British American Tobacco Group Research & Development

Method - Determination of hydrogen cyanide in mainstream smoke

1 SCOPE OF APPLICATION

The method is applicable to the quantitative determination of the yields of hydrogen cyanide in whole mainstream cigarette smoke, using continuous flow analysis.

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 – Sampling

3 PRINCIPLE

Five conditioned cigarettes are smoked per port for each cigarette sample tested using a linear 20 port smoking machine. The mainstream smoke is trapped in aqueous sodium hydroxide solution, where the hydrogen cyanide is converted to sodium cyanide. Determination of the cyanide ion is by reaction in the continuous flow analyser with chloramine-T and with a pyridine/pyrazolone reagent to form a stable chromophore, whose absorbance is measured at 540 nm.

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

Brij-35, 30% solution (GPR)

Chloramine-T, $CH_3.C_6H_4.SO_2.N(Na)Cl.3H_2O$ (GPR)
Potassium cyanide, KCN
Potassium dihydrogen orthophosphate, KH_2PO_4 HPLC Grade
Pyrazolone, (3-methyl-1-phenyl-5-pyrazolin-5-one), $C_{10}H_{10}N_2O$ (Purity >98%)
Bis-Pyrazolone, (3,3'-dimethyl-1,1'-diphenyl-(4,4'-bi-2-pyrazoline)-5,5'-dione, $C_{20}H_{18}N_4O_2$ (GPR)
Pyridine, C_5H_5N
di-Sodium Hydrogen Phosphate, Na_2HPO_4, (Purity >98%)
Sodium Hydroxide, NaOH, 1M Convol

All reagents are Analytical Grade or equivalent unless otherwise stated.

6 APPARATUS

Borgwaldt-KC LM20 linear 20 port smoking machine – 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

44mm Cambridge filter pads

Bran & Luebbe continuous flow analyser (AA III)

Auto-sampler

Proportioning pump

Colorimeter with 540nm filters

Pre-set dispenser

1L measuring cylinder

Sterilin pots

Magnetic stirrer

Filter funnel and paper

0.5mL glass pipettes (Class A)

2mL glass pipettes (Class A)

3mL glass pipettes (Class A)

5mL glass pipettes (Class A)

10mL glass pipettes (Class A)

15mL glass pipettes (Class A)

25mL glass pipettes (Class A)

50mL volumetric flasks (Class A)

100mL volumetric flasks (Class A)

200mL volumetric flasks (Class A)

500mL volumetric flasks (Class A)

1L volumetric flask

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 as specified in ISO 4387:2000 unless otherwise requested.

8 ANALYTICAL PROCEDURE –SOLUTION PREPARATION

8.1 Preparation of reagents

8.1.1 Buffer solution

Dissolve 13.6g (± 0.01g) of potassium dihydrogen phosphate and 0.28g (± 0.001g) of di-sodium hydrogen phosphate in approximately 600mL deionised water in a 1000mL beaker. Pour into a 1000mL volumetric flask using a funnel, make up accurately to 1000mL with deionised water in a volumetric flask, add 0.5mL of Brij-35 and mix thoroughly using a magnetic stirrer. Store in an amber bottle.

8.1.2 Stock Pyrazolone Solution

Add 2.5g (± 0.01g) of pyrazolone to about 800mL of deionised water. Leave stirring overnight until no more will dissolve. Make up accurately to 1000mL with deionised water in a volumetric flask and mix thoroughly. Filter and store in a amber bottle.

8.1.3 Pyridine / Pyrazolone Reagent

Dissolve 0.04g (± 0.001g) of bis-pyrazolone in 40mL of pyridine. Add 210mL of stock pyrazolone solution (7.1.2). Make up accurately to 250mL with deionised water in a volumetric flask and mix thoroughly. Store in an amber bottle. Check colour of reagent every day. If solution is pink discard the solution and prepare fresh solution. Do not keep solutions for longer than one week.

8.1.4 Chloramine-T Solution, 0.4%

Dissolve 2g (± 0.01g) chloramine-T in approximately 300mL of deionised water. Make up accurately to 500mL with deionised water in a volumetric flask and mix thoroughly. Prepare fresh weekly. Store in an amber bottle.

8.1.5 Sodium Hydroxide Solution (1M)

In a 1L volumetric flask containing approximately 100mL deionised water add one 1M Convol of sodium hydroxide. Rinse Convol through with deionised water and make up accurately to 1L with deionised water. Mix thoroughly and pour into dispenser.

8.1.6 Brij-35 Wash Solution

Add 0.5mL of Brij-35 to 1000mL of deionised water in a glass bottle.

8.2 Preparation of Standards

8.2.1 Potassium Cyanide Stock Solution (Equivalent HCN concentration = 2.0mg/mL)

Weigh 1.2056g (± 0.0001g) of potassium cyanide, transfer quantitatively to a 250mL volumetric flask and make up to volume with 1M sodium hydroxide solution.

[Note: The formula weight of potassium cyanide is 65.1, and that of hydrogen cyanide is 27. Thus the equivalent weight of hydrogen cyanide in the stock solution is

$$1.2056g \times \frac{27}{65.1} = 0.5000g$$

and the equivalent hydrogen cyanide concentration is 2.00mg/mL]

Store the potassium cyanide stock in a refrigerator**.** This is stable for six months.

8.2.2 Potassium Cyanide Intermediate Stock Solution (Equivalent HCN concentration 80µg/mL)

Pipette a 10mL aliquot of stock solution into a 250mL volumetric flask and make up accurately to volume with 1M sodium hydroxide solution. Store the cyanide intermediate solution in a refrigerator. This is stable for six months.

Hydrogen cyanide content = approximately 80µg/mL

8.2.3 Working Standards

Dilute the potassium cyanide intermediate solution as shown below into 50mL volumetric flasks and make each up to volume with 1M sodium hydroxide solution.

Calibration	Aliquot Volume mL	HCN µg/mL	HCN µg/cigarette *

Standard	(8.2.2)		
1	0.5	0.8	5.6
2	3	4.8	33.6
3	10	16.0	112.0
4	15	24.0	168.0
5	25	40.0	280.0

* When 5 cigarettes are smoked into a total of 35 mL trapping solution.

Store the cyanide solutions in a refrigerator and prepare new working standards every two months from the Stock solution.

8.2.4 Potassium Cyanide QC Stock Solution (HCN concentration 400 µg/mL)

Weigh 0.1929g (± 0.0001g) of potassium cyanide, quantitatively transfer to a 200mL volumetric flask and make up to volume with 1M sodium hydroxide solution. The hydrogen cyanide content of this solution is 400µg/mL.

8.2.5 Potassium Cyanide QC Solution (HCN concentration 20 µg/mL)

Pipette a 5mL aliquot of QC stock solution into a 100mL volumetric flask and dilute to volume with 1M sodium hydroxide solution. The hydrogen cyanide content of this solution is 20µg/mL.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

9.1 Sample Collection

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.

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).

Check the puff volume with a soap bubble manometer ensuring that it is 35mL (± 0.3mL).

9.1.1 Assembly of trapping system

Assemble the hydrogen cyanide trapping system behind the cigarette holder as follows:

i. Front trap containing 25mL 1M sodium hydroxide solution delivered from a pre-set dispenser.

ii. Rear trap containing 10mL 1M sodium hydroxide solution 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.

9.2 Sample Extraction

At the completion of smoking transfer the contents of both traps into a clean Sterilin pot. This is done by pouring the front trap into the pot then rinsing the front trap with the back trap, transfer to pot, shake and use the combined extract as the sample for analysis.

For high hydrogen cyanide delivery cigarettes, the smoke extract should be diluted quantitatively with 1M sodium hydroxide solution. The dilution factor must be recorded and correction made in the calculation of results.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Set up the continuous flow analyser. The sample time should be set at 90 seconds and the wash time 30 seconds. Aspirate the system with deionised water (with 1mL of Brij added) for approximately 15 minutes. Transfer the reagent pickup tubes to their appropriate reagents and aspirate until a steady baseline and uniform bubble patterns are observed. The bubbles should flow smoothly through the CFA and be uniform in shape and spacing with rounded ends.

10.2 System Suitability Criteria

The16µg/mL Standard should have an HCN content equivalent of between 100-120µg/cig

10.3 Run Order

Run a set of duplicate standards through the analyser followed by a QC standard, a blank trapping solution and then the smoke samples. After a maximum of 10 smoke samples run a QC standard and at the end of the sequence, run standard 4. When all samples have been run, transfer all reagent pick up tubes into deionised water and aspirate for approximately 20 minutes.

11 CALCULATIONS

This calculation is performed automatically by the analytical instrument.

Plot a graph of corrected absorbance units for each standard against hydrogen cyanide content (µg per cigarette). The calibration graph should have an R^2 value of >0.999.

Determine the hydrogen cyanide concentration (µg per cigarette) for each smoke sample from the calibration graph.
If a manual check of the calculation is required the following equation can be used:

$$\text{Hydrogen cyanide (µg per cigarette)} = \frac{C \times V \times D}{N}$$

Where:

C = concentration (µg per mL) of hydrogen cyanide determined from the calibration graph

V = volume (mL) of smoke extract (normally 35mL)

N = number of cigarettes smoked per port (normally 5)

D = dilution factor (only required if sample has been diluted)

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 1.94µg/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 5.60/µg/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 smoked per port may need to be reduced to avoid overloading the trapping solution.

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