British American Tobacco Group Research & Development

Method - Determination of ammonia in mainstream smoke

1 SCOPE OF APPLICATION

The method is applicable to quantitative determination of the yields of ammonia in whole mainstream cigarette smoke, using ion chromatography with conductometric 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 fourth edition

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 modified linear 20 port smoking machine to accommodate liquid trapping systems. The mainstream smoke is collected into 2 liquid impingers containing sulphuric acid. After smoking the impinger solutions are combined and diluted using 0.05M sulphuric acid. An aliquot of the resulting solution is submitted for analysis by ion chromatography using conductometric 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 x Ammonium ion Stock Standard solution 1000 µg NH_4^+/mL (must be different batches)

Sulphuric Acid, 0.05M Convol

De-ionised water at 18.2 MΩ

Methanol (HPLC grade)

Helium gas

Methanesulfonic Acid EluGen Cartridge

6 APPARATUS

Borgwaldt-KC LM20 linear 20 port smoke engine. – 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

1mL pipette (Class A)

2mL pipette (Class A)

4mL pipette (Class A)

5mL pipette (Class A)

1mL Pasteur pipettes

20mL borate glass sample tubes

Standard 2mL HPLC vials and caps

1L Volumetric Flask (Class A)

25mL Volumetric Flasks (Class A)

20mL Volumetric Flasks (Class A)

50mL Volumetric Flasks (Class A)

Dispenser suitable for weak acids

Dionex Ion Chromatograph

IonPac CS12A column (8.5µm x 250 x 4mm)

IonPac CG12A guard column (8.5µm x 50 x 4mm)

7 PRELIMINARY SAMPLE PREPARATION

Cigarettes should be conditioned according to normal procedures (ISO 3402:1999). Unless specifically requested, cigarettes are not subject 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

To a 1L volumetric flask (class A) containing approximately 50mL deionised water add one 0.05M Convol of sulphuric acid. Make up to volume with deionised water.

8.2 Needle wash solution

Deionised water containing 5% by volume of methanol.

8.3 Pre-mobile Phase

Deionised water, the mobile phase which passes through the column is produced *in-situ* by the eluent generator unit on the ion chromatograph instrument.

8.4 20µg/mL Stock standard solution

Pipette 1mL of the purchased 1000µg/mL ammonium ion solution into a 50mL volumetric flask (class A) and make up to volume with 0.05M sulphuric acid.

8.5 Calibration Standards

Dilute the 20µg/mL stock solution (see 8.4) as follows into 20 and 25mL volumetric flasks (class A). Make to volume using 0.05M Sulphuric Acid.

Calibration Standard	Concentration of NH_4^+ Standard	Volume of 20µg/mL stock solution (mL)	Final volume (mL)
6	5 µg/mL	5	20
5	3.2 µg/mL	4	25
4	2 µg/mL	2	20
3	1 µg/mL	1	20
2	0.5 µg/mL	2 from Std 6	20
1	0.1 µg/mL	2 from Std 3	20

8.6 QC Stock Solution

Pipette 1mL of the 2nd purchased 1000µg/mL ammonium ion solution into a 50mL volumetric flask (class A) and make up to volume with 0.05M sulphuric acid. This Stock is used to make up the working QC standard

8.7 QC Standard Solution (1.6µg/mL)

Pipette 2mL of the QC Stock Solution (see 8.6) into a 25mL volumetric flask and make to volume with 0.05M Sulphuric Acid.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

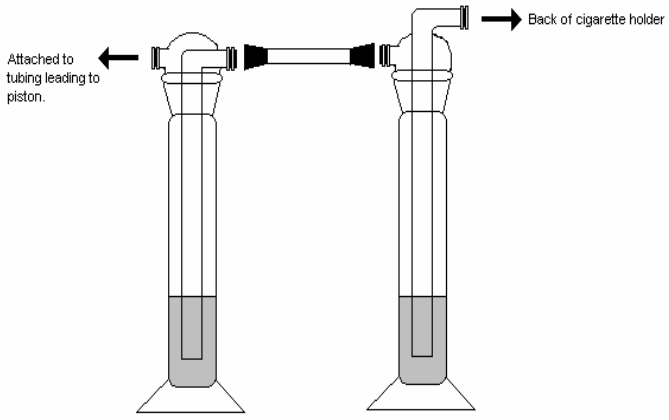
9.1 Sample Collection – Preparation of liquid traps

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 (±50mm/sec) and the average airflow across the measured ports should be 200mm/sec (± 30mm/sec).

9.1.1 Assembly of trapping system

Assemble the ammonia trapping system behind the cigarette holder as shown below, with each trap containing 20mL 0.05M sulphuric acid 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 ± 0.3mL (for ISO smoking).

9.1.2 Smoking

Smoke 5 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

The two impingers are combined and an aliquot of approximately 1mL is added to an HPLC vial and capped.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Analysis is performed on a Dionex Ion Chromatograph fitted with autosampler, eluant generator and conductometric detector.

A 25µL injection loop is used and the injection volume of all samples is 25µL. The gradient profile is shown in the table below:

Time (min)	Conc. Of MSA (mM)	Eluant Generator Curve	Flow Rate (mL/min)	Suppressor Current (mA)
0.0	18	5	1.00	88
7.0	18	5	1.00	88
7.1	40	5	1.00	88
13.0	40	5	1.00	88
13.1	18	5	1.00	88
18.00	18	5	1.00	88

Auto-sampler tray temperature	10°C
Column temperature	30°C
Syringe speed	5
Pressure range	200 psi (min) and 3000 (max)
Flush volume	250µL
Needle height	2mm

10.2 System Suitability Criteria

- R^2 of calibration curve should be greater than 0.98
- The peak area of the lowest standard should be not less than 0.015µSiemens*minutes.
- The peak area of the blank should not exceed 25% of that of the lowest standard.

- Check that the peak shapes, especially those of sodium and ammonium, are well-resolved. peak. If peak shapes are significantly non-ideal, e.g. tailing, consider replacing guard or column.
- The ammonium ion peak will elute in the range 5.00 – 5.60 mins (18mM methanesulphonic acid mobile phase) and will be the second prominent peak (after sodium ion elution at approximately 4.5 min).
- Reference cigarette – results within defined limits
- Quality control solution – results within defined limits

10.3 Run Order

Start the run with 2 blank 0.05M sulphuric acid samples

Calibration standards in desceding order

QC standard

Blank 0.05M Sulphuric Acid

10 samples (including a reference cigarette sample)

QC standard

Blank 0.05M Sulphuric Acid

10 samples (including a reference cigarette sample)

QC standard

etc

11 CALCULATIONS

In order to obtain the value of the amount of ammonium ions released per cigarette, the concentration of the cigarette measurement is multiplied by 40 (volume in mL of trapping solution) and divided by 5 (number of cigarettes smoked). To convert µg/mL of *ammonium ions* into µg/mL of *ammonia* it is necessary to multiply by the factor 17/18.

Calculate the concentration of ammonia (µg/cigarette) from the formula: -

Ammonia (µg/cigarette) = C * V * 17 / N * 18

Where C = Concentration of ammonia (µg/mL) as determined by ion chromatograph software

V = Total volume of sample (normally 40 mL)

N = Number of cigarettes smoked

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 is defined by the concentration of the lowest calibration standard, and equates to 0.8µg/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 regimes the number of cigarettes or the volume of acid in the liquid impingers may need to be adjusted in order to avoid saturating the liquid traps with smoke condensate.

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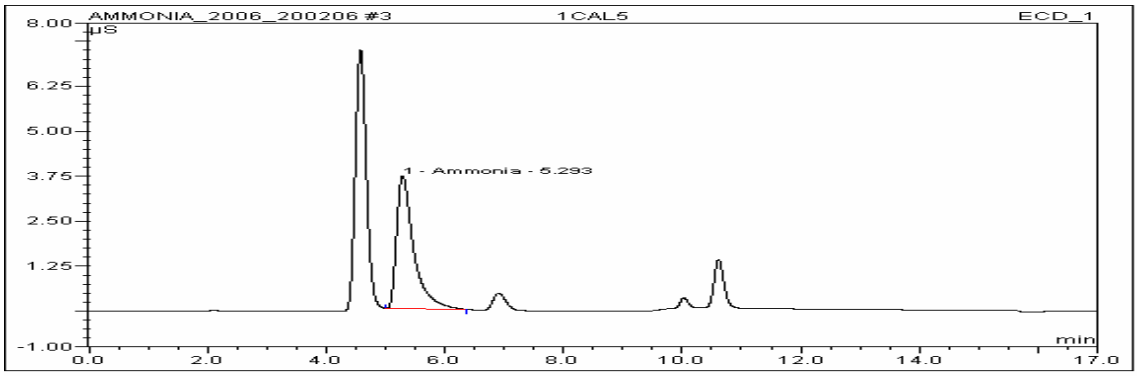
APPENDIX A SAMPLE CHROMATOGRAMS

Typical standard chromatogram



Typical smoke sample chromatogram