

CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

ANNEX A

Part 6 Method: Determination of hydrogen cyanide yields in mainstream cigarette smoke by UV/Visible spectroscopy

COMMISSIONED BY :

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*Setting standards
in analytical science*

1 Scope of the method

- 1.1 This method is suitable for the determination of hydrogen cyanide yields in mainstream tobacco smoke by UV/Visible Spectroscopy.

2 Principle of the method

- 2.1 For each sample, two conditioned cigarettes are smoked on a 20 channel linear smoking machine. The mainstream smoke is passed through a Cambridge filter holder and then a glass tube containing silica gel. The hydrogen cyanide is extracted from the filter pad and silica gel into dilute sodium hydroxide solution. The smoke solutions are then treated to a two stage process which complexes the cyanide ions present so that the absorbance might be measured. First, an aliquot of the smoke solution is put into a test tube, buffer solution added followed by chloramine-T to generate cyanogen chloride. Secondly, pyridine/barbituric acid reagent is added to produce a visibly coloured complex. The solutions are analysed using a UV/visible spectrophotometer.
- 2.2 The instrument is calibrated by preparing a set of standards of potassium cyanide and analysing using the method. A blank is analysed with each set of samples.

3 Apparatus

- 3.1 20 Channel linear Filtrona smoking machine and ancillary equipment
- 3.2 Cambridge 44 mm glass fibre filter pads and holders
- 3.3 Glass 'manifold' tube to act as a silica gel trap. (see Figure 1). The internal diameter of the tube is such that the back of the Cambridge filter holder (3.2) fits securely (i.e. no leaks).
- 3.4 Glass wool
- 3.5 1000 mL amber bottles (for storing stock solution)
- 3.6 250 mL conical flasks (or equivalent).
- 3.7 Automatic pipettes (1 ml and 5 mL)
- 3.8 UV/Visible spectrophotometer (fitted with flow through cell) – or “manual” instrument
- 3.9 10 mm glass cells or disposable plastic cuvettes
- 3.10 Test tubes and stoppers
- 3.11 Analytical balance – 4 decimal place
- 3.12 Glass pipettes (1 mL, 2 mL, 5mL, 10mL, 15mL, 20mL)
- 3.13 Orbital shaker

4 Reagents

Reagents are Analytical Grade or equivalent unless otherwise stated.

- 4.1 Non indicating silica gel, granular 2.5- 6 mm - (dry for a minimum of 24 hours at 105°C and store in a dessicator before use).
- 4.2 0.1 M Sodium Hydroxide solution
- 4.3 Water (Elgastat – UHP) or equivalent

- 4.4 Potassium dihydrogen orthophosphate
- 4.5 *di*-sodium hydrogen orthophosphate
- 4.6 Chloramine-T
- 4.7 Pyridine
- 4.8 Barbituric Acid
- 4.9 Concentrated Hydrochloric acid
- 4.10 Potassium Cyanide (relative molar mass 65.12*, \equiv HCN relative molar mass 27.03*)

5 Preparation of standards and reagents

Preparing concentrated solutions of Potassium Cyanide must be performed by a trained competent operator. Solutions are prepared in a spill tray within a fume cupboard.

- 5.1 **Stock solution 1** (nominal concentration 2 mg mL⁻¹ HCN): Using a weighing boat, weigh out 2.4000 g \pm 0.1000 g potassium cyanide (4.10) – record the weight. Quantitatively transfer to a 500 mL volumetric flask half full of sodium hydroxide solution (4.2) and make up to the mark with NaOH solution. Stopper securely and shake gently to mix. Store in a labelled amber glass bottle (3.2) – expiry date 1 month.
- 5.2 **Stock solution 2**: (nominal concentration 32 μ g mL⁻¹ HCN): Pipette 4 mL (3.12) of stock solution 1 (5.1) into a 250 mL volumetric flask half full of sodium hydroxide solution (4.2) and dilute to volume with NaOH soln. Stopper securely and shake gently to mix. The solution is stable for 1 week.
- 5.3 Using glass pipettes, pipette the following volumes of stock solution 2 (5.2) into a series of 100 mL volumetric flasks and dilute to volume with sodium hydroxide solution (4.2). Stopper and shake to mix. Prepare daily.

Volume of stock solution (5.2) added (mL)	Nominal concentration of cyanide solution (as HCN μ g mL ⁻¹)	Nominal concentration of HCN in cigarette smoke (μ g cig ⁻¹)
1	0.32	24
2	0.64	48
5	1.6	120
10	3.2	240
15	4.8	320
20	6.4	480

- 5.4 **Phosphate Buffer Solution** – Weigh out 27.20 \pm 0.30g potassium dihydrogen orthophosphate (4.4) and 0.56 \pm 0.05g *di*-sodium hydrogen orthophosphate (4.5). Quantitatively transfer to a 1000mL volumetric flask and dilute to volume with water (4.3). Stopper and shake to mix. Store in a glass bottle in a cupboard – the solution is stable for six months but discard if there is any algae growth present in the solution.

* NIST – www.nist.gov

- 5.5 **Chloramine-T** – Weigh out 0.40 ± 0.03 g of Chloramine-T and quantitatively transfer to a 100 mL volumetric flask, make up to the mark with water (4.6). Prepare fresh each day.
- 5.6 **Pyridine-barbituric acid reagent** –In a 250 mL beaker weigh out 7.50 ± 0.08 g barbituric acid and dissolve in 50 mL water (4.3). In a fume cupboard, while constantly stirring with a glass rod, add 38mL pyridine (4.7), while at the same time slowly adding 5 mL hydrochloric acid (4.9). Make up to 500mL in a volumetric flask with water. Store in an amber bottle and keep refrigerated - solution is stable for two days.

6 Method

- 6.1 Cigarettes are conditioned¹ at a temperature of $22 \pm 1^\circ\text{C}$ and 60 ± 2 % relative humidity for a minimum of 48 hours but not exceeding 10 days.
- 6.2 Butt marking is to ISO butt length specifications². Filtered cigarettes are smoked to a measured butt length equal to either the tipping paper + 3 mm or filter length + 8 mm whichever is longer. The minimum butt length is 23 mm and this is also used for non filter brands. All smoking is conducted in an environment of temperature $22 \pm 2^\circ\text{C}$ and 60 ± 5 % relative humidity¹.
- 6.3 As far as practicable, ISO conditions³ for smoking cigarettes apply. The smoking machine puffing parameters are 35 ± 0.2 cm³ puff volume with 2.0 ± 0.05 second puff duration once every 60.0 ± 0.5 seconds. The smoking machine may have to be adjusted slightly to achieve a 35 cm³ puff volume.
- 6.4 A minimum of five determinations are performed for each brand. The smoking of the cigarette brands are randomised so that samples from the same brand are smoked on different days on different channels.
- 6.5 For each sample one silica gel trap is required (6.5). NB The back of the Cambridge filter holder is connected to the glass trap which replaces the metal port on the channel of the smoking machine.
- 6.6 Place a small piece of glass wool (3.4) at the point where the glass tube narrows (3.3). Weigh out 4 ± 0.1 g of silica gel (4.1), transfer to the tube and shake gently to make sure the gel has settled (i.e. no large gaps). Tease out a piece of glass wool and using tweezers insert into the tube – this keeps the silica gel in place (Figure 1). Assemble the traps on the smoking machine, and fit the labyrinth holders⁴.

Figure 1



- 6.7 It is practicable to smoke twenty samples in a batch. Additionally a blank determination should be included with every batch. The sample blank will be a trap assembly containing silica gel and filter pad or prepared by puffing an unlit 1R4F cigarette through the trap under identical conditions to those used for smoking the cigarettes.

- 6.8 Two cigarettes are smoked using ISO specifications.
- 6.9 After each cigarette has been smoked, a clearing puff is taken and five clearing puffs at the end of the smoking run.
- 6.10 Disconnect the silica gel traps and holders from the smoking machine.
- 6.11 For each trap, transfer the pad into to a labelled 250 mL conical flask (3.6). Wipe the inside of the trap with two quarter pads and also add this to the flask. Transfer the glass wool portions and silica gel to the appropriate flask.
- 6.12 Using a measuring cylinder, add 150 mL of NaOH solution (4.2) to the flask.
- 6.13 Place flasks on an orbital shaker (3.13) and shake for 20 minutes.

7 Analytical procedure

- 7.1 Switch on the UV/Spectrophotometer and allow to warm up - wavelength - 575nm
- 7.2 Pipette (3.7) 0.5 mL of each sample/standard/blank into labelled test tubes.
- 7.3 Pipette (3.7) 5 mL of buffer (5.4) into each tube.
- 7.4 Pipette 2 mL (3.7) Chloramine-T (4.6) into each tube, shake gently to mix and allow to stand for 3 minutes.
- 7.5 Pipette 8 mL of the pyridine-barbituric acid mixture (5.6) into each tube. Stopper the tube and invert several times to ensure thorough mixing. Allow a minimum of 5 minutes for the magenta colour to develop. NB Before analysis, invert the stoppered tube to ensure the contents are mixed thoroughly.
- 7.6 Measure the absorbances with the spectrophotometer (3.8) – if using the autoflow cell then do two consecutive readings. Solutions should be analysed within 5 to 15 minutes of the addition of the pyridine-barbituric mixture (7.5). Collect the waste in a labelled waste solvent bottle.

8 Validation of method

- 8.1 The following results were obtained for 1R4F and 1R5F using the method

Brand	No of determinations	Mean Ammonia yield $\mu\text{g cig}^{-1}$	Comments (anticipated values)
1R4F	5	123 ± 10.1	Same day
1R4F	5	125 ± 9.88	Results from study (different days)
1R5F	5	23.0 ± 2.04	Same day
1R5F	5	22.8 ± 1.07	Results from study (different days)

- 8.2 The blank sample gives a small absorbance compared to a water sample. Therefore, to determine a limit of quantitation, a blank sample solution was put through the analysis procedure ten times and a limit of quantitation (and reporting limit) determined. The mean of the blank plus 5 sample standard deviations gave a concentration of $0.10 \mu\text{g mL}^{-1}$. Therefore the reporting limit was set at $<8 \mu\text{g cig}^{-1}$ (2 cigarettes and 150 mL volume)

8.3 Normal laboratory QC procedures should be in place as summarised in the table below:

Aim	How achieved
To show smoking is to ISO conditions	<ul style="list-style-type: none"> • Check puff volumes • Check puff profiles • Check environmental conditions • Establish correct butt mark lengths • Determine CO yields
To show calibration is satisfactory	<ul style="list-style-type: none"> • Print graph and “look at curve” - check for linearity • Check R² (>0.99), intercept close to Zero/ Zero.
To show analytical instrument is calibrated and operating satisfactorily	<ul style="list-style-type: none"> • Use analytical standards which are in date • Run standard at end of run to check calibration • Analysis of blank matrix
To show standard laboratory equipment is functioning satisfactorily	<ul style="list-style-type: none"> • Balance – check weight • Temperature check of refrigerators and freezers • Pipettes – volume check • Conditioning cabinet – temperature and relative humidity • Smoking environment – temperature and relative humidity • Air flows – within specification

9 Results

9.1 Results should be reported as µg cig⁻¹

9.2 Mean, standard deviation and relative standard deviation will be determined for each brand of cigarettes and Dixon’s outlier test will be performed (5% level).

10 Calculations

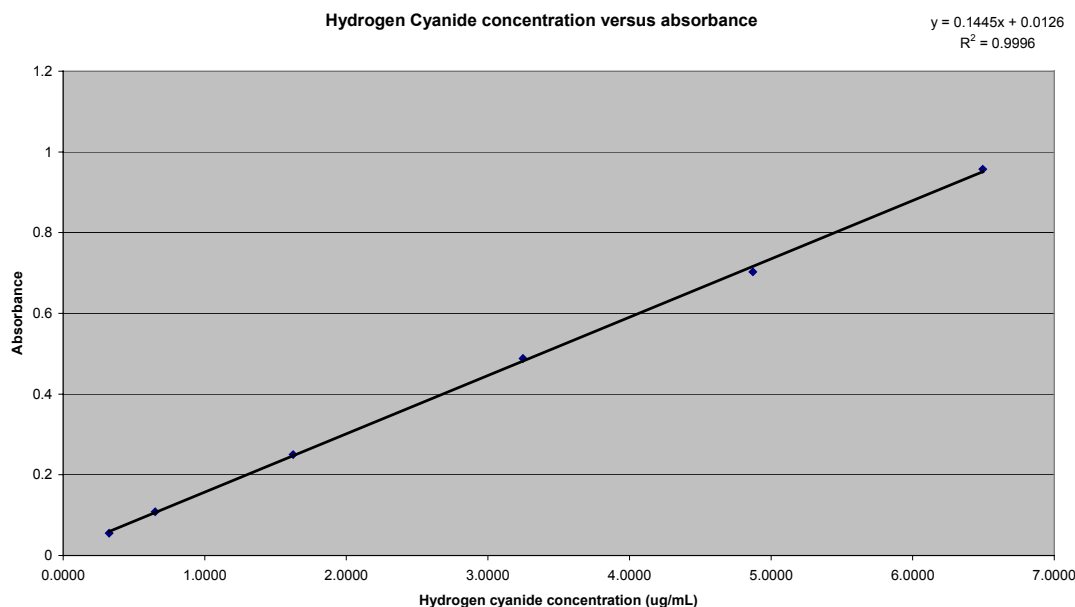
10.1 Using a spread sheet package, determine the concentration of hydrogen cyanide in the stock solutions. The calculation for stock solution 1 is shown below:

$$\text{concentration of HCN in stock solution 1 (mg mL}^{-1}\text{)} = \frac{\text{weight of KCN (g)} \times 27.03 \times 1000}{65.12 \times 500 \text{ (mL)}}$$

10.2 From this calculate the concentration in µg mL⁻¹ of each of the working standards.

10.3 Take an average of the two absorbance readings for each standard. Plot a calibration graph of concentration (µg mL⁻¹) versus absorbance.

Figure 2



10.4 A typical example of a calibration curve is shown in Figure 2.

10.5 Calculate the linear regression equation without forcing the line through zero. Check the plots, coefficient of determination (r^2) and intercept before accepting the calibration (see validation section for acceptance criteria).

10.6 Use the calibration curve to determine the concentration of HCN in the extract solution for each sample plus the blank

10.7 Calculate the HCN yield per cigarette for each sample using the following equation:

$$\text{HCN yield}(\mu\text{g/cig}) = [\text{sample solution concentration} (\mu\text{g/mL})] \times \frac{\text{volume of NaOH soln (mL)}}{\text{no. cigarettes smoked (cig)}}$$

¹ ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing

² ISO :4387: 2000 - Methods for chemical analysis of tobacco and tobacco products – Part 14: Determination of total and nicotine- free dry particulate matter using a routine analytical smoking machine

³ ISO 3308:2000 – Routine analytical cigarette smoking machine – Part 1: Definitions and standard conditions

⁴ HEA/B1-0001 (standard operating procedure) : Total Particulate Matter (and NFDPM) yields of cigarette smoke using a linear smoking machine.