CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

ANNEX A

Part 5 Method: Determination of ammonia yields in mainstream cigarette smoke using the Dionex DX-500 ion chromatograph

COMMISSIONED BY :

Tobacco Manufacturers Association

55 Tufton Street

LONDON SW1P 3QL

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Contact Point: IAN AXFORD

Tel: 020 8943 7375

Prepared by: Pete Houlgate

Consumer Safety & Tobacco Products Group

1 Scope of the method

1.1 This method is applicable to the determination of ammonia yield in mainstream tobacco smoke using the Dionex DX-500 ion chromatograph.

2 Principle of the method

2.1 For each sample, eight conditioned cigarettes are smoked on a 20 channel linear smoking machine. The mainstream smoke is passed through a Cambridge filter pad into a bubbler containing 0.1 mol L^{-1} Malic acid. The filter pads are extracted with the malic acid from the bubbler. The solutions are filtered through syringe filters before analysing within 12 hours using the Dionex ion chromatograph fitted with a conductivity cell.

2.2 The instrument is calibrated with a set of ammonium chloride standards. A blank is analysed with each set of samples.

3 Apparatus

3.1 20 Channel linear Filtrona smoking machine and ancillary equipment

3.2 Bubblers

3.3 44 mm Cambridge filters pads

3.4 Cambridge filter trap consisting of pad (3.3) plus holder

3.5 125 mL conical flasks and stoppers

3.6 Orbital shaker

3.7 Glass pipette

3.8 Automatic pipette (0 – 5 mL)

3.9 Dionex DX-500 ion chromatograph

3.10 IonPac CS12 Analytical Column 4 × 250 mm

3.11 IonPac CG12 Guard 4x50mm

3.12 Self Regenerating Suppressor 4mm

3.13 Disposable 10mL syringes & Acrodisc Syringe Filters

3.14 Analytical balance – 4 decimal place

4 Reagents

Reagents are Analytical Grade or equivalent unless otherwise stated.

4.1 0.1 mol L^{-1} Malic Acid

4.2 Ammonium Chloride

4.3 Water (Elgastat – UHP) or equivalent

4.4 0.5 mol L^{-1} Sulfuric acid

4.5 Mobile phase - using a measuring cylinder add 44 mL of sulfuric acid (4.4) to a 2 litre reservoir. Add 1960 mL of water (4.3) to the reservoir and swirl to mix.

5 Preparation of standards and reagents

5.1 **Stock solution 1** (nominal concentration 1000 mg mL^{-1} NH_4 ions)**:** Using a weighing boat, weigh out 0.2970 g ± 0.0010 g ammonium chloride (4.2). Quantitatively transfer to a 100 mL volumetric flask and dilute to volume with 0.1M Malic acid (4.1). Stopper securely and shake gently to mix. Expiry date 1 month.

5.2 **Stock solution 2** (nominal concentration 100 mg mL^{-1} NH_4 ions)**:** Take a 10ml aliquot of stock 1 with a glass pipette (3.7) and pipette into a 100mL volumetric flask. Dilute to volume with 0.1M Malic acid (4.1). Stopper securely and shake gently to mix. Expiry date 1 month.

5.3 Using the automatic pipette (3.8), pipette the following volumes of stock solution 2 (5.2) into a series of 100 mL volumetric flasks and dilute to volume with malic acid solution (4.1). Stopper and shake to mix. Prepare daily.

Standard No.	Volume of stock solution (5.2) added (mL)	Nominal concentration of ammonium solution (as NH_4^+ mg mL^{-1})	Nominal concentration* of NH_3 in cigarette smoke[i] (mg cig^{-1})
1	2.5	2.5	12.5
2	2	2	10
3	1.5	1.5	7.5
4	1	1	5
5	0.5	0.5	2.5
6	0.25	0.25	1.25

6 Method

6.1 Cigarettes are conditioned[ii] at a temperature of 22 ± 1°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 specifications[iii]. 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 ± 2°C and 60 ± 5 % relative humidity [ii].

6.3 As far as practicable, ISO conditions[iv] for smoking cigarettes apply. The smoking machine puffing parameters are 35 ± 0.2 cm^3 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 cm^3 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 Cigarettes are conditioned [ii] at a temperature of 22 ± 1°C and 60 ± 2 % relative humidity for a minimum of 48 hours but not exceeding 10 days.

6.6 It is practicable to smoke ten samples in a batch. Additionally a blank determination should be included with every batch. The sample blank can be either a Cambridge filter pad or will be prepared by puffing an unlit 1R4F cigarette through the assembly under identical conditions to those used for smoking the cigarettes.

* Based on total sample volume of 50 mL and eight cigarettes smoked

6.7 Assemble a Cambridge filter trap (3.4), bubbler and back up filter pad holder for each channel.

6.8 Add 30 mL malic acid (4.1) to each bubbler with a measuring cylinder. Complete the assembly on the smoke machine. See Figure 1.

Figure 1

6.9 Eight cigarettes are smoked using ISO specifications.

6.10 After each cigarette has been smoked, a clearing puff is taken. At the end of the smoking run five clearing puffs are taken.

6.11 For each trap, transfer the pad from the trap (6.7) to a labelled flask (3.5). Wipe the inside of the holder with a quarter piece of pad and also add to the flask. Stopper the flask

6.12 Place the first stoppered flask on a balance (3.14) and tare. Add the bubbler contents to the flask. Rinse the bubbler with 2 lots of malic acid (4.1), ca 15 – 20 mL in total and add these washings to the flask. Reweigh the flask and record the weight of malic acid added. For low tar cigarettes (NFDPM yield <4 mg cig^{-1}), keep the washings to a minimum (e.g. 10 mL).

6.13 For the purposes of the study, the density of malic acid 0.1 mol L^{-1} is taken to be 1.

6.14 Repeat 6.12 for the other flasks. NB for the blank, add 50 mL by measuring cylinder if it was not set up on the smoking machine.

6.15 Place the flasks on a shaker (3.6) and shake for 20 minutes.

6.16 Filter the extract into 5 mL sample vial using a syringe and filter (3.13) and place in an auto-sampler tray. Analysis should take place within 12 hours.

7 Setting up the Dionex

7.1 The following parameters were used

7.1.1 Mobile phase (4.5) flow rate 1 mL min^{-1}

7.1.2 Run time 18 minutes

7.1.3 Method – cations.net

7.2 The following run sequence should be followed:

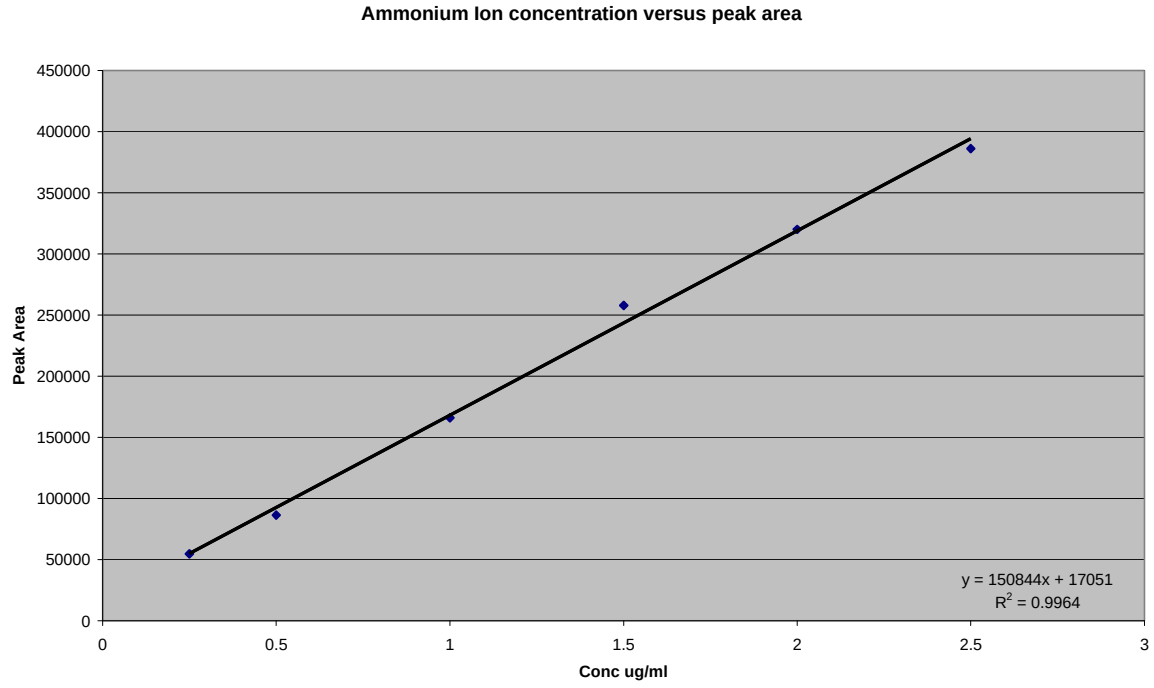
Standards 6 to 1
Samples 1 to 10
Blank (random position between 2 samples)
Standards 1 to 6

Note it is practicable to smoke two runs in a day, and analyse on the Dionex in one batch – in this case include the 2 µg mL^{-1} ammonium standard in the middle of a sequence as a check on the performance.

8 Validation of method

8.1 The following results were obtained using the method

8.2 A typical calibration curve is shown below



8.3 The following data was obtained for 1R4F and 1R5F

Brand	No of determinations	Mean Ammonia yield mg cig^{-1}
IR4F	5	8.01 ± 0.39
1R5F	5	1.90 ± 0.20

8.4 Stability - A solution of 1R4F was analysed after ca 36 hours and the ammonium ion concentration was found to have increased significantly (ca 30%). Therefore, solutions are analysed within 12 hours of smoking[†].

8.5 Trapping efficiency - two samples (Brand A and B) were analysed separately (pad and bubbler solution). Brand A gave 5.4 μg cig^{-1} (pad) and 0.3 μg cig^{-1} in the bubbler. Brand B gave 2.9 μg cig^{-1} (pad) and no ammonia detected in the bubbler.

8.6 Spiking - Two sample solutions (Brand A and B) were spiked (50/50) with a 1 mg mL^{-1} ammonium standard. Brand A gave 5.8 μg cig^{-1} and Brand B gave 2.8 μg cig^{-1} after deduction of the contribution of the spike.

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

Aim	**How achieved**
To show smoking is to ISO conditions	• Check puff volumes • Check puff profiles • Check environmental conditions • Establish correct butt mark lengths • Determine CO yields
To show calibration is satisfactory	• Print graph and "look at curve" - check for linearity • Check R^2 (>0.99) • Monitor intercept for significant change
To show analytical instrument is calibrated and operating satisfactorily	• Use analytical standards which are in date • Quality control sample • Analysis of blank matrix
To show standard laboratory equipment is functioning satisfactorily	• 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

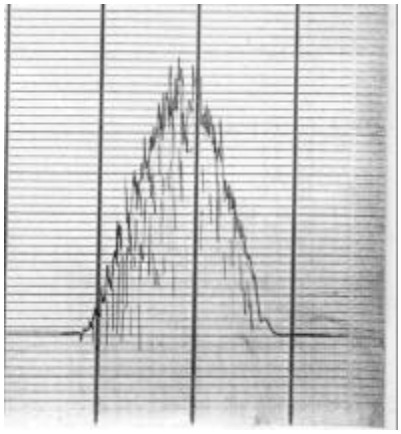
9.1 Results should be reported as μg cig^{-1}

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).

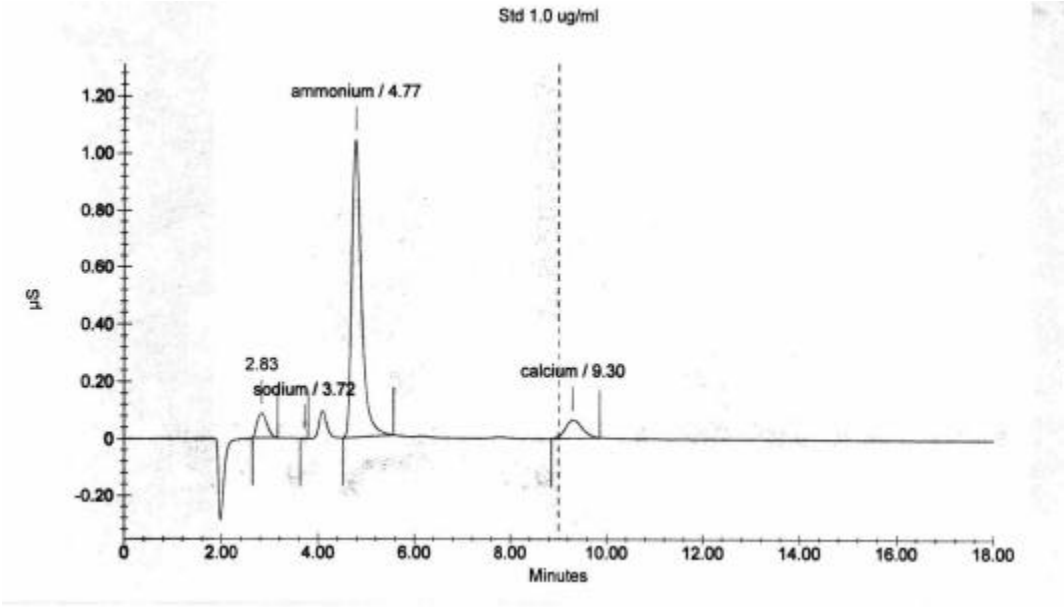
[†] *Note – In practice sample solutions should be analysed asap after smoking.*

10 Appendix

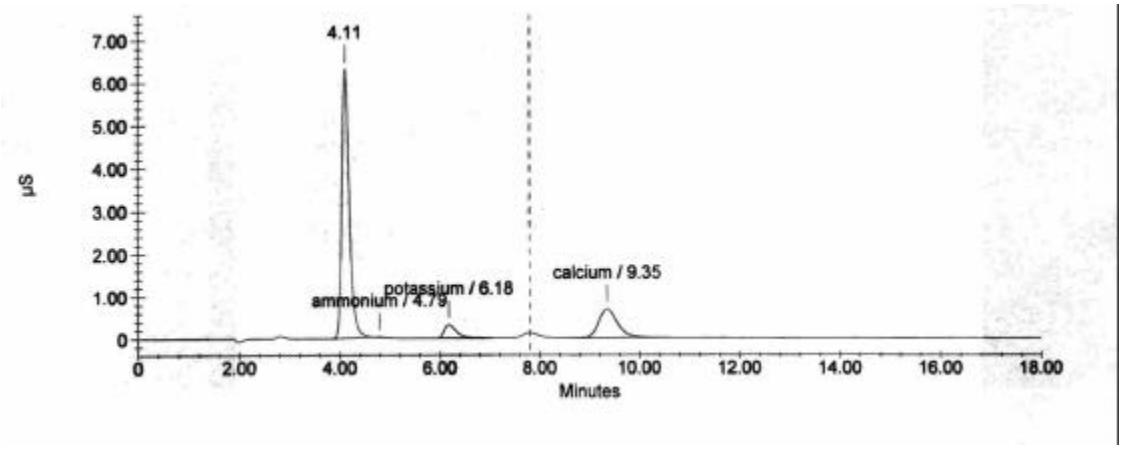
10.1 Puff Profile



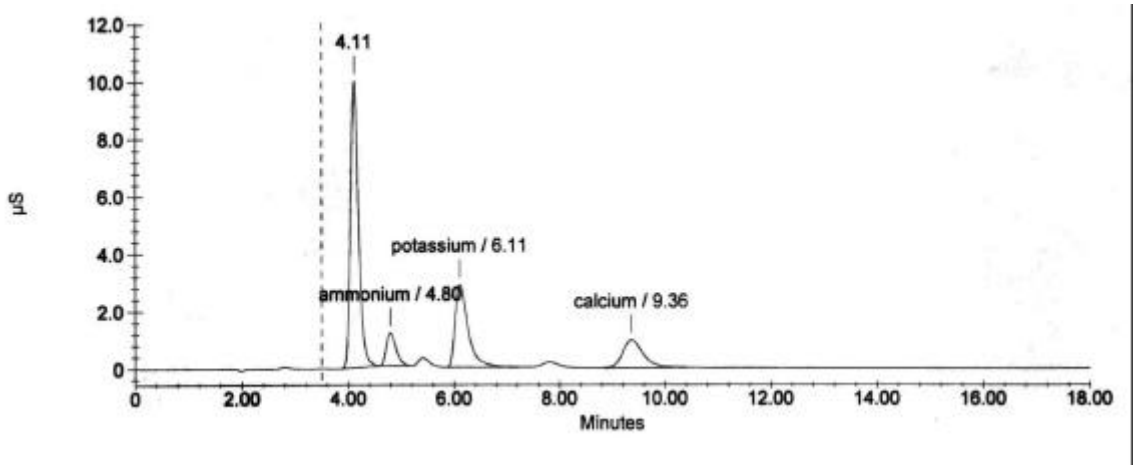
10.2 Chromatogram of a Standard



10.3 Chromatogram of a Blank



10.4 Chromatogram of Reference Cigarette 1R4F



11 Calculations

11.1 Using a spread sheet package, calculate the concentration of ammonium ions in µg mL^{-1} in the stock and working standards.

11.2 Calculate the peak area average for each standard. Plot a calibration graph of ammonium concentration (µg mL^{-1}) versus mean peak area.

11.3 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).

11.4 Use the calibration curve to determine the concentration of ammonium ions in the extract solution for each sample plus the blank

11.5 Calculate the ammonia yield per cigarette for each sample using the following equation:

$$\text{Ammonia yield } \left(\mu g\, cig^{-1}\right) = \frac{\text{sample } NH_4 \text{ solution conc.} \left(\mu g\, mL^{-1}\right) \times \text{volume of malic acid } (mL) \times 17.03}{\text{no. cigarettes smoked } (cig) \times 18.04}$$

[i] Relative molar mass of ammonia = 17.03, hydrogen = 1.01 - source www.nist.gov
[ii] ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing
[iii] 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
[iv] ISO 3308:2000 – Routine analytical cigarette smoking machine – Part 1: Definitions and standard conditions