British American Tobacco Group Research & Development

Method - Determination of phenols in mainstream cigarette smoke

1 SCOPE OF APPLICATION

The method is applicable to the quantitative determination of the yields of phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol and hydroquinone 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

Five conditioned cigarettes are smoked using a 20 port rotary Borgwaldt smoking machine. The mainstream smoke is collected on to a 44mm Cambridge filter pad (CFP). After smoking, the CFP is extracted with *tert*-butyl methyl ether (TBME) containing the internal standard, *o*-chlorophenol. The extract is derivatised with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 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.

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)

Tert-butyl methyl ether (TBME) (SLR grade)

o-Chlorophenol

Phenol

o-Cresol

m-Cresol

p-Cresol

Catechol

Resorcinol

Hydroquinone

6 APPARATUS

20 port rotary Borgwaldt smoking machine

Soap bubble manometer to measure puff volume

Analytical balance capable of measuring to at least four decimal places

44mm Cambridge filter pads

Wrist action shaker

Heating block capable of holding 2mL GC vials and incubating to 76°C

Certified thermometer

Agilent GC/MS with autosampler

2mL amber GC crimp top vials with Teflon/Silicone caps

5L dispenser bottle

5L volumetric flask (class A)

100mL amber volumetric flask

0.5mL pipette (class A)

1mL pipette (class A)

2mL pipette (class A)

3mL pipette (class A)

50mL amber volumetric flasks (class A) with stoppers

multidispenser pipette capable of dispensing 250µL and 500µL

500µL gas tight syringe

J&W DB5 MS 60m x 0.25mm i.d x 0.25µm Column

5m of 0.25mm deactivated retention gap

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

Weigh 80mg (±1mg) of o-chlorophenol and transfer to a 5L volumetric flask (class A) and make to volume with TBME. Transfer to a 5L dispenser bottle.

8.2 Stock Standard Solution

To a 100mL amber volumetric flask (class A) add the following amounts of each compound and make to volume with extracting solution (8.1).

Compound	Target Weight (mg)
Phenol	40

o-Cresol	10
m-Cresol	10
p-Cresol	10
Catechol	100
Resorcinol	10
Hydroquinone	80

8.3 Calibration Standards

Dilute the Stock Standard Solution (8.2) as follows in a 50mL amber volumetric flask (class A) and make to volume with extracting solution.

Calibration Standard	Volume (mL) of Stock Solution	Final Volume (mL)
1	0.10	50
2	0.25	50
3	0.50	50
4	1.00	50
5	1.50	50
6	2.50	50
7	3.50	50

The calibration standards will contain the following concentrations of each compound:

Compound	Concentration (µg/mL)						
	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Phenol	0.8	2.0	4.0	8.0	12.0	20.0	28.0
o-Cresol	0.2	0.5	1.0	2.0	3.0	5.0	7.0
m-Cresol	0.2	0.5	1.0	2.0	3.0	5.0	7.0
p-Cresol	0.2	0.5	1.0	2.0	3.0	5.0	7.0
Catechol	2.0	5.0	10.0	20.0	30.0	50.0	70.0
Resorcinol	0.2	0.5	1.0	2.0	3.0	5.0	7.0
Hydroquinone	1.6	4.0	8.0	16.0	24.0	40.0	56.0

8.4 QC Stock Standard Solution

To a 100mL amber volumetric flask (class A) add the following amounts of each compound and make to volume with extracting solution (8.1).

Compound	Target Weight (mg)
Phenol	40
o-Cresol	10
m-Cresol	10
p-Cresol	10
Catechol	100
Resorcinol	10
Hydroquinone	80

8.5 QC Standard

Dilute 1.0mL of the QC Stock Standard Solution (8.4) with extracting solution in a 50mL amber volumetric flask (class A).

The QC standard will contain the following concentrations:

Compound	QC Standard concentration (µg/mL)
Phenol	8
o-Cresol	2
m-Cresol	2
p-Cresol	2
Catechol	20
Resorcinol	2
Hydroquinone	16

All standards are stored in a freezer until required and defrosted thoroughly prior to use. Expiry date: 3 months from date of preparation.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

9.1 Sample Collection

Cigarettes are smoked on a Borgwaldt Rotary 20 port smoking machine. Warm-up the smoking machine for 20 minutes before smoking.

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

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

9.2 Sample Extraction

Place the CFP into a 50mL conical flask and stopper immediately. The CFP holder is wiped with two clean CFP quarters and these are added to the flask. To the flask add 10mL of extracting solution using the preset dispenser, seal with parafilm and shake for 20 minutes (180 revs/minute).

9.3 Sample Clean Up

9.3.1

Dispense a 250µL aliquot of the sample extract or standard followed by 250µL of BSTFA and 500µL of TBME into a 2mL amber autosampler vial.

9.3.2

Cap the vial using Teflon/Silicone 11mm crimp caps and invert to mix. Place the vial in a pre-heated heating block for 30 minutes at 76°C (±5°C). The sample is ready 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 DB5 MS 60m x 0.25mm i.d x 0.25µm, preceded by 5m of 0.25mm deactivated retention gap
Injection type and temperature	Splitless / 250°C
Column temperature programme	Initial 60°C hold for 6 minutes Ramp 10°C/min to 180°C 15°C/min to 300°C;hold for 2 minutes Total run time is 28.0 minutes
Carrier gas	Helium
Transfer line temperature	280°C
Injection Volume	1µL
Column Flow	1mL/min (constant flow)
Solvent Delay	14 minutes
MS Source temperature	230°C
MS Quadrupole temperature	150°C
MS Mode	SIM
Ion Dwell time	100ms

The following ions are used as target and qualifier ions

Compound	Group	m/z	Approximate Retention time (minutes)
Phenol	1	166*(100%) 151(350-380%), 152 (15-25%)	15.4
o-Cresol	2	180*(100%), 165(140-160%), 91(110-130%)	16.8
m-Cresol	2	165*(100%), 180(25-40%), 91(10-20%)	16.9
p-Cresol	2	180*(100%), 165(240-270%), 91(45-60%)	17.1
o-Chlorophenol	3	200*(100%), 185(300-340%), 149(240-265%), 93(260-310%)	18.0
Catechol	4	254*(100%), 239(25-35%), 73(440-520%)	19.6
Resorcinol	4	254*(100%), 239(135-155%), 73(75-110%)	20.4
Hydroquinone	4	254*(100%), 239(120-140%), 73(55-75%)	20.6

* quantitation ion

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 prior to every run. Check the following criteria are met on the tune report:

Air and water peaks <10%

EM Volts 1000 – 3000

Ion ratios of m/z 69:219:502 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 m-cresol peak and the o-cresol peak. The valley should be >90% of the *m*-cresol peak height. If the valley is ≤90%, 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 1, and use the instrument software to assess the phenol peak shape. The value for tailing, as defined in the instrument software, should be <3.26. If the result is >3.26 the problem should be investigated before further analysis.

10.2.4 Ion Ratio Check

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

10.2.5 Calibration Linearity

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

10.3 Run Order

Start with two conditioning samples*

Calibration standards in ascending order

QC standard

10 samples (including the reference cigarette sample)

QC standard

10 samples (including the reference cigarette sample)

etc

Calibration standards in ascending order

*NB: These conditioning samples should be underivatised smoke extracts.

11 CALCULATIONS

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

Peak area ratio = compound peak area/o-chlorophenol peak area

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

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

$$\text{Individual compound concentration (µg/cigarette)} = \frac{C \times V}{N}$$

Where: C = Individual compound concentration in extract (µg/mL)

V = Volume of extracting solution (usually 10mL)

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

Compound	Quantitation Limit (µg/cigarette)	Lower Reporting Limit (µg/cigarette)
Phenol	0.11	1.6
o-Cresol	0.03	0.4
m-Cresol	0.03	0.4
p-Cresol	0.07	0.4
Catechol	0.24	4.0
Resorcinol	0.06	0.4
Hydroquinone	0.19	3.2

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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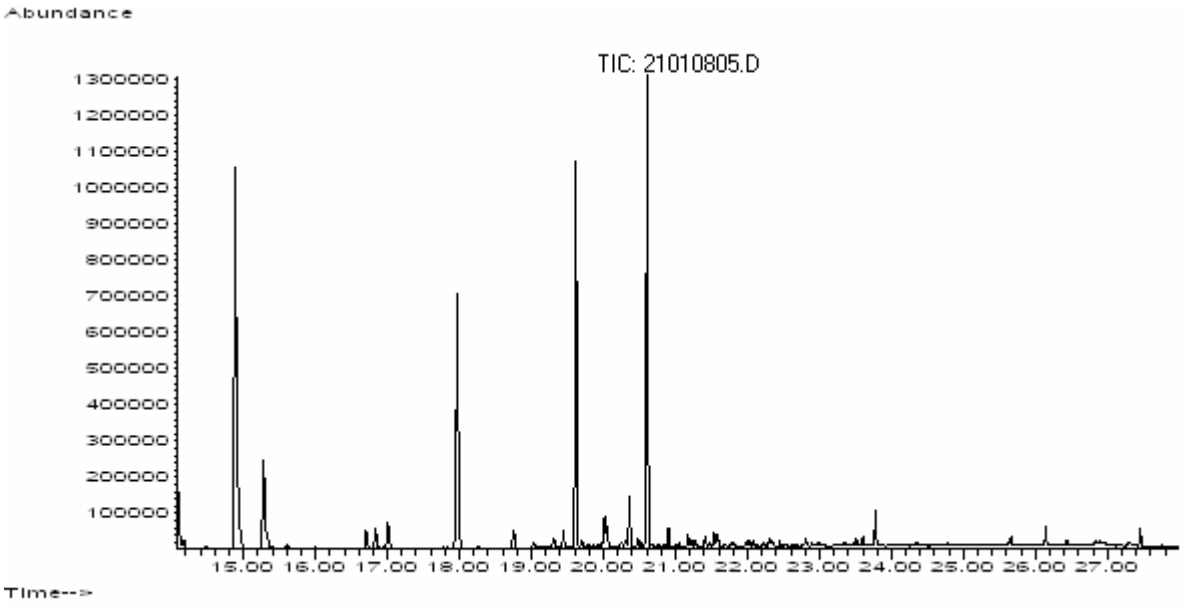
APPENDIX A SAMPLE CHROMATOGRAMS

Calibration Standard - Total ion chromatogram



Smoke Extract - Total ion chromatogram