

CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

ANNEX B

Part 2 Validation Data : Determination of eight carbonyls yields in cigarette smoke by High Performance Liquid Chromatography

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*Setting standards
in analytical science*

Validation data for Carbonyls

In this document the findings from the validation exercise are summarised.

1. Trapping system

A liquid trap has been used consisting of a Gas washing bottle – 40 mL solvent (capacity ca 70 mL), Dreschel head, Grade 0 sinter. The trap was chosen as a result of the findings from our validation exercises. The exercise showed that this was the best compromise in terms of effect on puff profile versus trapping efficiency out of the possibilities examined. There is some break through (<10%) and some effect on puff profile with this set up.

NB, the alternatives looked at included using less solvent, two traps in series and a simple impinger type bubbler.

2. Number of cigarettes

The validation exercise might have indicated that there was a slightly higher concentration of carbonyls found when smoking one cigarette compared to two cigarettes. However, the results were significantly more variable (carbon monoxide and formaldehyde yields) when smoking one cigarette. Therefore, in the method, two cigarettes were smoked to obtain better precision.

3. Stability

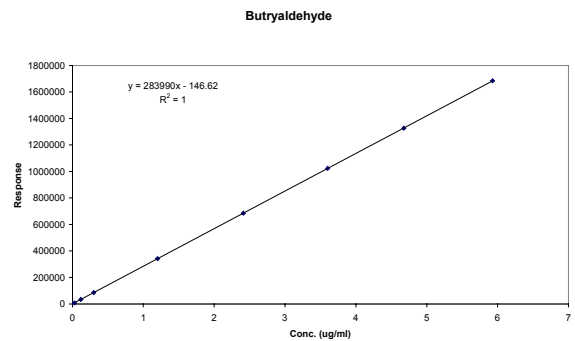
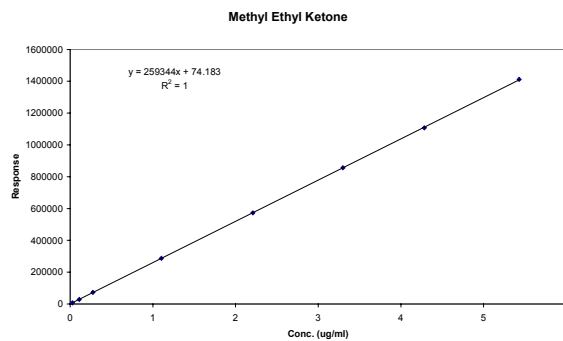
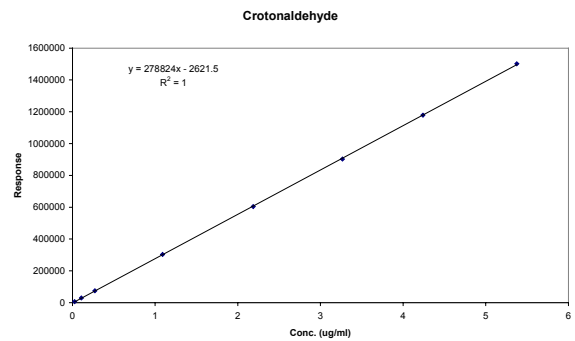
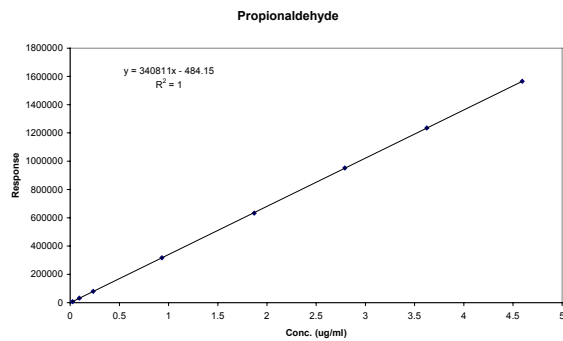
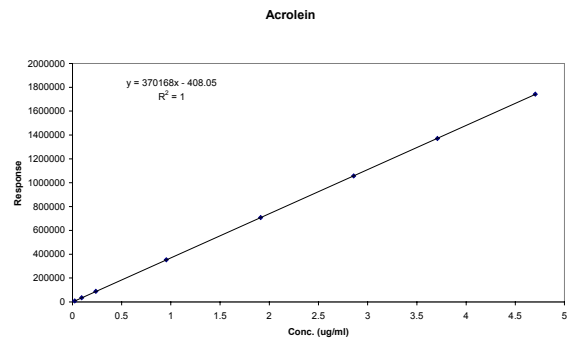
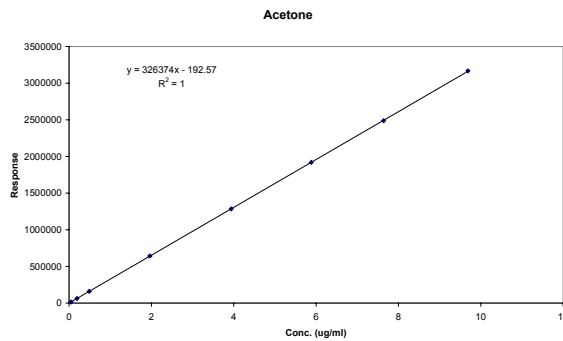
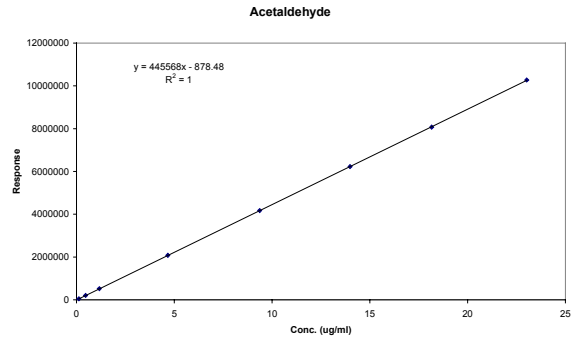
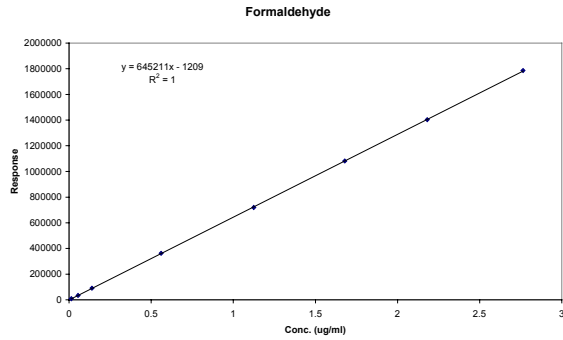
	Formaldehyde- 2,4DNPH	Acetaldehyde- 2,4DNPH	Acetone- 2,4DNPH	Acrolein- 2,4DNPH	Propionaldehyde- 2,4DNPH	Crotonaldehyde- 2,4DNPH	Methyl ethyl ketone-2,4DNPH	Butryaldehyde- 2,4DNPH
1R4F/1, 40ml Solvent, 2Cig	17.1	687.8	291.6	55.5	52.0	16.3	72.9	68.1
1R4F/1, 40ml Solvent, 2Cig after 24 hours	17.9	702.9	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

4. Calibration

Below is a set of calibration curves obtained with the method during the study. NB Concentration is $\mu\text{g mL}^{-1}$ of analyte.

The range needed to be extended for cigarettes containing high yields of carbonyls. This was achieved by diluting sample solutions with acetonitrile (1:1).



5. Blank correction

Analysis of blank samples (i.e. the trapping solution) gave positive results. This is due to contamination of the 2,4 DNPH solid/solution by the laboratory atmosphere (e.g. acetone). The levels varied each time a new batch of trapping solution was prepared.

The only analyte present in significant concentrations (concentration greater than the reporting limit) was acetone. During the study, the levels of acetone ranged from 5 µg cig⁻¹ to 10 µg cig⁻¹.

6. Limit of detection/quantitation/reporting

Analysis of the blank trapping solutions gave detectable peaks (varied with each batch). Analysis of the lowest standard gave peaks significantly greater than that detected in trapping solution. Therefore the bottom standard was used to determine the reporting limits as shown below.

Analyte	Analyte yield ug per cig.
Formaldehyde	<2
Acetaldehyde	<5
Acetone	<4
Acrolein	<2
Propionaldehyde	<3
Crotonaldehyde	<2
Butryaldehyde	<2
Methyl ethyl ketone	<2

7. Standards (precision and accuracy)

A check was made on the accuracy/precision of the system by 6 repeat injections of a QC - all CVs were less than 0.5% except butan-2-one where there was one rogue result.

Formaldehyde

R²= 0.9998
Calc. Conc. 2.216

Injection	Value ug/ml
1	2.188
2	2.185
3	2.192
4	2.194
5	2.190
6	2.198
Average	2.1912
StDev	0.005
%CV	0.209

Acetaldehyde

R²= 0.9998
Calc. Conc. 4.687

Injection	Value ug/ml
1	4.643
2	4.631
3	4.645
4	4.656
5	4.649
6	4.652
Average	4.646
StDev	0.009
%CV	0.188

Acetone

R²= 0.9998
Calc. Conc. 3.85

Injection	Value ug/ml
1	3.806
2	3.803
3	3.816
4	3.808
5	3.812
6	3.817
Average	3.810
StDev	0.006
%CV	0.147

2-Butanone

R²= 0.9999
Calc. Conc. 2.258

Injection	Value ug/ml
1	2.238
2	2.226
3	2.241
4	2.233
5	2.478
6	2.235
Average	2.275
StDev	0.099
%CV	4.373

Acrolein

R²= 0.9999
Calc. Conc. 1.885

Injection	Value ug/ml
1	1.865
2	1.863
3	1.867
4	1.867
5	1.871
6	1.865
Average	1.866
StDev	0.003
%CV	0.146

Propionaldehyde

R²= 0.9998
Calc. Conc. 3.110

Injection	Value ug/ml
1	3.071
2	3.065
3	3.077
4	3.076
5	3.074
6	3.083
Average	3.074
StDev	0.006
%CV	0.197

Crotonaldehyde

R²= 0.9998
Calc. Conc. 2.188

Injection	Value ug/ml
1	2.171
2	2.167
3	2.181
4	2.177
5	2.176
6	2.170
Average	2.174
StDev	0.005
%CV	0.239

Butryaldehyde

R²= 0.9998
Calc. Conc. 4.298

Injection	Value ug/ml
1	4.241
2	4.241
3	4.258
4	4.258
5	4.283
6	4.258
Average	4.257
StDev	0.015
%CV	0.362

A check was made on the repeatability of the standards ($\mu\text{g mL}^{-1}$) at the high and low end.

Low Level

	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
Low Calibration Standard Inj. 1	0.218	0.461	0.381	0.184	0.305	0.212	0.220	0.423
Low Calibration Standard Inj. 2	0.217	0.458	0.380	0.186	0.305	0.213	0.220	0.416
Low Calibration Standard Inj. 3	0.218	0.461	0.378	0.183	0.306	0.214	0.225	0.433
Low Calibration Standard Inj. 4	0.217	0.459	0.379	0.186	0.304	0.209	0.217	0.423
Low Calibration Standard Inj. 5	0.218	0.459	0.379	0.188	0.305	0.213	0.224	0.425
Low Calibration Standard Inj. 6	0.218	0.458	0.379	0.185	0.306	0.212	0.218	0.418
Average	0.218	0.459	0.379	0.185	0.305	0.212	0.221	0.423
Standard Deviation	0.000516	0.00137	0.00103	0.00175	0.000753	0.00172	0.00320	0.00597
%CV	0.237	0.297	0.272	0.945	0.247	0.812	1.452	1.411

High Level

High Calibration Standard Inj. 1	10.938	23.194	19.047	9.325	15.365	10.830	11.131	21.237
High Calibration Standard Inj. 2	10.966	23.264	19.098	9.349	15.409	10.874	11.214	21.283
High Calibration Standard Inj. 3	10.966	23.241	19.088	9.348	15.399	10.874	11.221	21.300
High Calibration Standard Inj. 4	10.967	23.279	19.095	9.346	15.400	10.865	11.157	21.276
High Calibration Standard Inj. 5	10.948	23.216	19.070	9.338	15.387	10.920	11.142	21.261
High Calibration Standard Inj. 6	10.978	23.287	19.126	9.369	15.442	10.899	11.236	21.328
Average	10.961	23.247	19.087	9.346	15.400	10.877	11.184	21.281
Standard Deviation	0.0146	0.03666	0.02682	0.01447	0.025453	0.03067	0.04533	0.03142
%CV	0.134	0.158	0.140	0.155	0.165	0.282	0.405	0.148

As a check that the standards (all purchased from one supplier) were satisfactory, we purchased a couple of carbonyls from another manufacturer and used a formaldehyde-2,4 DNPH derivative prepared in house. Results are given in the following table

	Weight of derivative g	Purity %	Percentage Analyte	Calculated concentration of analyte ug/ml	Measured analyte concentration ug/ml	Difference ug/ml	% Difference from calculated value
Formaldehyde-2,4DNPH LGC	0.00280	99.0	14.29	7.92	7.85	-0.07	-0.90
Formaldehyde-2,4DNPH Sigma	0.00207	99.8	14.29	5.90	5.75	-0.16	-2.70
Acetaldehyde-2,4DNPH Sigma	0.00190	99.9	19.65	7.46	7.60	0.14	1.88

8. Precision and accuracy

The table below shows the results for 5 determinations of 1R4F and 1R5F (single run). The most variable carbonyl concentration was consistently formaldehyde. Additionally, it is expected from the chromatograms that poorer precision would be obtained with low carbonyl yields, e.g. crotonaldehyde.

	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

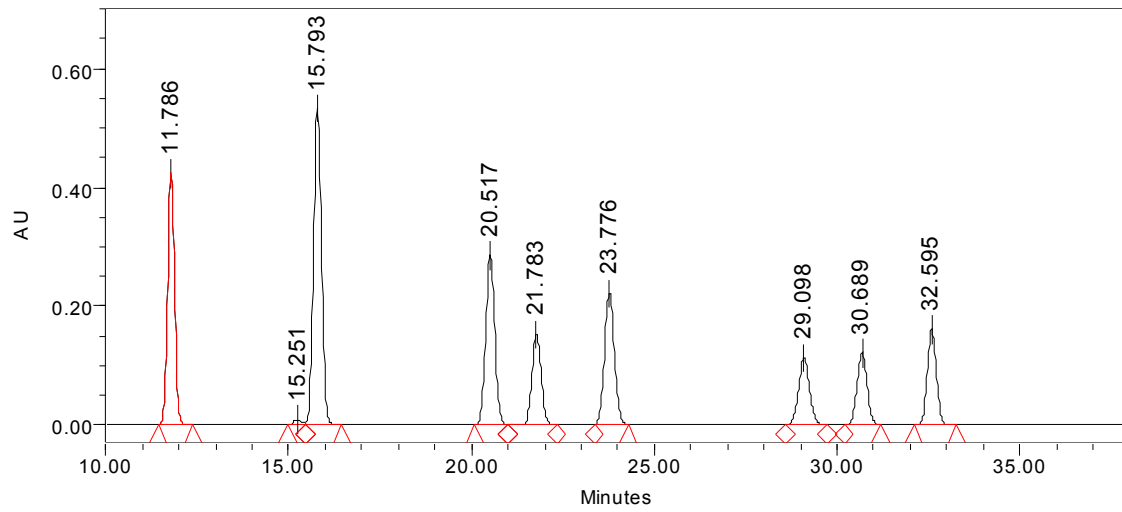
	Formaldehyde- 2,4DNPH	Acetaldehyde- 2,4DNPH	Acetone- 2,4DNPH	Acrolein- 2,4DNPH	Propionaldehyd e-2,4DNPH	Crotonaldehyde- 2,4DNPH	Methyl ethyl ketone-2,4DNPH	Butryaldehyde- 2,4DNPH
Blank DNPH			5.32*					
1R5F/1, 40ml Solvent, 2Cig	2.8	185.4	84.5	13.4	13.8	2.9	18.2	16.3
1R5F/2, 40ml Solvent, 2Cig	2.6	175.9	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. Acetaldehyde

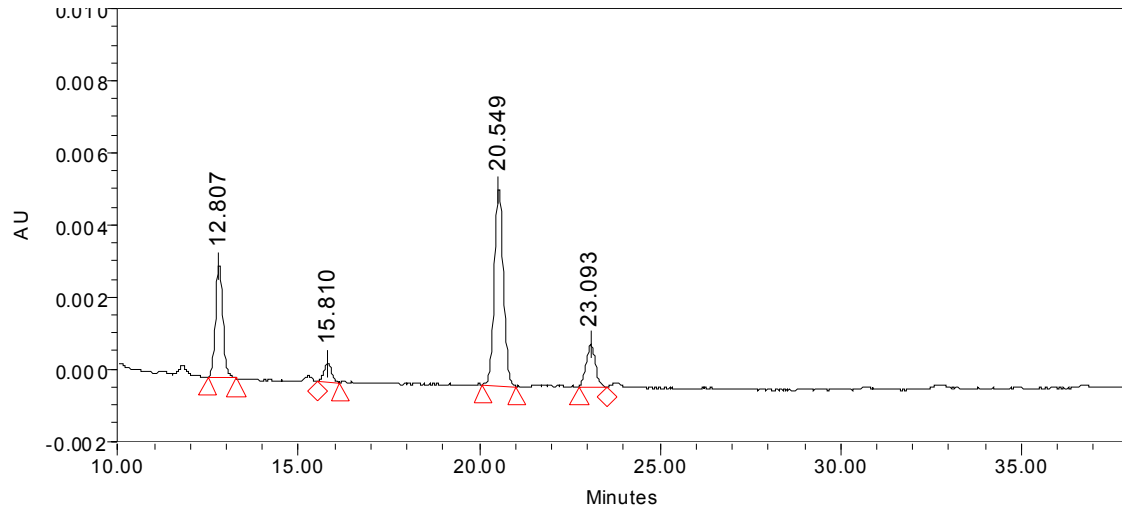
Two peaks were detected for acetaldehyde. This has been reported by others as being due to the cis and trans isomers. Therefore, peak areas were summed before calculating the acetaldehyde concentration.

10. Chromatograms

Example of standard chromatogram



Example of Blank DNPH chromatogram



Example of 1R4F Chromatogram

