

## **British American Tobacco Group Research & Development**

### **Method - Determination of semi-volatiles in mainstream cigarette smoke.**

#### **1 SCOPE OF APPLICATION**

The method is applicable to quantitative determination of the yields of pyridine, styrene and quinoline in whole mainstream cigarette smoke, using gas chromatography-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**

Five conditioned cigarettes are smoked using a 20 port rotary Borgwaldt smoking machine. The mainstream smoke is collected on a 44mm Cambridge filter pad (CFP) with a XAD-4 sorbent tube behind the pad. After smoking, the CFP and the contents of the XAD-4 tube are combined and extracted using methanol. The samples are analysed by GC/MS, and quantified by 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.

Pyridine – anhydrous 99.8%

Quinoline – 98%

Styrene – 99%

Pyridine-d<sub>5</sub>

Quinoline-d<sub>7</sub>

Styrene-d<sub>8</sub>

Methanol

#### **6 APPARATUS**

Borgwaldt-KC RM20CSR rotary 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

500µL syringe

10mL pipette  
 4mL pipette  
 2mL pipettes  
 1mL pipette  
 XAD-4 tubes  
 100mL volumetric flasks (class A) with stoppers  
 50mL volumetric flask (class A) with stopper  
 25mL volumetric flask (class A) with stopper  
 20mL volumetric flasks (class A) with stoppers  
 10mL volumetric flasks (class A) with stoppers  
 100mL conical flasks with stoppers  
 DB-WAX Column 30m x 0.25mm x 0.25µm  
 Guard column  
 Agilent GC/MS with autosampler

## 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 3308:2000 unless otherwise requested.

## 8 ANALYTICAL PROCEDURE –SOLUTION PREPARATION

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

### 8.1 Pyridine, Quinoline and Styrene stock standards

Prepare standard solutions of the analytes according to the table below. Weigh to the nearest mg, 100mg of each of the standard materials and transfer to separate volumetric flasks (class A). Make up to volume with methanol.

Analyte	Weight (mg)	Final Volume (mL) in MeOH	Stock Concentration
Pyridine	100	10	10mg/mL
Quinoline	100	100	1mg/mL
Styrene	100	10	10mg/mL

This stock standard is stable for at least six months if stored at -20°C.

### 8.2 Mixed primary standard (pyridine and styrene 20µg/mL, Quinoline 2µg/mL)

Using a syringe measure 100µL of each individual stock (8.1) into a 50mL volumetric flask (class A) and make up to volume with methanol.

### 8.3 Deuterated internal standards (ISTD)

#### Pyridine, Quinoline and Styrene deuterated internal stock (pyridine and styrene 10mg/mL, quinoline 1mg/mL)

Prepare standard solutions of the analytes according to the table below. Weigh to the nearest mg, the required mass of each of the standard materials and transfer to separate volumetric flasks (class A). Make up to volume with methanol.

Analyte	Weight (mg)	Final Volume (mL) in MeOH	Stock Concentration
Pyridine-d <sub>5</sub>	100	10	10mg/mL
Quinoline-d <sub>7</sub>	25	25	1mg/mL
Styrene-d <sub>8</sub>	100	10	10mg/mL

#### 8.4 Mixed deuterated internal standard spiking solution (pyridine-d<sub>5</sub> and styrene-d<sub>8</sub> 200µg/mL, quinoline-d<sub>7</sub> 20µg/mL)

Pipette 2.0mL of each internal standard stock solution (8.3) into a 100mL volumetric flask (class A) and make to volume with methanol.

#### 8.5 Calibration standards

Diute the mixed primary standard (8.2) as follows and add the internal standard (8.4) then make up to volume with methanol.

Calibration Standard	Volume of primary standard (mL)	Volume of ISTD (µL)	Concentration (µg/mL)		
			Pyridine	Quinoline	Styrene
6	10.0	200	10	1	10
5	4.0	200	4	0.4	4
4	2.0	200	2	0.2	2
3	1.0	200	1	0.1	1
2	0.5	200	0.5	0.05	0.5
1	0.2	200	0.2	0.02	0.2

The calibration standards are stable for three months if stores at -20°C.

#### 8.6 Quality control standard stock solution (pyridine and styrene 10mg/mL, quinoline 1mg/mL)

Weigh to the nearest 0.1mg 100mg of pyridine and styrene and 10mg quinoline in a volumetric flask and make up to volume with methanol.

This stock standard is stable for at least six months if stored at -20°C

#### 8.7 Quality control standard (pyridine and styrene 4µg/mL, quinoline 0.4µg/mL)

Pipette 400µL of the QC stock solution (8.6), plus 1mL of the ISTD (8.4) into a 100mL volumetric flask (class A), make up to volume with methanol.

This QC standard is stable for three months if stored at -20°C.

## 9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

### 9.1 Sample Collection

Cigarettes are smoked on a Borgwaldt Rotary 20 port smoking machine. Typically the RM20 CSR is used. Warm-up the smoking machine for 20 minutes before smoking.

Check the linear airflow is 200 mm/s ( $\pm$  30mm/s). Directly behind the Cambridge filter pad insert the XAD-4 tube. Check that the system has no leaks and puff volume is 35mL ( $\pm$  0.3mL) (for ISO smoking).

5 cigarettes are loaded and the Cambridge filter pad is spiked with 200 $\mu$ L of internal standard spiking solution (8.4) and smoked. Record the number of lit puffs.

### 9.2 Sample Extraction

Add 20mL ( $\pm$  1mL) of methanol to each conical flask via dispenser (check delivery with a volumetric flask) and seal flask and shake at 180rpm for 20 minutes.

Transfer an aliquot of approximately 2mL of extract to a crimp top GC vial.

## 10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

### 10.1 Instrument Set Up Parameters

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

Column type	J&W DB-WAX 30m x 0.25mmID x 0.25 $\mu$ m film, or equivalent, with retention gap (approximately 1m)
Injection type and temperature	Splitless/ 250°C
Column temperature programme	40°C (2 minutes)/10°C per minute to 150°C/20°C per minute to 240°C Hold for 20 minutes.
Carrier gas	Helium (1.0 mL/minute)
Transfer line Temperature	240°C
Injection Volume:	1 $\mu$ L
Solvent Delay	5.50 minutes
MS Source temperature	230°C
MS Quadrupole temperature	150°C
MS Mode	SIM
Ion Dwell time	50 ms

The following ions should be used as target and qualifier ions

	Target	Qualifier1	Qualifier 2
Pyridine	79.0(100%)	51.0(25-35%)	53.0(5-15%)
Pyridine-d <sub>5</sub>	84.0(100%)	56.0(45-58%)	54.0(15-25%)
Styrene	104.0(100%)	103.0(40-50%)	78.0(30-40%)
Styrene-d <sub>8</sub>	112.0(100%)	84.0(35-45%)	86.0(0-10%)
Quinoline	129.0(100%)	128.0(16-20%)	102.0(20-28%)
Quinoline-d <sub>7</sub>	136.0(100%)	108.0(20-30%)	109.0(1.5-5%)

These ion ratios should be used to confirm that the peaks in the standards are the correct compounds.

The ion ratios of the sample peaks should be within 20% of the standard ion ratios in that run. There may be exceptions when the qualifier ion ratios fall outside these limits, it may be that there are other compounds present in the sample that have the same ion. The compound can be reported as present if the retention time and one of the qualifier ions matches the corresponding standard peak.

## **10.2 System Suitability Criteria**

### **10.2.1 MS Tuning**

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

air and water peaks <10%

EM volts 1000 – 3000

Ion ratio of 502:219:69 is 10:4:1 respectively.

Peak width approx. 0.6 ( $\pm$  0.1)

### **10.2.2 Peak shape check**

Open a chromatogram of calibration standard 3, and use the instrument software to assess the pyridine-d<sub>5</sub> peak shape. The value for USP tailing, as defined in the instrument software should be <3.5. If the result is >3.5 the problem should be investigated before further analysis.

### **10.2.3 Ion ratio check**

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

### **10.2.4 Calibration linearity**

The R<sup>2</sup> value of the calibration graphs must be >0.99.

## **10.3 Run Order**

Two conditioning samples

Calibration Standards in ascending order

10 samples

QC standard

10 samples

QC standard

Continue until all samples are analysed

Calibration Standards in ascending order

## **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 example:

Peak area ratio = (Pyridine peak area)/(Pyridine-d<sub>5</sub> peak area)

Check the plots, coefficient of determination (R<sup>2</sup>) and intercept before accepting the calibrations. Calculate the concentration of pyridine, styrene and quinoline in the sample solutions.

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

$$\text{Yield per cig } (\mu\text{g/cigarette}) = \frac{\text{concentration in extract } (\mu\text{g/mL}) \times V}{N}$$

N = Number of cigarettes smoked (normally 5).

V = Extract volume (normally 20mL).

## 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 limits are defined as ten times the standard deviation of the lowest calibration standard analysed ten times. The practical reporting limits are defined by the concentration of the lowest calibration standard and are as follows:

Compound	Detection Limit ( $\mu\text{g/cigarette}$ )	Lower Reporting Limit ( $\mu\text{g/cigarette}$ )
Pyridine	0.15	0.8
Styrene	0.04	0.08
Quinoline	0.02	0.8

## 13 QUALITY ASSURANCE AND CONTROL

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

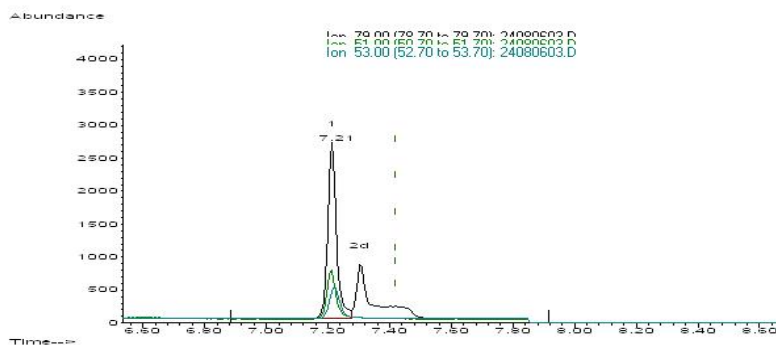
## 14 SPECIAL CASES

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

Standard 1 - extracted ion chromatogram of pyridine



Ky2R4F - extracted ion chromatogram of styrene

