

British American Tobacco Group Research & Development

Method - Determination of carbonyls in mainstream cigarette smoke.

1 SCOPE OF APPLICATION

The method is applicable to quantitative determination of the yields of formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone and butyraldehyde in whole mainstream cigarette smoke, using high performance liquid chromatography with ultra-violet detection.

2 NORMATIVE REFERENCES

- ISO 3308:2000 – Routine analytical cigarette smoking machine – definitions and standard conditions
- ISO 3402:1999 – Tobacco and tobacco products – atmospheres for conditioning and testing
- ISO 4387:2000 – Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine
- ISO 8243:2006 – Cigarettes - Sampling

3 PRINCIPLE

Two conditioned cigarettes are smoked using a modified linear 20 port smoking machine to accommodate liquid trapping systems. The mainstream smoke is collected into 2 liquid impingers containing a solution of 2,4-dinitrophenylhydrazine (DNPH). After smoking the impinger solutions are combined and diluted with buffer solution. An aliquot of the resulting solution is submitted for analysis via high performance liquid chromatography with ultra-violet detection.

4 HEALTH & SAFETY

Read and understand the Material Safety Data Sheets for the chemicals used in this method. Read and understand the method risk assessment. Ensure that you understand the hazards and follow control measures relevant to the operation of this method. All preparation of standards and extraction of samples must be performed in a fume cupboard.

5 REAGENTS AND MATERIALS

All reagents are Analytical Grade or equivalent unless otherwise stated.

Deionised water (18.2MΩ.cm)

Acetonitrile (HPLC grade)

Tetrahydrofuran (HPLC grade)

Isopropanol (HPLC grade)

2,4-Dinitrophenylhydrazine (DNPH)

Formaldehyde-DNPH derivative

Acetaldehyde-DNPH derivative

Acetone-DNPH derivative

Acrolein-DNPH derivative

Propionaldehyde-DNPH derivative

Crotonaldehyde-DNPH derivative

2-Butanone-DNPH derivative (methyl ethyl ketone-DNPH derivative)

Butyraldehyde-DNPH derivative

10M Sodium hydroxide solution

Glacial acetic acid

6 APPARATUS

Borgwaldt-KC LM20 linear 20 port smoking machine – adapted for use with liquid traps

Smoke collection system for each port: A suitable trapping system has been constructed from Quickfit® tubes and Dreschel heads.

Soap bubble manometer to measure puff volume

Analytical balance capable of measuring to at least four decimal places

Anemometer

pH sticks

Glass weighing boats

150mL conical flasks

Pipette capable of dispensing 40µL

0.1mL pipettes (class A)

0.5mL pipettes (class A)

1mL pipettes (class A)

5mL pipettes (class A)

15mL pipettes(class A)

2L solvent bottle

1L solvent bottle

1L amber volumetric flask (class A) with stopper

200mL volumetric flask (class A) with stopper

100mL volumetric flask (class A) with stopper

50mL volumetric flask (class A) with stopper

25mL volumetric flask (class A) with stopper

Ultrasonic bath capable of operating at 30°C

2mL capacity amber crimp top vials and caps

Agilent 1100 HPLC with UV detector and autosampler

Luna 5µm C₁₈, 250mm x 4.6mm

C₁₈ packed Guard Column

7 PRELIMINARY SAMPLE PREPARATION

Cigarettes should be conditioned according to normal procedures (ISO 3402:1999). Unless specifically requested, cigarettes are not subjected to any selection criteria other than the rejection of any obviously defective or damaged cigarettes. Butt marking is as specified in ISO 4387:2000 unless otherwise requested.

8 ANALYTICAL PROCEDURE – SOLUTION PREPARATION

8.1 Extracting Solutions

8.1.1 DNPH Trapping Solution

Weigh 2.5g (\pm 0.1g) of DNPH and transfer to a 1L amber volumetric flask and make up to volume with acetonitrile (do not sonicate).

8.1.2 Mobile Phase A

To a 2L solvent bottle add 1180mL water, 600mL acetonitrile, 200mL tetrahydrofuran and 20mL isopropanol. Sonicate for 10 minutes at 30°C (\pm 5°C) prior to use.

8.1.3 Mobile Phase B

To a 1L solvent bottle add 330mL water, 650mL acetonitrile, 10mL tetrahydrofuran and 10mL isopropanol. Sonicate for 10 minutes at 30°C (\pm 5°C) prior to use.

8.1.4 Buffer Solution (pH 4)

To a 200mL volumetric flask add 60mL deionised water, 20mL of 10M sodium hydroxide solution and 100mL glacial acetic acid. Make up to volume with water and check the pH of the solution.

8.2 Stock Standard Solution

To a 100mL amber volumetric flask add the following amounts of the carbonyl-DNPH derivatives to acetonitrile and make up to volume.

Compound	Target Weight (mg)	Purity (%)	Volume (mL)	Free carbonyl concentration (μ g/mL)
Formaldehyde-DNPH	60	99	100	84.88
Acetaldehyde-DNPH	60	99	100	116.72
Acetone-DNPH	60	99	100	144.83
Acrolein-DNPH	40	99	100	93.99
Propionaldehyde-DNPH	40	99	100	96.56
Crotonaldehyde-DNPH	40	99	100	110.93
Methyl ethyl ketone-DNPH	40	99	100	113.21
Butyraldehyde-DNPH	40	99	100	113.21

8.3 Calibration Standards

Dilute the Stock Standard Solution with acetonitrile as follows in 200mL amber volumetric flasks (class A).

Calibration Standard	Volume (mL) of Stock Standard	Final Volume (mL)
1	0.04	200
2	0.4	200
3	2.0	200
4	4.0	200
5	20.0	200

The calibration standards will contain the following free carbonyl concentrations:

Compound	Concentration ($\mu\text{g/mL}$)				
	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Formaldehyde	0.017	0.170	0.849	1.698	8.488
Acetaldehyde	0.023	0.233	1.167	2.334	11.672
Acetone	0.029	0.290	1.448	2.897	14.483
Acrolein	0.019	0.186	0.930	1.861	9.304
Propionaldehyde	0.019	0.191	0.956	1.912	9.558
Crotonaldehyde	0.022	0.222	1.109	2.219	11.093
Methyl ethyl ketone	0.022	0.226	1.132	2.264	11.321
Butyraldehyde	0.023	0.226	1.132	2.264	11.321

8.4 QC Stock Standard Solution

Prepare the solution as described for Stock Standard Solution (8.2).

8.5 QC Standard

Dilute 2.0mL of the QC Stock Standard Solution with acetonitrile in a 200mL amber volumetric flask (class A).

The QC standard will contain the following free carbonyl concentrations:

Compound	QC Standard concentration ($\mu\text{g/mL}$)
Formaldehyde	0.849
Acetaldehyde	1.167
Acetone	1.448
Acrolein	0.930
Propionaldehyde	0.956
Crotonaldehyde	1.109
Methyl ethyl ketone	1.132
Butyraldehyde	1.132

All standards are stored in a freezer until required and defrosted thoroughly prior to use. Expiry date: 6 months from date of preparation.

8.6 Sample Blank

To measure the background concentration of the carbonyl compounds a sample blank is prepared by adding 0.5mL of DNPH trapping solution and 0.5mL of buffer solution to a 2mL amber vial.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

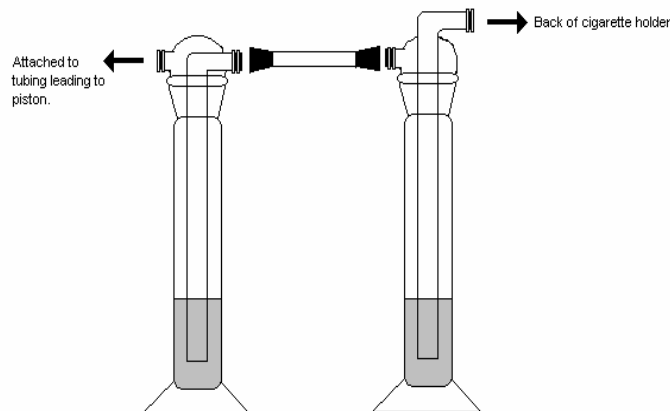
9.1 Sample Collection – Preparation of Liquid Traps

This method utilises two liquid impingers attached in series to the back of each smoking port. This is a deviation from the ISO Method 3308:2000.

Prior to smoking allow the smoking machine to warm up for 30 minutes and measure airflow in ports 1, 10 and 20 with an airflow probe. Individual port readings should be 200mm/sec (\pm 50mm/sec) and the average airflow across the measured ports should be 200mm/sec (\pm 30mm/sec).

9.1.1 Assembly of trapping system

Assemble the carbonyl trapping system behind the cigarette holder as shown below, with each trap containing 25mL of DNPH trapping solution (see 8.1.1), delivered from a pre-set dispenser.



N.B. Take great care to ensure that the traps are connected correctly otherwise instrument damage can occur.

Check the system for leaks by taking a puff and ensuring liquid does not siphon back up the impinger tube. Check the puff volumes with the traps in position and adjust if necessary. Puff volumes should be 35ml \pm 0.3mL (for ISO smoking).

9.1.2 Smoking

Smoke 2 conditioned cigarettes through each trapping system. One clearing puff is taken between each cigarette run, and a further five clearing puffs are taken at the end of the run to ensure any residual smoke has been collected in the trapping solution. Record the number of lit puffs.

9.2 Sample Extraction

Transfer the contents of the first impinger to a 100mL conical flask. Transfer the contents of the second impinger to the first impinger and add to the conical flask. Pipette 0.5mL of the sample extract and 0.5mL of buffer solution into a 2mL amber vial.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Analysis is performed on an Agilent 1100 High Performance Liquid Chromatograph (HPLC) fitted with autosampler and UV detector.

Column type	Luna 5µm C ₁₈ , 250mm x 4.6mm
Guard column	C ₁₈ packing
Mobile phase	Gradient – see table below
Flow rate	1.5mL/min
Injection volume	20µL
Column oven temperature	30°C
Autosampler tray temperature	5°C
Detector	UV 380nm
Run time	35 minutes
Peak height/area	Area

Time (min)	Mobile phase A	Mobile phase B	Flow rate (mL/min)	Profile
0	100	0	1.5	Linear
8	70	30	1.5	Linear
20	47	53	1.5	Linear
25	15	85	1.5	Linear
27	100	0	1.5	Linear
35	100	0	1.5	Linear

10.2 System Suitability Criteria

- R² of calibration curve should be greater than 0.999
- Peak height for crotonaldehyde standard 1 should be ≥ 0.20
- Peak area for crotonaldehyde standard 1 should be ≥ 5.00
- Reference cigarette – results within defined limits
- Quality control solution – results within defined limits

10.3 Run Order

Acetonitrile blank

Calibration standards in ascending order

Extraction blank

QC standard

10 samples (including a reference cigarette sample)

Acetonitrile blank

QC standard

10 samples (including a reference cigarette sample)

etc

QC standard

11 CALCULATIONS

Use the instrument software to determine the concentrations of each carbonyl as µg/mL. To convert results to µg/cigarette use the following equation:

$$\text{Carbonyl concentration (}\mu\text{g/cigarette)} = \frac{\text{carbonyl concentration (}\mu\text{g/mL)} \times V}{N}$$

Where: V = Total volume (100mL, based on extracting solution diluted 1:1 with buffer solution)

N = Number of cigarettes smoked (normally 2)

12 PRECISION AND REPORTING LIMITS

Five replicate smokings and analyses are performed to determine the precision of the analysis. Longer term precision is monitored through the maintenance of control charts.

The lower reporting limits are defined by the concentrations of the lowest calibration standard, and are as follows:

Compound	Lower reporting limit (µg/cigarette)
Formaldehyde	0.85
Acetaldehyde	1.15
Acetone	1.45
Acrolein	0.95
Propionaldehyde	0.95
Crotonaldehyde	1.10
Methyl ethyl ketone	1.10
Butyraldehyde	1.15

13 QUALITY ASSURANCE AND CONTROL

Control charts of the QC standard and the reference cigarette are maintained to allow inspection of the method performance.

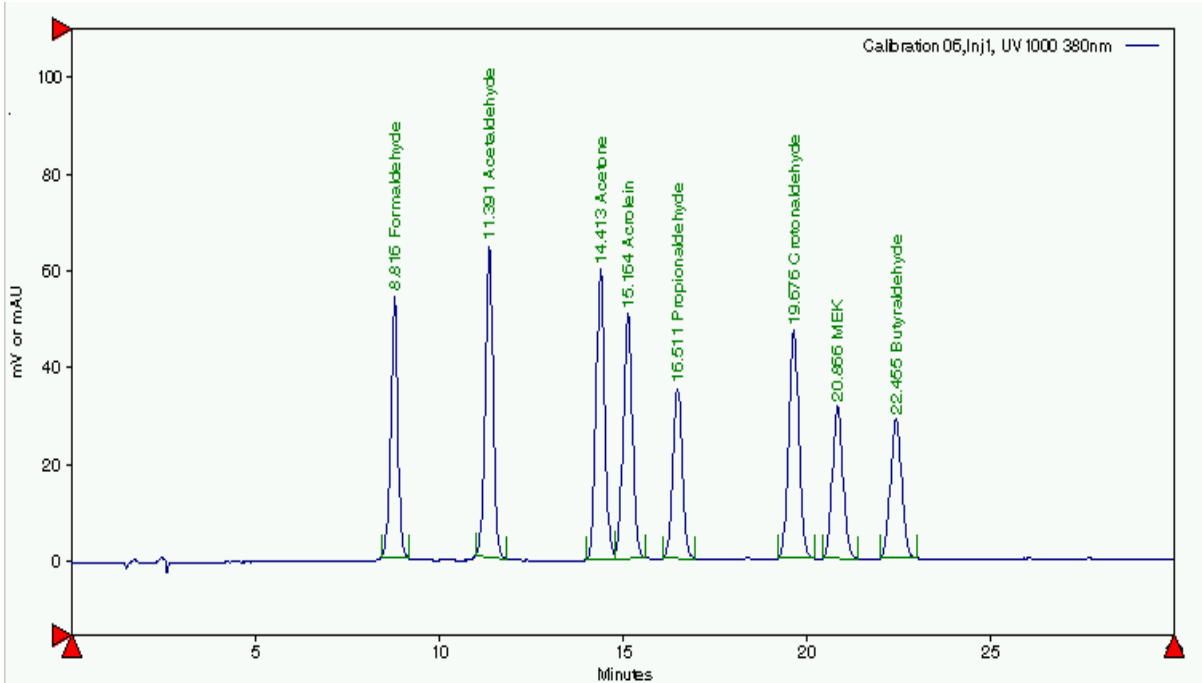
14 SPECIAL CASES

Under more intense smoking regimes, the number of cigarettes smoked per port may need to be reduced to avoid overloading the trapping solution.

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APPENDIX A SAMPLE CHROMATOGRAMS

Calibration standard



Kentucky Reference Cigarette 1R4F

