CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

ANNEX B

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

COMMISSIONED BY :

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Setting standards in analytical science

Validation data for ammonia

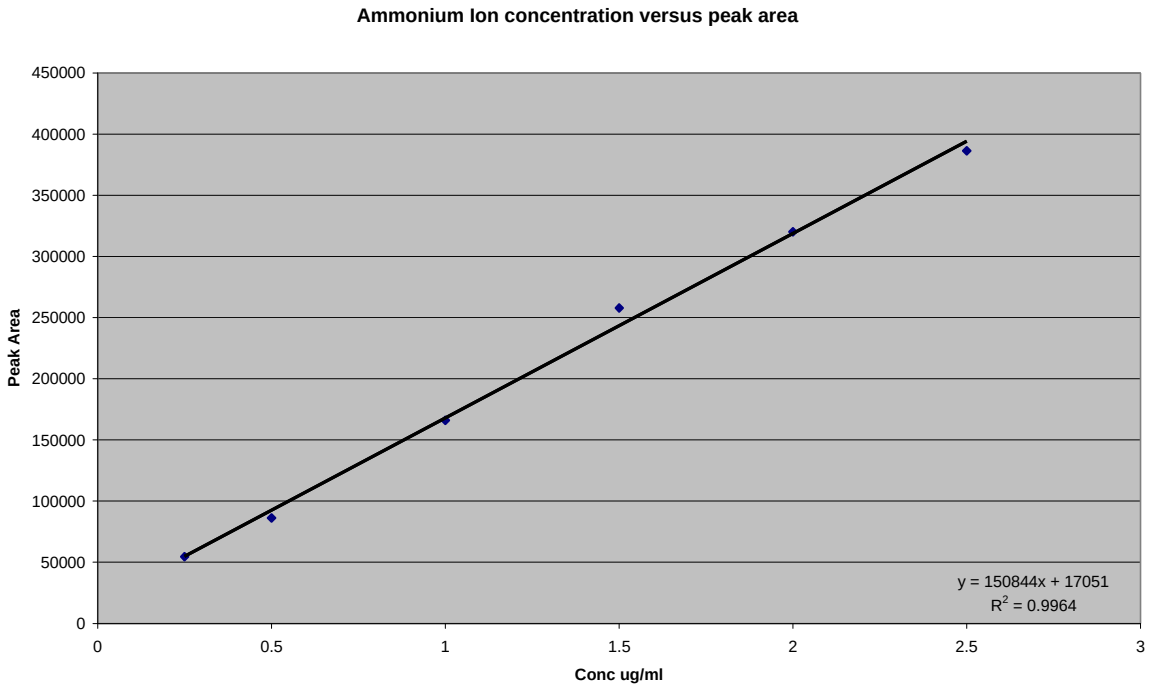
The following is a summary of the validation data obtained when validating the ammonia method.

1. Overview

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 using the Dionex ion chromatograph fitted with a conductivity cell.

2. Calibration

Six standards are used to calibrate the instrument. They are run at the beginning and end of the run – mean peak area calculated and plotted against standard concentration. A typical calibration curve is shown below.



The intercept and slope were found to be consistent over time, i.e. calibrations carried out using 'new' standards on different days. NB If the intercept is too large (i.e. close to the value for the bottom standard) then the calibration is rejected as being unsatisfactory.

As a check on the calibration, a blank sample is run with every batch of samples and one of the standards is run as a QC in the middle of the sequence.

3. Limit of detection/quantitation/reporting

The blank sample gave a small peak area, consistently below the value for the intercept. Therefore, the lowest standard was used as the limit of quantitation (and reporting limit) for the method. Most brands of cigarettes gave values significantly above this limit. For low tar cigarettes, the volume of extract solution was kept to ca 40 mL by limiting the volume of solvent used for washing the bubbler. This resulted in a reporting limit of <1.3 μg cig^{-1} (see also the section on method sensitivity in Appendix 1 of the report)

4. Precision, repeatability and accuracy

1R4F and 1R5F were smoked on the same day to show repeatability within a run. Brand A and B were brands which had recently been used in an inter comparison exercise for a range of analytes including ammonia.

Brand	No of determinations	Mean Ammonia yield μg cig^{-1}	Comments (anticipated values)
IR4F	5	8.01 ± 0.39	Same day
1R4F	5	7.22 ± 0.31	Old calibration range & 5 cigs*
1R4F	5	6.2 ± 1.10	Results from study (different days)
1R5F	5	1.90 ± 0.20	Same day
1R5F	5	0.59 ± 0.19	Old calibration range & 5 cigs *
Brand A	4	5.6 ± 0.4	Same day (6.2 –16.6) †
Brand B	4	2.6 ± 0.1	Same day (3.0 - 6.4)†
Brand C	2	7.7 ± 0.5	Plain cigarette (UK blend – high tar) Old calibration range & 5 cigs*
Brand D	2	23.7 ± 0.4	Filter cigarette (dark air cured tobacco) Old calibration range & 5 cigs*
Brand E	2	2.4 ± 0.2	Filter cigarette (menthol UK blend medium/low tar) Old calibration range & 5 cigs*
Brand F	2	10.4 ± 0.1	Filter cigarette (USA blend – high tar) Old calibration range & 5 cigs*
Brand G	2	1.5 ± 0.4	Filter cigarette (UK blend – low tar) Old calibration range & 5 cigs*

* Initially calibration range was from 4 to 0.1 mg mL^{-1} and five cigarettes were smoked. Since most of the sample solutions were near the bottom standard which was also close to the intercept, the calibration range was altered to 2.5 to 0.25 mg mL^{-1} and the number of cigarettes increased from 5 to 8.

† Results from inter comparison exercise used different smoking regimes and analytical methods. Our smoking regime/analytical method is comparable with bottom end of range of results.

5. Stability

A solution of 1R4F was analysed after ca 36 hours and the ammonium concentration was found to have increased significantly (ca 30%). Therefore, all solutions were analysed within 12 hours of smoking.

Problems with the autosampler during the study resulted in some additional data being generated about the lifetime of the solutions. There is evidence that the ammonium concentration has increased during the first 12 hours and therefore solutions should be analysed asap after smoking.

6. Break through

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.

7. Spiking

Two sample solutions (Brand A and B) were spiked (50/50) with a 1 μg 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.