

CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

ANNEX A

Part 2 Method: Determination of eight carbonyl yields in cigarette smoke by High Performance Liquid Chromatography

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*Setting standards
in analytical science*

1 Scope of the method

- 1.1 This method is applicable to the extraction and determination of eight carbonyl compounds in mainstream tobacco smoke by High Performance Liquid Chromatography (HPLC).

2 Principle of the method

- 2.1 For each sample, two conditioned cigarettes are smoked on a 20 channel linear smoking machine. The mainstream smoke is collected (and reacted) in a scintered bubbler containing a solution of acidified 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile. After smoking the derivatised carbonyls are stabilised by the addition of pyridine. If appropriate, the solutions are diluted by 50% with acetonitrile to reduce the carbonyl concentration so that it is within the calibration range. The solutions are analysed by high performance liquid chromatography with diode array detection. Individual carbonyl concentrations are determined by an external standard calibration method. A blank is analysed with each batch of samples.

3 Apparatus

- 3.1 20 Channel linear Filtrona smoking machine and ancillary equipment
- 3.2 Artificial lips to hold cigarettes
- 3.3 Scintered bubblers (gas washing bottle – ca 75 mL, dreschel head, Grade 0 sinter) and connecting pieces (see Figure 1)
- 3.4 HPLC system (see Section 8)
- 3.5 60 mL screw top amber vials (or equivalent) – used for storing standards.
- 3.6 2 decimal place top pan balance
- 3.7 Five figure balance
- 3.8 2 mL capacity amber vials and teflon lined caps
- 3.9 Positive displacement pipettes (250 μ L and 50 μ L)
- 3.10 1000 mL mobile phase reservoirs

4 Reagents

Reagents are Analytical Grade or equivalent unless otherwise stated.

- 4.1 2,4 Dinitrophenylhydrazine
- 4.2 Acetonitrile (HPLC grade)
- 4.3 Perchloric acid (60%)
- 4.4 Water (Elgastat – UHP)
- 4.5 Pyridine
- 4.6 Tetrahydrofuran (HPLC grade)
- 4.7 Propan-2-ol (HPLC grade)

5 Standards

- 5.1 Formaldehyde (methanal) DNPH derivative, 99% purity
- 5.2 Acetaldehyde (ethanal) DNPH derivative, 99% purity
- 5.3 Acetone (propanone) DNPH derivative, 99% purity
- 5.4 Acrolein (propenal) DNPH derivative, 99% purity
- 5.5 Propionaldehyde (propanal) DNPH derivative, 99% purity
- 5.6 Crotonaldehyde (2-butenal) DNPH derivative, 99% purity
- 5.7 Butyraldehyde (butanal) DNPH derivative, 99% purity
- 5.8 Methyl Ethyl Ketone (2-butanone) DNPH derivative
- 5.9 The standards should be stored in a cool dry environment and have a manufacturers expiry date.

6 Preparation of solutions

- 6.1 **Perchloric acid solution (1.82 mol L⁻¹)** – Using a measuring cylinder, add 10 mL of perchloric acid (4.3) to a 50 mL volumetric flask half full of water (4.4). Make up to the mark with water, stopper and shake to mix. Store in a cupboard, the solution is stable for 4 weeks.
- 6.2 **Trapping (& derivatisation) solvent (5 g L⁻¹)** – Weigh out 5.00 g ± 0.10 g of DNPH (4.1) and quantitatively transfer to a 1000 mL volumetric flask containing approximately 700 mL of acetonitrile (4.2). Using a measuring cylinder, add 15 mL of perchloric acid solution (6.1) and swirl the contents of the flask until all the crystals have dissolved. Make up to the mark with acetonitrile. Store in an amber glass bottle. The solution is stable for six weeks but should be checked on a regular basis for contamination (see 7.15).
- 6.3 **Stock mixed carbonyl standard (nominal 600 - 200 µg mL⁻¹ derivative)** – Using a weighing boat, weigh out accurately (3.7) the following to within ± 3 mg of the target weight. Each material is transferred to a 50 mL volumetric flask (containing ca 25 mL of acetonitrile) and the weighing boat re-weighed to determine the amount of hydrazone.

Compound	Target Weight /mg	Percentage analyte (e.g. acetone)* /%
Formaldehyde 2,4 dinitrophenyl hydrazone (5.1)	20.00	14.29
Acetaldehyde 2,4 dinitrophenyl hydrazone (5.2)	30.00	19.65
Acetone 2,4 dinitrophenyl hydrazone (5.3)	20.00	24.38
Acrolein 2,4 dinitrophenyl hydrazone (5.4)	10.00	23.74
Propionaldehyde 2,4 dinitrophenyl hydrazone (5.5)	15.00	24.38
Crotonaldehyde 2,4 dinitrophenyl hydrazone (5.6)	10.00	28.01
Butyraldehyde 2,4 dinitrophenyl hydrazone (5.7)	20.00	28.59
Methyl ethyl ketone dinitrophenyl hydrazone (5.8)	10.00	28.59

* Calculated using molar masses

- 6.4 Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 3 months, stored in a freezer at -20 °C. NB The solution needs to warm up to room temperature before it can be used to prepare calibration solutions. NB Potentially eight calibration solutions may have to be prepared to cover the wide range of carbonyl concentrations from different cigarette brands. In practice five or six standards may be sufficient to prepare a calibration curve to encompass the range of concentrations in the samples.
- 6.5 **HPLC Calibration standard 1 (nominal 120 - 40 $\mu\text{g mL}^{-1}$ derivative)** – Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 5 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.6 **HPLC Calibration standard 2 (nominal 96 - 32 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 4 mL of acetonitrile (4.2). Using an automatic pipette, add 4 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.7 **HPLC Calibration standard 3 (nominal 72 - 24 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 3 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.8 **HPLC Calibration standard 4 (nominal 48 – 16 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 2 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.9 **HPLC Calibration standard 5 (nominal 24 - 8 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 1 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.10 **HPLC Calibration standard 6 (nominal 6 – 0.2 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 250 μL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.11 **HPLC Calibration standard 7 (nominal 2.4 – 0.8 $\mu\text{g mL}^{-1}$)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 100 μL of the stock carbonyl solution (6.3), stopper the

- flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.12 **HPLC Calibration standard 8 (nominal 0.6 – 0.2 µg mL⁻¹)** - Tare a balance with a stoppered 25 mL volumetric flask containing approximately 10 mL of acetonitrile (4.2). Using an automatic pipette, add 25 µL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.13 **QC solution** - Tare a balance with a stoppered 100 mL volumetric flask containing approximately 25 mL of acetonitrile (4.2). Using an automatic pipette, add 8 mL of the stock carbonyl solution (6.3), stopper the flask and reweigh. Make up to the mark with acetonitrile, stopper and shake to mix. Transfer to an appropriately labelled vial (3.5). The standard is stable for 6 weeks, stored in a freezer at -20 °C.
- 6.14 **Mobile Phase A.** Add 235.8 ± 0.5 g (300 mL) of acetonitrile (4.2), 590.0 ± 1.0 g of water (4.4), 88.9 ± 0.2 g (100 mL) of tetrahydrofuran (4.6) and 7.85 ± 0.05 g (10 mL) of propan-2-ol (4.7) to a mobile phase reservoir (3.10). Swirl to mix, place in an ultrasonic bath for a minimum of five minutes and then install in the HPLC system. NB The system removes dissolved gases in the mobile phase by a process of vacuum degassing.
- 6.15 **Mobile Phase B.** Add 510.9 ± 1 g (650 mL) of acetonitrile (4.2) and 350 ± 0.7 g of water (4.4) to a mobile phase reservoir (3.10). Swirl to mix, place in an ultrasonic bath for a minimum of five minutes and then install in the HPLC system.
- 6.16 **Mobile Phase C.** Add approximately 500 mL of acetonitrile (4.2) to a mobile phase reservoir (3.10) and install in the HPLC system.

7 Method

- 7.1 Cigarettes are conditioned¹ at a temperature of 22 ± 1°C and 60 ± 2 % relative humidity for a minimum of 48 hours but not exceeding 10 days.
- 7.2 Butt marking is to ISO butt length specifications². Filtered cigarettes are smoked to a measured butt length equal to either the tipping paper + 3 mm or filter length + 8 mm whichever is longer. The minimum butt length is 23 mm and this is also used for non filter brands. All smoking is conducted in an environment of temperature 22 ± 2°C and 60 ± 5 % relative humidity¹.
- 7.3 As far as practicable, ISO conditions³ for smoking cigarettes apply. The smoking machine puffing parameters are 35 ± 0.2 cm³ puff volume with 2.0 ± 0.05 second puff duration once every 60.0 ± 0.5 seconds. The smoking machine may have to be adjusted slightly to achieve a 35 cm³ puff volume.
- 7.4 A minimum of five determinations are performed for each brand. The smoking of the cigarette brands are randomised so that samples from the same brand are smoked on different days on different channels.
- 7.5 As a check that cigarettes have been smoked in accordance with ISO standard conditions, carbon monoxide concentration is determined and the yield compared with that normally achieved. Results for cigarettes that give significantly low or high CO yields (± 3 × standard deviation) will be discarded.

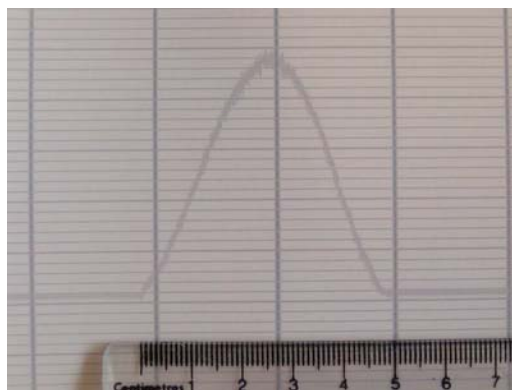
- 7.6 It is practicable to smoke ten samples in a batch. Additionally a blank determination should be included with every batch. The sample blank can be either the trapping solution (6.2) or prepared by puffing an unlit 1R4F cigarette through the trap assembly under identical conditions to those used for smoking the cigarettes.



Figure 1

- 7.7 For each sample, one bubbler is required. An “artificial lip” assembly is connected to the inlet port of the smoking machine. The bubbler assembly is connected to the rear of the smoking machine as shown in Figure 1. NB The distance between the bubbler assembly and the cigarette should be kept to a minimum. The bubbler design is shown in Figure 1. NB The distance between the inlet tube and base of the bubbler should be kept small, <1 cm.
- 7.8 Place the bottom section of the bubbler on a top pan balance (3.6) and tare. Using a measuring cylinder, add 40 mL of the trapping solution (6.2). Reweigh the bubbler to determine the weight of solution.
- 7.9 Assemble the traps as shown in Figure 1. The bubblers are connected to a channel of the smoking machine via Cambridge filter pad assemblies.
- 7.10 Two cigarettes are smoked using ISO specifications as far as practicable. It is not possible to get a perfect puff profile with a scintered bubbler. Figure 2 shows the puff profile that can be obtained with this set up. 2.5 cm is equivalent to 1 second duration.

Figure 2



7.11 After each cigarette has been smoked, a clearing puff is taken and two clearing puffs at the end of the smoking run.

Steps 7.12 to 7.16 need to be carried out immediately after smoking as some of the DNPH derivatives are unstable.

7.12 Disconnect the bubbler and shake gently to rinse the inside wall. Using, e.g., a pipette filler, apply pressure to the exhaust side of the bubbler so that the inside of the inlet tube is rinsed with the extract solution.

7.13 Using automatic pipettes (3.9), transfer 1000 μL ($4 \times 250 \mu\text{L}$) of each solution to appropriately labelled amber vials and then add 50 μL of pyridine.

7.14 For samples with high concentration of carbonyls (e.g. NFDPM yield is $>8 \text{ mg cig}^{-1}$), dilute the sample by 50%. Transfer 500 μL ($2 \times 250 \mu\text{L}$) of each solution to appropriately labelled amber vials, add 500 μL ($2 \times 250 \mu\text{L}$) of acetonitrile (4.2) and then 50 μL of pyridine.

7.15 Prepare sample blanks by adding 1000 μL of the trapping solution (6.2) and/or 500 μL ($2 \times 250 \mu\text{L}$) of the trapping solution followed by 500 μL ($2 \times 250 \mu\text{L}$) of acetonitrile (4.2) to labelled amber vials, then add 50 μL of pyridine.

7.16 Crimp the vials.

7.17 Set up a run on the HPLC to analyse (a) solvent blank, (b) HPLC calibration standards (6.5, 6.6, 6.7, 6.8, 6.9, 6.10, 6.11 & 6.12), (c) samples, (d) sample blanks, (e) QC solution (6.13) run after every six sample solutions to check calibration has not altered. Examples of a sample (IR4F) and standard chromatogram are given in Section 12.

7.18 Standards and solvent blank (4.2) are prepared for the HPLC by following step 7.13 in the method. The standards need to be allowed to warm up to room temperature before use.

8 Instrument conditions for analysis of DNPH derivatives by HPLC

8.1 HPLC gradient elution pump with diode array detector (DAD).

8.2 The instrument conditions are as follows

Column	5 μm C18, 250 mm \times 4.6 mm
Guard Column	2 \times C18 securiguard
Injection sample loop	20 μL
Column Oven Temperature	30 $^{\circ}\text{C}$
Sample Tray temperature	5 $^{\circ}\text{C}$
DAD settings	355 nm with bandwidth 4 nm
Flow rate	1.2 mL min^{-1}
Run time & interval time	42 min & 5 min

8.3 The HPLC mobile phase conditions for the run are as follows

Time (min)	A (%)	B (%)	C (%)	Gradient
0	100	0	0	
0 → 20	100 → 60	0 → 40		Linear
20 → 25	60	40	0	
25 → 35	60 → 0	40 → 100	0	Linear
35 → 37	0	100 → 0	0 → 100	Linear
37 → 42	0	0	100	
2 minutes	0 → 100	0	100 → 0	Linear
3 minutes (minimum)	100	0	0	

9 Validation data

9.1 *Accuracy:* The following table contains published data⁴ for carbonyl compounds yields from 1R4F.

	Yield/ µg cig ⁻¹
Formaldehyde	25
Acetaldehyde	752

9.2 It was found that one cigarette gave slightly higher results than for two cigarettes, but precision was significantly improved with two cigarettes. For the bench mark study, it was most appropriate to smoke 2 cigarettes to achieve satisfactory precision. The following tables gives results for 5 determinations of 1R4F and IR5F (within a run).

	Formaldehyde- 2,4DNPH	Acetaldehyde- 2,4DNPH	Acetone- 2,4DNPH	Acrolein- 2,4DNPH	Propionaldehyd e-2,4DNPH	Crotonaldehyde- 2,4DNPH	Methyl ethyl ketone-2,4DNPH	Butryaldehyde- 2,4DNPH
Blank DNPH			5.32*					
1R4F/1, 40ml Solvent, 2Cig	17.1	687.8	291.6	55.5	52.0	16.3	72.9	68.1
1R4F/2, 40ml Solvent, 2Cig	18.4	664.8	270.5	51.8	49.9	15.2	68.4	64.7
1R4F/3 40ml Solvent, 2Cig	17.1	738.1	310.0	58.6	54.2	17.3	69.0	72.1
1R4F/4, 40ml Solvent, 2Cig	16.2	752.2	309.0	58.6	56.4	16.5	77.3	66.0
1R4F/5, 40ml Solvent, 2Cig	18.5	725.7	305.8	60.0	53.4	16.7	76.6	72.6
Average	17.5	713.8	297.4	56.9	53.2	16.4	72.8	68.7
Standard Deviation	1.0	36.3	16.7	3.3	2.4	0.8	4.2	3.6
%CV	5.6	5.1	5.6	5.8	4.6	4.7	5.7	5.2

	Formaldehyde-2,4DNPH	Acetaldehyde-2,4DNPH	Acetone-2,4DNPH	Acrolein-2,4DNPH	Propionaldehyde-2,4DNPH	Crotonaldehyde-2,4DNPH	Methyl ethyl ketone-2,4DNPH	Butryaldehyde-2,4DNPH
Blank DNPH			5.32*					
1R5F/1, 40ml Solvent, 2Cig	2.8	185.4	84.5	13.4	13.8	2.9	18.2	16.3
1R5F/2, 40ml Solvent, 2Cig	2.6	175.9	80.5	12.6	13.0	2.6	16.7	15.2
1R5F/3, 40ml Solvent, 2Cig	2.8	144.9	67.3	10.4	10.8	2.1	13.8	12.8
1R5F/4, 40ml Solvent, 2Cig	2.8	191.2	86.9	13.3	14.3	2.8	18.5	16.2
1R5F/5, 40ml Solvent, 2Cig	3.5	204.0	94.3	15.1	15.2	3.3	20.2	17.4
Average	2.9	180.3	82.7	13.0	13.4	2.8	17.5	15.5
Standard Deviation	0.4	22.2	9.9	1.7	1.7	0.5	2.4	1.7
%CV	12.8	12.3	12.0	13.0	12.4	16.8	13.9	11.2

9.3 There is a balance between trapping efficiency and effect on puff profile. Validation data showed that 40 mL of trapping solution with a Grade 0 sinter gave acceptable trapping efficiencies (>90%) without having a major effect on the puff profile. The puff profile using this trap is shown in Figure 2

9.4 A check was carried out on the stability of a sample solution of 1R4F. All the carbonyl derivatives were stable for 48 hours. However significant peak interference was observed around the formaldehyde peak after 48 hours. Therefore all sample solutions are analysed within 24 hours of generation.

	Formaldehyde-2,4DNPH	Acetaldehyde-2,4DNPH	Acetone-2,4DNPH	Acrolein-2,4DNPH	Propionaldehyde-2,4DNPH	Crotonaldehyde-2,4DNPH	Methyl ethyl ketone-2,4DNPH	Butryaldehyde-2,4DNPH
1R4F/1, 40ml Solvent, 2Cig	17.1	687.8	291.6	55.5	52.0	16.3	72.9	68.1
1R4F/1, 40ml Solvent, 2Cig after 24 hours	17.9	702.9	300.1	56.3	53.1	16.2	75.5	69.5
1R4F/1, 40ml Solvent, 2Cig after 48hours	34.5	716.0	302.1	56.1	54.3	16.8	76.1	70.2

Peak interference

9.5 Normal laboratory QC procedures should be in place as summarised in the table below:

Aim	How achieved
To show smoking is to ISO conditions	<ul style="list-style-type: none"> Check puff volumes Check puff profiles Check CO yields (See paragraph 7.5) Establish correct butt mark lengths
To show standards are stable	<ul style="list-style-type: none"> Split one of the calibration standards between two 50 mL vials (e.g. Calibration solution 8). Use one solution and keep the other to act as a check
To show calibration is satisfactory	<ul style="list-style-type: none"> Print graph and “look at curve” - check for linearity Check R² (>0.99), intercept close to Zero/ Zero.
To show analytical instrument is calibrated and operating satisfactorily	<ul style="list-style-type: none"> Use certified standards which are in date Quality control solution Analysis of blank matrix

Aim	How achieved
To show standard laboratory equipment is functioning satisfactorily	<ul style="list-style-type: none"> • Balance – check weight • Temperature check of refrigerators and freezers • Pipettes – volume check • Conditioning cabinet – temperature and relative humidity • Smoking environment – temperature and relative humidity • Air flows – within specification

10 Results

10.1 Results should be reported as $\mu\text{g cig}^{-1}$

10.2 Mean, standard deviation and relative standard deviation will be determined for each brand of cigarettes and Dixon's outlier test will be performed (5% level).

11 Calculation

11.1 Using a spreadsheet package, determine the concentration of each carbonyl (e.g. acetone) in the stock standard using the following formula:

$$\text{Carbonyl conc. } (\mu\text{g mL}^{-1}) = \frac{\text{carbonyl DNPH wt. (g)} \times 1000000 \times \text{percentage analyte (\%)} \times \text{purity (\%)}^*}{\text{stock standard volume (mL)} \times 100 (\%) \times 100 (\%)}^*$$

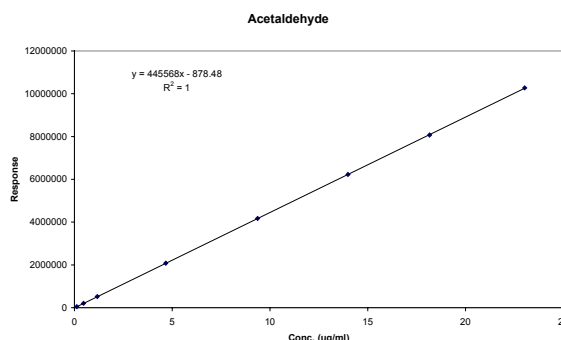
* If the purity of the standard is known then the carbonyl concentration is adjusted.

11.2 The concentration of each carbonyl in each calibration standard is calculated:

$$\text{Carbonyl conc. of calib. soln. } (\mu\text{g mL}^{-1}) = \frac{\text{carbonyl conc. of stock soln. } (\mu\text{g mL}^{-1}) \times \text{volume of stock soln. pipetted (mL)}}{\text{volume of calibration solution (mL)}}$$

11.3 Plot eight calibration graphs of concentration versus peak area for each carbonyl. NB the peak area of the two isomers are combined for acetaldehyde. Calculate the linear regression equation for each analyte ($y = mx + c$) without forcing the line through zero. Check the plots, coefficient of determination (r^2) and intercept before accepting the calibrations (see validation section for acceptance criteria). Figure 3 shows a typical example of a calibration curve for acetaldehyde (ethanal) – NB Concentrations are $\mu\text{g mL}^{-1}$ analyte

Figure 3



11.4 Using the spreadsheet and the sample peak areas, calculate the concentration of each analyte in the sample solutions including the blank determination.

11.5 Calculate the yield of each carbonyl per cigarette:

$$\text{Carbonyl yield per cigarette } (\mu\text{g cig}^{-1}) = \frac{\text{Carbonyl sample soln. conc. } (\mu\text{g mL}^{-1}) \times \text{sample solution weight (g)}}{0.786 (\text{g mL}^{-1})^* \times \text{number of cigarettes (cig)}}$$

(* Density of acetonitrile = 0.786 - Aldrich)

11.6 If appropriate, multiply the yield by 2 to allow for the 50% dilution in paragraph 7.14.

12 Appendix

Figure 4 Example of standard chromatogram

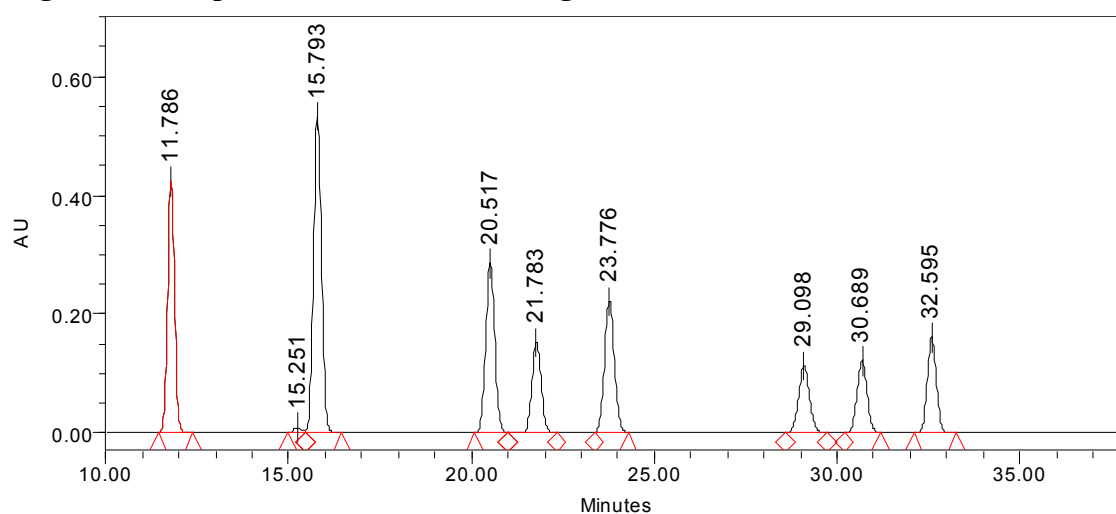


Figure 5 Example of Blank DNPH chromatogram

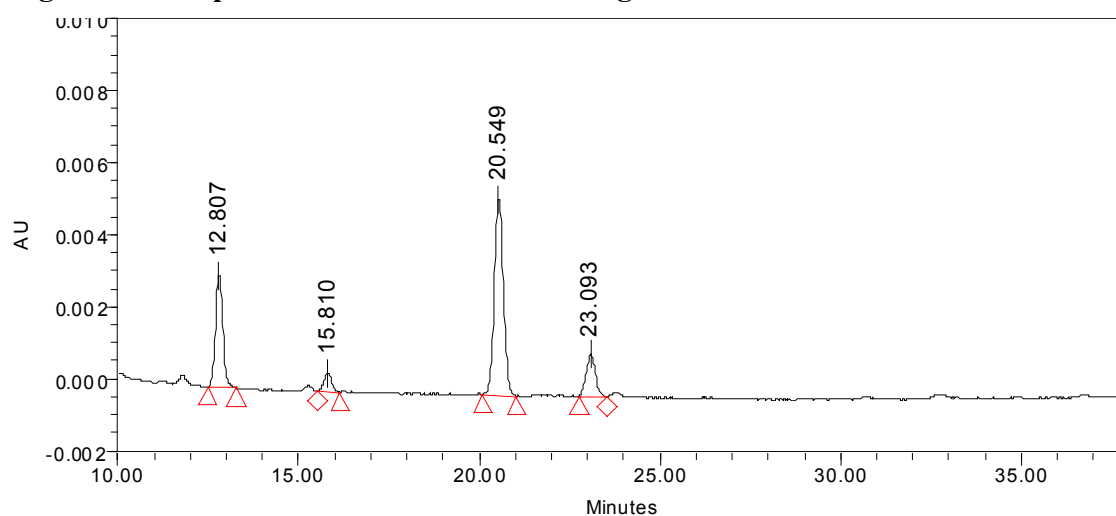
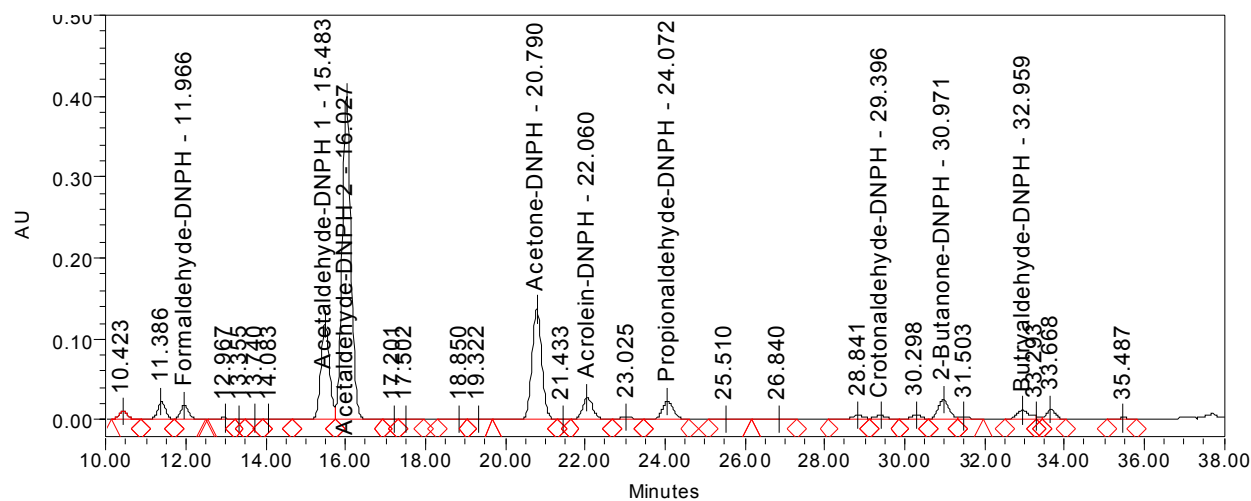


Figure 6 Example of 1R4F Chromatogram



¹ ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing

² ISO :4387: 2000 - Methods for chemical analysis of tobacco and tobacco products – Part 14: Determination of total and nicotine- free dry particulate matter using a routine analytical smoking machine

³ ISO 3308:2000 – Routine analytical cigarette smoking machine – Part 1: Definitions and standard conditions

⁴ Evans W H et al, Determination of formaldehyde and acetaldehyde in mainstream cigarette smoke by HPLC, *Analyst*, 1989, **114**, 355-360