British American Tobacco Group Research & Development

Method - Determination of benzo(a)pyrene in mainstream smoke

1 SCOPE OF APPLICATION

The method is applicable to quantitative determination of the yields of benzo(a)pyrene in mainstream cigarette smoke, using gas chromatography with mass selective detection.

2 NORMATIVE REFERENCES

ISO 3308:2000 – Cigarettes - 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

Twenty conditioned cigarettes are smoked using a 20 port rotary Borgwaldt smoking machine. The mainstream smoke is collected on to a 92mm Cambridge filter pad (CFP). After smoking, the CFP is extracted with cyclohexane. The extract is passed through a PAH Solid Phase Extraction (SPE) cartridge and eluted with cyclohexane. The eluent is evaporated to 1mL. The samples are analysed by GC/MS using Selective Ion Monitoring (SIM).

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.

Benzo(a)pyrene (CAS Number: 50-32-8) 100ng/µL in cyclohexane

Benzo(a)pyrene-d_{12} (CAS Number: 63466-71-7) 100ng/µL in cyclohexane

Cyclohexane

6 APPARATUS

Borgwaldt-KC RM200 rotary 20 port smoking machine

Soap bubble manometer to measure puff volume

Analytical balance capable of measuring to at least four decimal places

92mm Cambridge Filter Pads

500µL syringe

10mL pipette (Class A)

2 x 5mL pipette (Class A)

3mL pipette (Class A)

2mL pipette (Class A)

3 x 1mL pipettes (Class A)

2 x 0.5mL pipettes (Class A)

2x 100mL volumetric flasks (amber, Class A) with stoppers

7 x 50mL volumetric flasks (amber, Class A) with stoppers

100mL conical flasks with stoppers

40mL vials

7mL vials

Flask shaker

PAH - S.P.E. cartridges (1.5g layered column, NH_2/C_{18})

Zymark Turbovap

Ultrasonic bath

2mL capacity amber GC vials and caps

Agilent GC/MS with autosampler

ASPEC^{TM} XL Sample Processor for Solid Phase Extraction

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

All standards must be clearly and permanently labelled, including expiry date, and stored in a freezer.

8.1 Benzo(a)pyrene Stock Solution (1000 ng/mL)

Pipette 0.5mL (class A) of the 100ng/µL B(a)P in cyclohexane standard into an amber 50mL volumetric flask (class A) and make up to volume with cyclohexane.

8.2 Benzo(a)pyrene-d_{12} Internal Spike Solution (ISTD) (2000 ng/mL)

Pipette 1.0mL (class A) of the 100ng/µL Benzo(a)pyrene-d_{12} in cyclohexane solution into an amber 50mL volumetric flask (class A) and make up to volume with cyclohexane.

8.3 Calibration Standards

Dilute the benzo(a)pyrene Stock solution (see 8.1) and add the Internal standard spike solution (ISTD – see 8.2) as follows in amber 50mL volumetric flasks (class A).

Calibration Standard	Volume (mL) of B(a)P stock	Volume (mL) ISTD	B(a)P Concentration. (ng/mL)
7	5.0	1	100.00
6	3.75	1	75.0
5	2.5	1	50.0
4	1.25	1	25.0
3	0.5	1	10.0
2	0.25	1	5.0
1	0	1	0

8.4 QC - Benzo(a)pyrene Stock Solution (500ng/mL)

Pipette 0.5mL (class A) of the 100ng/µL B(a)P in cyclohexane into an amber 100mL volumetric flask (class A) and make up to volume with cyclohexane.

8.5 QC - Benzo(a)pyrene Solution (40ng/mL)

Pipette 4mL (class A) of the Benzo(a)Pyrene stock solution (see 8.1) into an amber 50mL volumetric flask (class A), add 1mL of ISTD (see 8.2) and make up to volume with cyclohexane.

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 check no background benzo(a)pyrene is present throughout the extraction and analysis procedures, a sample blank is prepared by adding 25mL cyclohexane to a 92mm Cambridge filter pad and running through the extraction process as detailed in section 9.3. A sample blank is prepared for each GC/MS run.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

9.1 Sample Collection

Cigarettes are smoked on a Borgwaldt rotary 20 port smoking machine. Typically the RM200 is used, but if the cigarette style is not suitable, the RM20 CSR may be used. Warm-up the smoking machine for 20 minutes before smoking.

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

20 cigarettes are loaded and smoked. Record the number of lit puffs and the weight of Total Particulate Matter (TPM).

9.2 Sample Extraction

Cut the pad into quarters and place into a ≥100mL conical flask and stopper immediately. Clean scissors with solvent between pads. Spike the CFP with 100µL of benzo(a)pyrene-d_{12} Internal Spike Solution (see 8.2). To the flask add 25mL of cyclohexane using the preset dispenser. Seal the flask with a stopper and parafilm and shake for 1 hour on a flatbed shaker (180 revs/minute).

9.3 Sample Clean Up

Steps 9.3.1 and 9.3.2 are automated using the ASPEC™ XL**.** Refer to ASPEC™ XL Sample Processor for Solid Phase Extraction Users Guide, for operating instructions.

9.3.1

Condition a PAH Solid Phase Extraction (SPE) cartridge with 5mL of cyclohexane and discard the eluate. Do not let the cartridge run dry. Place a labelled 40mL vial under the SPE cartridge.

9.3.2

Pipette 5mL of sample extract on to the cartridge and allow this to pass through the cartridge collecting the eluate. Elute sample with a further two 5mL aliquots of cyclohexane allowing the cartridge to run dry after passing the last aliquot through the cartridge.

9.3.3

Evaporate the combined eluate to approximately 1mL (± 0.5mL) in a steady stream of nitrogen using the Turbovap at 30°C (approximately 10 minutes). Sonicate for 5 minutes. Transfer the total eluate to a GC autosampler vial and submit for analysis by GC/MS.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Analysis is performed on an Agilent 6890 Gas Chromatograph (GC) fitted with autosampler and 5973 Mass Selective Detector (MSD).

Column type	J&W DB-5MS 30m x 0.32mmID x 0.25μm film, with retention gap (approximately 1m) or equivalent
Injection type and temperature	Splitless/ 290°C
Column temperature programme	60°C (2 minutes)/8°C per minute to 290°C/Hold at 290°C for 14 minutes. Total run time is ca. 45 minutes
Carrier gas	Helium (1.5mL/minute)
Transfer line Temperature	280°C
Injection Volume:	1μL
Solvent Delay	6 minutes
MS Source temperature	230°C
MS Quadrupole temperature	150°C
MS Mode	SIM
Ion Dwell time	50ms

The following ions are used as target and qualifier ions

	Target	Qualifier 1	Qualifier 2
B(a)P	252.0 (100%)	250.0 (18-24%)	253.0 (17-22%)
B(a)P-d_{12}	264.0 (100%)	260.0 (15-21%)	265.0(17-23%)

Standards and samples are quantified on the target ions stated above. The qualifier ion ratios are used to confirm that the peaks in the standards are correctly identified. In addition, the ion ratios of the sample peaks should be within ±20% of the standard ion ratios in that run.

10.2 System Suitability Criteria

10.2.1 MS Tuning

Tune the MS weekly, or if the system has been vented. Check the following criteria are met on the tune report:

air and water peaks <10%

EM volts 1000 – 3000

Ion ratios of m/z 502:219:69 are approximately 10:4:1

Peak width approximately 0.6 (± 10%)

10.2.2 Peak resolution check

Open a chromatogram for the control cigarette smoke extract, and use the instrument software to integrate the B(a)P peak and the following peak. The valley should be ≥60% of the B(a)P peak height. If the valley is <60%, investigate the problem before further analysis takes place. Record the value in the maintenance log.

10.2.3 Peak shape check

Open a chromatogram of calibration standard 3, and use the instrument software to assess the B(a)P peak shape. The value for tailing, as defined in the instrument software, should be <1.6. If the result is >1.6 the problem should be investigated before further analysis.

10.2.4 Ion ratio check

Check ion ratios are within the limits shown in section 10.1.

10.2.5 Calibration linearity

The R^2 value of the calibration graph must be >0.99.

10.3 Run Order

Start run with two conditioning* samples

Sample blank (see 8.6)

Calibration standards in ascending order

10 samples (including a reference cigarette sample)

QC standard

10 samples (including a reference cigarette sample)

QC standard

etc

Calibration standards in ascending order

* NB: These conditioning samples should be smoke extracts

11 CALCULATION(S)

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

Peak area ratio = (B(a)P peak area)/(B(a)P-d_{12} peak area)

Check the plots, coefficient of determination (R^2) and intercept before accepting the calibrations. Calculate the concentration of Benzo(a)pyrene in the sample solutions.

The results obtained from the GCMS are in ng/mL. To convert results to ng/cigarette, use the following equation:

$$\text{B(a)P (ng/cigarette)} = \frac{\text{B(a)P in extract (ng/mL)} \times E \times FV}{N \times C}$$

Where: E = Volume of extraction solution used to extract pad (normally 25mL)
FV = Final volume of sample prior to GC (1mL)
N = Number of cigarettes smoked (normally 20)
C = Volume of extract taken for SPE clean up (normally 5mL)

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 method detection limit is 0.42ng/cigarette, defined as ten times the standard deviation of the lowest calibration standard analysed ten times. The practical reporting limit is defined by the concentration of the lowest calibration standard, and equates to 1.25/ng/cigarette.

13 QUALITY ASSURANCE AND CONTROL

Cambridge filter pads containing smoke particulate matter were spiked with B(a)P at various levels. Typical recoveries after the work-up and analysis procedures ranged from 85 – 106%. No B(a)P is typically detected in laboratory reagent blanks.

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

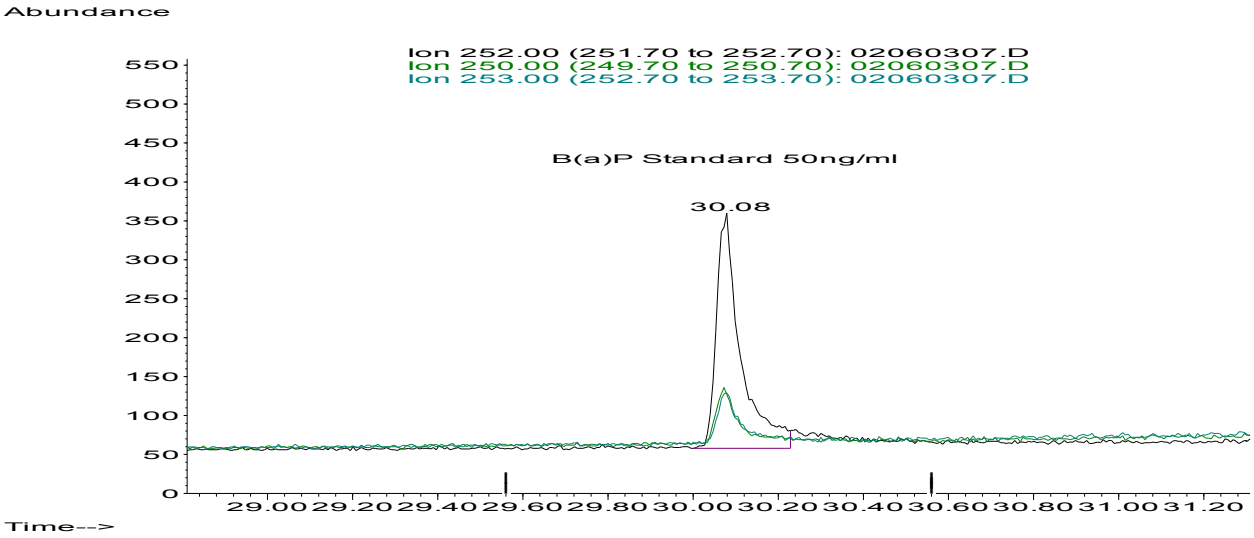
14 SPECIAL CASES

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.

Date of Issue: 31 March 2008

APPENDIX A SAMPLE CHROMATOGRAMS

50 ng/ml B(a)P Standard



Kentucky Reference Cigarette 2R4F