent and may have to be modified in order to achieve a linear response over the range of concentrations for the analyte of interest.

13.2.2. 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).

Note: Adjustments to the mobile phase may be required depending upon column differences or resolution of the analytes.

- Sample Wash: Solvent A.

13.2.3. Mobile Phase: Gradient

- Flow rate: 1.5 mL/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			
Action	100 % A	0 % B	0 % C

- Equilibrate 10 minutes.

Note: Adjustments to the gradient may be required, depending on instrument and column conditions as well as the resolution of the analyte peak.

- Inject 20 μ L of each sample onto the HPLC column and analyze as per the chromatographic conditions listed above.

Note: The choice of chromatographic conditions may be different for different instrument configurations and columns applied for separation.

- Elution pattern should be similar to the example chromatograms shown in Figure 2.

13.3. Calculations

13.3.1. Calibration Curve:

- Twenty μ L of each calibration standard is injected onto the HPLC column and analyzed as per the chromatographic conditions listed above.

13.3.2. Determination of Response Factor

- A calibration curve for each individual carbonyl is prepared by plotting the concentration of the standards versus their respective peak areas.
- Response factors are calculated for each individual carbonyl compound from the calibration curves.

13.3.3. Sample Quantification

- The amount of the various carbonyl compounds in smoke samples is quantified by the external standard method. A typical chromatogram is shown in Figure 3. The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.
- Carbonyl concentrations are reported in (μ g/mL) by the chromatography software.
- Determination of Mainstream Carbonyl Deliveries in [μ g/cigarette]

e.g. **Carbonyl [$\mu\text{g}/\text{cigarette}$] = (Peak Area / Resp. Factor) X (DF / No. of Cigarettes)**

where:

- DF is the dilution factor = Impinger volume X (Final Volume / Aliquot Volume).
- The Resp. Factor shall be determined from the calibration curve.

Note: It was observed that under the conditions chosen for the derivatisation an isomerization of acetaldehyde hydrazone occurs. The resulting additional isomer can be separated by HPLC and elutes under the described separation conditions in front of the main isomer (Figure 3). For the calculation of acetaldehyde yield the area of both isomers should be calculated to obtain correct results.

14. REPEATABILITY AND REPRODUCIBILITY

These were determined from a major international study involving 15 laboratories and eight cigarette samples including the reference cigarettes Ky3R4F and Ky1R5F (both available from the University of Kentucky) and the CORESTA Monitor Test CM 6. The five commercial available cigarettes and the references cover a wide range of blends and constructions and were carried out in 2010. The statistical evaluation of this collaborative study was conducted according the ISO 5725 part 1 and part 2.

14.1. Outlier and Data Analysis for Selected Volatiles Collaborative Study

- Calculations were made according to ISO 5725-2: Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability (r) and reproducibility (R) of a standard measurement method.
- For this carbonyls study, ISO 5725-2 can be applied directly in determining estimates of r and R since the data was collected under repeatability and reproducibility conditions as defined by ISO 5725-1 (i.e. the participating laboratories all used the same method as opposed to their own internal method, thus satisfying the reproducibility condition).

14.2. Data Removal

Individual data points that were reported as non-numeric (i.e. below the LoQ) had to be removed prior to evaluation of numeric data for outliers.

14.3. Among-Laboratory Variability

- For evaluation of agreement among laboratories, the Grubbs' test was applied as per ISO 5725-2 section 7.3.4 to detect single or multiple outlying laboratories.
- If the Grubbs statistic was significant at the 5% level, the sample data from that laboratory(s) was removed.
- In case the Grubbs statistic for a single high outlier was significant for certain analytes, samples and laboratories the data were removed prior to the calculations of the general mean, repeatability and reproducibility variance.

14.4. Determination of Among-Laboratory Repeatability and Reproducibility Variance

- For each of the j test samples ($j = 1, \dots, 8$), the general mean (m_j) was determined as per ISO 5725-2 section 7.4.4 across the p participating laboratories whose data remained following the removal of outliers.
- Repeatability variance (sr_j) was determined as per ISO 5725-2 section 7.4.5.1 and reproducibility variance (sR_j) was determined as per ISO 5725-2 section 7.4.5.5.
- Repeatability (r) and Reproducibility (R) figures calculated for 95% confidence level were also indicated for the individual compounds and sample cigarettes.

14.5. Results

Calculated statistical data of the individual selected volatile compound are indicated in the tables below.

Formaldehyde [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sR_j	R
1	14	15,1	1,9	5,4	4,8	13,6
2	14	8,39	1,09	3,08	2,33	6,60
3	14	22,8	2,7	7,6	6,1	17,4
4	14	2,35	0,46	1,29	1,05	2,97
5	13	27,7	2,9	8,2	7,9	22,3
CM 6	14	42,7	4,3	12,1	10,2	28,7
Ky 1R5F	15	3,00	0,44	1,24	1,29	3,66
Ky 3R4F	15	18,8	1,7	4,9	4,6	13,0
% Reported	98,3					
% Removed	1,7					

Acetaldehyde [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sR_j	R
1	13	485	27	77	56	158
2	13	335	21	60	47	134
3	14	445	28	79	46	131
4	14	86,4	7,8	22,1	16,6	46,8
5	11	502	20	56	49	139
CM 6	13	651	27	77	55	156
Ky 1R5F	15	141	13	37	30	86
Ky 3R4F	15	538	28	79	63	177
% Reported	93,9					
% Removed	6,1					

Acetone [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	14	199	11	31	35	99
2	13	141	9	26	28	78
3	14	171	12	33	30	85
4	14	36,5	4,0	11,4	14,1	39,8
5	13	180	12	33	31	88
CM 6	15	251	17	48	37	104
Ky 1R5F	15	62,0	5,8	16,3	18,4	51,9
Ky 3R4F	15	206	12	35	35	99
% Reported	98,3					
% Removed	1,7					

Acrolein [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	14	42,0	2,6	7,3	8,5	24,1
2	14	29,9	2,5	7,0	6,4	18,0
3	14	39,6	3,0	8,4	7,1	20,1
4	14	6,18	0,77	2,19	1,60	4,53
5	13	44,4	2,8	7,8	9,0	25,5
CM 6	15	63,1	4,2	11,8	10,1	28,6
KR 1R5F	14	9,17	0,97	2,74	2,32	6,56
KR 3R4F	15	47,6	2,9	8,1	8,4	23,7
% Reported	98,3					
% Removed	1,7					

Propionaldehyde [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	13	36,2	2,4	6,7	5,1	14,4
2	13	26,9	1,7	4,7	3,9	11,1
3	13	33,2	2,5	7,2	4,3	12,2
4	14	6,68	0,73	2,06	1,76	4,99
5	11	36,9	1,9	5,3	3,7	10,4
CM 6	14	48,5	3,3	9,3	5,3	15,1
KR 1R5F	14	11,3	1,0	2,9	2,8	8,0
KR 3R4F	15	39,8	2,2	6,3	5,5	15,7
% Reported	93,0					
% Removed	7,0					

Crotonaldehyde [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	14	16,9	1,4	3,9	5,3	15,1
2	12	7,18	0,75	2,13	3,09	8,75
3	12	12,2	1,3	3,6	4,0	11,2
4	13	1,49	0,31,	0,89	0,73	2,07
5	13	11,1	1,1	3,2	4,8	13,5
CM 6	11	19,1	1,2	3,5	4,3	12,1
KR 1R5F	14	2,15	0,37	1,04	1,05	2,97
KR 3R4F	14	12,1	1,0	2,7	5,1	14,4
% Reported	91,0					
% Removed	9,0					

n-Butyraldehyde [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	14	23,4	2,0	5,8	3,8	10,8
2	13	18,4	1,5	4,3	2,4	6,9
3	13	24,1	2,5	5,7	3,2	9,2
4	14	4,4	0,5	1,42	0,91	2,58
5	12	23,3	1,5	4,3	4,1	11,6
CM 6	15	36,5	3,0	8,6	5,4	15,2
KR 1R5F	14	7,62	0,66	1,85	1,62	4,59
KR 3R4F	14	26,9	1,6	4,6	4,4	12,3
% Reported	93,7					
% Removed	6,3					

2-Butanone [$\mu\text{g}/\text{cigarette}$]						
Sample j	# laboratories	m_j	sr_j	r	sRj	R
1	14	45,9	3,4	9,6	10,4	29,5
2	13	31,9	2,2	6,2	8,1	23,0
3	14	40,5	3,3	9,3	9,6	27,0
4	14	7,16	0,91	2,58	2,30	6,51
5	14	37,5	4,0	11,3	8,6	24,4
CM 6	15	58,3	6,0	16,9	12,7	35,9
KR 1R5F	15	12,6	1,4	4,1	4,0	11,4
KR 3R4F	15	48,0	3,4	9,5	10,6	30,0
% Reported	99,1					
% Removed	0,9					

15. REPORT

15.1. Test results

- The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Confidence limits were calculated and expressed on the basis of the laboratory data before any rounding has taken place.
- Carbonyl yields in the mainstream smoke of cigarette in µg/cig were rounded to the nearest 0,1 µg

16. BIBLIOGRAPHY

- Health Canada Official Method T 104
- Houlgate, P.R., Dhingra, K.S., Nash, J.S., and Evans, W.H., 1989: Determination of Formaldehyde and Acetaldehyde in Mainstream Cigarette Smoke by high-performance Liquid Chromatography; *Analyst* 114, p. 355-360.
- 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.
- "Method - Determination of carbonyls in mainstream cigarette smoke." 31 Mar. 2008. British American Tobacco Group Research & Development. See <<http://www.bat-science.com/>>

Figure 2 - HPLC Chromatogram of a Typical Combined Carbonyl Calibration Standard using Column AQ RP C 18 5µm, 250mm x 4,6mm

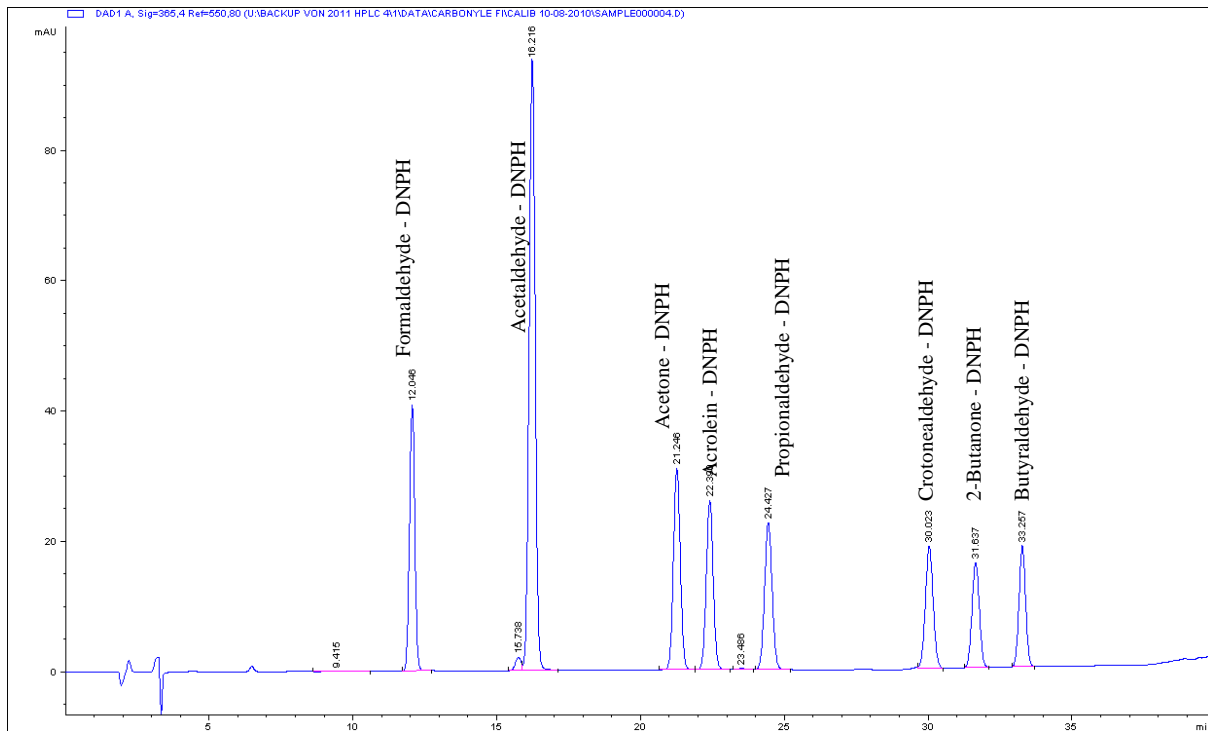
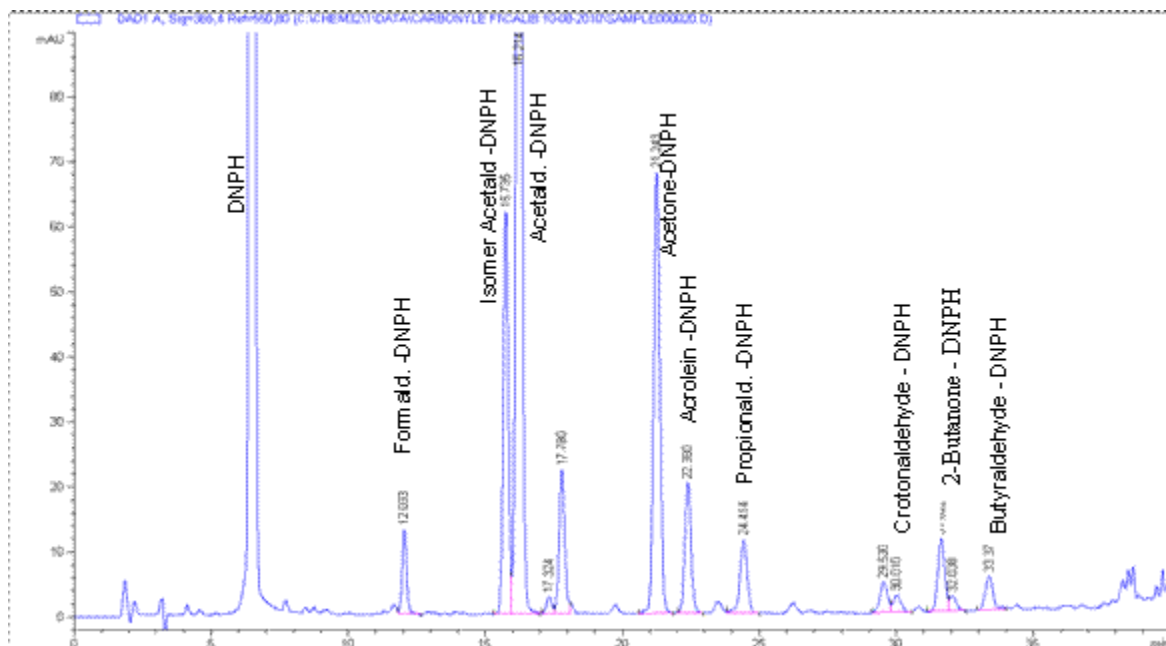


Figure 3 - HPLC Chromatogram of Carbonyls in DNPH Extract of Mainstream Tobacco Smoke of KR 3R4F using Column AQ RP C18, 5µm, 250mm x 4,6 mm



APPENDIX I – Example of Preparation of Calibration Standards

(a): Stock Standards

Carbonyl Hydrazone	Formula Wt Hydrazone	Primary Standards					Secondary Standard *		
		Formula Wt Carbonyl	Weight	Purity	Volume	Stock	Vol of 1° Stock (µL)	Dilute to Volume (mL)	Stock (µg/mL)
			(mg)	(%)	(mL)	(µg/mL)			
Formaldehyde	210,15	30,03	50,6	99,9	25	288,9	500	25	5,779
Acetaldehyde	224,18	44,05	33,9	99,9	25	266,2	1800	25	19,16
Acetone	238,21	58,08	40,6	99,9	25	395,6	800	25	12,66
Acrolein	236,18	56,06	17,7	99,9	25	167,9	850	25	5,708
Propionaldehyde	238,21	58,08	35,8	99,9	25	348,8	500	25	6,976
Crotonaldehyde	250,22	70,09	36,1	99,9	25	404,1	500	25	8,082
MEK	252,23	72,11	33,4	99,9	25	381,6	500	25	7,631
Butyraldehyde	252,23	72,11	42,8	99,9	25	489,0	1000	25	19,56

* In a single 25mL volumetric flask, made to volume with acetonitrile

(b): Carbonyl Working Standards **

Carbonyl	Volume of Secondary Standard (mL)							
	10,0	7,0	4,0	2,0	0,80	0,40	0,20	0,05
	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
Formaldehyde	5,779	4,045	2,311	1,156	0,4623	0,2311	0,1156	0,0289
Acetaldehyde	19,16	13,42	7,666	3,833	1,533	0,7666	0,3833	0,0958
Acetone	12,66	8,861	5,063	2,532	1,013	0,5063	0,2532	0,0633
Acrolein	5,708	3,996	2,283	1,142	0,4566	0,2283	0,1142	0,0285
Propionaldehyde	6,976	4,883	2,790	1,395	0,5581	0,2790	0,1395	0,0349
Crotonaldehyde	8,082	5,657	3,233	1,616	0,6465	0,3233	0,1616	0,0404
MEK	7,631	5,342	3,053	1,526	0,6105	0,3053	0,1526	0,0382
Butyraldehyde	19,56	13,69	7,823	3,912	1,565	0,7823	0,3912	0,0978

** In a single 10mL volumetric flask, made to volume with acetonitrile

APPENDIX II – Recrystallisation of 2,4-Dinitrophenylhydrazine

- Weigh approximately 35g of DNPH into a weigh boat. Transfer the DNPH into a clean 2L Erlenmeyer flask and add a stirrer.
- Add 750mL of anhydrous reagent grade ethanol to the flask. Place the flask on a hot plate equipped with a stirrer. Gently heat the solution with constant stirring.
- When the solution is warm, slowly add 1000mL of ethyl acetate. Continue to heat and stir (making sure not to boil) until all of the DNPH is completely dissolved. The solution should be clear and a very dark red.
- Vacuum filter the hot solution.
- Transfer the filtrate to a 2L Erlenmeyer flask.
- If crystallization does not start to occur, scratch the inside of the flask with a glass rod. Cover the Erlenmeyer flask with a watch glass and allow the solution to cool overnight in a cupboard.
- Vacuum filter the recrystallized DNPH.
- Transfer the crystals into a clean weigh boat that is labeled with the date of recrystallization and the Lot# of the DNPH. Weigh the recrystallized DNPH. Place the crystals in a dessicator to remove any moisture.
- The filtrate can be evaporated down with a rotovap and vacuum filtered again to recover more crystals.

Note: If making a larger quantity of DNPH (more than 2 days' worth), the DNPH must be wetted with 30% water. After adding the water, place in an airtight container and label it as having 30% water.