

British American Tobacco Group Research & Development

Method - Determination of tar, nicotine and carbon monoxide in mainstream cigarette smoke.

1 SCOPE OF APPLICATION

The method is applicable to quantitative determination of the yields of tar, nicotine and carbon monoxide in mainstream cigarette smoke, using gas chromatography with thermal conductivity and flame ionisation 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
- ISO 8454:2007 – Cigarettes – Determination of carbon monoxide in the vapour phase of cigarette smoke – NDIR method
- ISO 10315:2000 – Cigarettes – Determination of nicotine in smoke condensates – Gas Chromatographic method
- ISO 10362-1:1999 – Cigarettes – Determination of water in smoke condensates - Gas Chromatographic method

3 PRINCIPLE

Five conditioned cigarettes are smoked using a 20 port linear smoking machine equipped with a carbon monoxide analyser. The vapour phase is collected in a vapour phase collection bag and the carbon monoxide content analysed using a non-dispersive infra-red analyser. The mainstream smoke is collected on a 44mm Cambridge filter pad (CFP). After smoking, the CFP is extracted with propan-2-ol containing internal standards. The samples are analysed by gas chromatography using thermal conductivity and flame ionisation 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.

2% carbon monoxide in nitrogen

4% carbon monoxide in nitrogen

6% carbon monoxide in nitrogen

Ethanol, absolute

n-Heptadecane (minimum purity 99%)

Nicotine (minimum purity 99%)

Propan-2-ol (GPR grade)

Deionised water (18.2M Ω .cm)

6 APPARATUS

Cerulean SM450 linear 20 port smoking machine

Soap bubble manometer to measure puff volume

Analytical balance capable of measuring to at least four decimal places

44mm Cambridge filter pads

Hot-air oven at 105°C

20mL solvent dispenser (accuracy \pm 0.1mL)

2, 5, 10, 20, 25 and 44 μ L precision syringes

1, 2, 10, 20, 25 and 40 mL pipettes

150mL conical flasks

Flask shaker

2mL capacity amber crimp top GC vials and caps

Dual injection Agilent GC with thermal conductivity and flame ionisation detectors

200mL volumetric flask (class A)

100mL volumetric flask (class A)

5L volumetric flask

Poraplot Q 10m x 0.53mm column

CP-WAX 52CB 25m x 0.53mm 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 Solution

8.1.1 Internal Standard Stock Solution

Weigh 25g (\pm 1mg) of n-heptadecane into a 200mL volumetric flask (class A) and make to volume with propan-2-ol.

8.1.2 Extracting Solution

Pipette 10mL of the internal standard stock solution and 25mL of ethanol into a 5L volumetric flask and make to volume with propan-2-ol.

8.2 Nicotine Standards

8.2.1 Stock 1A

Weigh 0.5g (\pm 1mg) of nicotine into a 100mL volume flask (class A) and make to volume with extracting solution (8.1.2).

Store in a refrigerator. Expiry date: 3 months from date of preparation.

8.2.2 Stock 1B

Weigh 0.5g (± 1 mg) of nicotine into a 100mL volume flask (class A) and make to volume with extracting solution.

Store in a refrigerator. Expiry date: 3 months from date of preparation.

8.2.3 Stock 2A

Pipette 25mL of Stock 1A into a 100mL volumetric flask (class A) and make to volume with extracting solution.

Store in a refrigerator. Expiry date: 1 month from date of preparation.

8.2.4 Stock 2B

Pipette 25mL of Stock 1A into a 100mL volumetric flask (class A) and make to volume with extracting solution.

Store in a refrigerator. Expiry date: 1 month from date of preparation.

8.2.5 Nicotine Calibration Standards

Dilute the Stock 2A solution as follows in 50mL volumetric flasks (class A) using extracting solution.

Calibration Standard	Volume of Stock 2A (mL)	Nicotine Concentration (mg/mL)
1	1.0	0.025
2	2.0	0.050
3	*	0.250
4	20.0	0.500
5	25.0	0.625

*NB: Standard 3 is same solution used for the QC standard (see 8.2.6).

Store in a refrigerator. Expiry date: 1 month from date of preparation.

8.2.6 QC Standard

Pipette 40mL of Stock 2B into a 200mL volumetric flask (class A) and make to volume with extracting solution. The nicotine concentration in the QC standard will be 0.250mg/mL.

Store in a refrigerator. Expiry date: 1 month from date of preparation.

8.3 Water Calibration Standards

To six 150mL conical flasks add one whole and 2 quarters of clean Cambridge filter pads and stopper immediately. Using calibrated microlitre precision syringes add the following amounts of deionised water to the conical flasks followed by 20mL of extracting solution immediately after the addition of the water. Stopper each flask immediately.

Calibration Standard	Volume of Water (μL)	Water Concentration (mg/mL)
1	2	0.10
2	5	0.25
3	10	0.50
4	25	1.25
5	44	2.20

Shake the flasks for 20 minutes at a moderate speed. The standards are prepared daily.

NB: Flasks should be dried in an oven at 105°C for a minimum of 1 hour and stored in a suitable desiccator. Additions to, and removal from these flasks should be performed as rapidly as possible. The exact weight of water added is required by data handling system.

8.4 Water QC Standard

Pipette 20 μL of deionised water onto one whole and two quarters of Cambridge filter pad. Add 20mL of extracting solution, stopper and shake for 20 minutes at moderate speed. The standard is prepared daily.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

9.1 Sample Collection

Cigarettes are smoked on a 20 port linear smoking engine. Warm-up the smoking engine for 15 minutes before smoking.

Check the linear airflow is 200 mm/s (\pm 30mm/s), the system has no leaks and puff volume is 35mL (\pm 0.3mL) (for ISO smoking).

5 cigarettes per port are smoked. Take one clearing puff between each “sub run” and a further five clearing puffs after the final cigarette is smoked. On completion of smoking the carbon monoxide content of the vapour phase bags is determined using the calibrated non-dispersive infra-red analyser. Record the number of lit puffs and the weight of the Total Particulate Matter (TPM).

9.2 Sample Extraction

Transfer the CFP to a 150mL conical flask and stopper. Using a quarter of a CFP wipe the front of the CFP holder and with a second quarter of CFP wipe the back and front of the CFP holder, adding these to conical flask. To the flask add 20mL of extracting solution and stopper immediately. Prepare two water blanks by adding one whole and two quarters of CFP to a 150mL conical flask followed by 20mL of extracting solutions. One blank is prepared prior to sample extraction and one on completion. Shake for 20 minutes at moderate speed.

9.3 Sample Clean Up

Transfer an aliquot of each extract to a dry GC autosampler vial and cap immediately. The sample is ready for analysis.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Analysis is performed on an Agilent 6890 Gas Chromatograph fitted with autosampler and thermal conductivity and flame ionisation detectors.

Column Type	For water analysis: fused silica column containing Poraplot Q 10m x 0.53mm For nicotine analysis: WCOT fused silica coating (1 micron) CP-WAX 52CB 25m x 0.53mm
Injection type and temperature	Split 20mL/minute / 250°C
Column temperature programme	160°C isocratic
Carrier gas	Helium
Injection Volume	1µL
Flame ionisation temperature	250°C
Thermal conductivity temperature	300°C
Column Flow	14mL/min

10.2 System Suitability Criteria

The R² value of the calibration graphs must be >0.99.

10.3 Run Order

Nicotine calibration standards in ascending order

Water calibration standards in ascending order

Nicotine QC standard

Water QC standard

4 water blanks

20 samples (including reference sample)

11 CALCULATIONS

Using the instrument software, plot a calibration graph of calibration standards concentration against peak area ratio, without forcing the line through zero.

For nicotine:

Peak area ratio = Nicotine peak area/heptadecane peak area

For water:

Peak area ration = Water peak area/ethanol peak area

Check the plots, coefficient of determination (R²) and intercept before accepting the calibrations. Calculate the concentration of nicotine and water in the samples.

The results obtained from the instrument are converted to mg/cigarette using the following equation:

$$\text{Sample concentration (mg/cigarette)} = \frac{\text{sample concentration (mg/mL)} \times V}{N}$$

Where: V = Volume of extracting solution (usually 20mL)
 N = Number of cigarettes smoked (usually 5)

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 limit for nicotine is defined by the concentration of the lowest calibration standard, and equates to 0.1mg/cigarette.

13 QUALITY ASSURANCE AND CONTROL

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

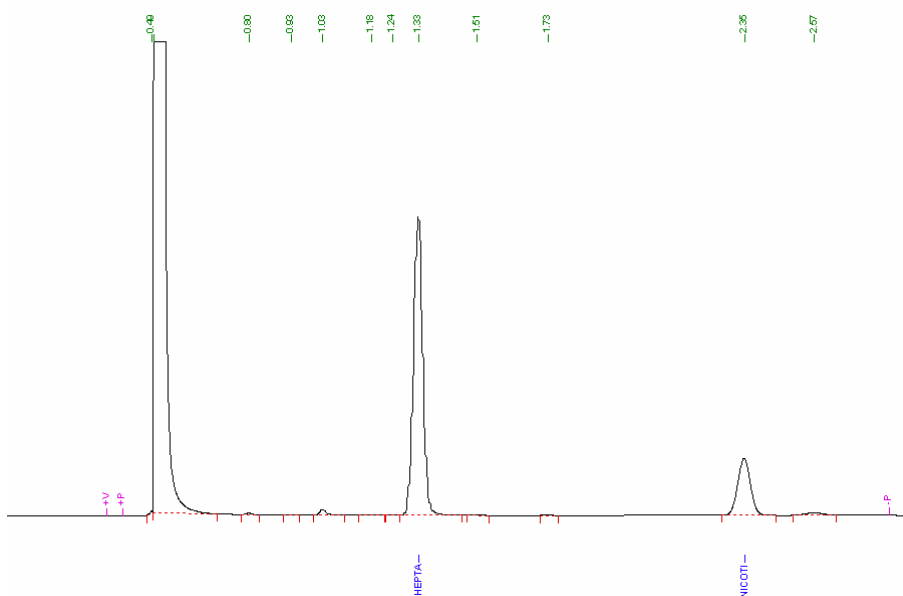
14 SPECIAL CASES

Under more intense smoking regimes, the number of cigarettes per smoking run may need to be reduced in order to avoid smoke breakthrough on the Cambridge filter pad.

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APPENDIX A SAMPLE CHROMATOGRAMS

Smoke Extract - Nicotine



Smoke Extract - Water

