

Validation data for hydrogen cyanide

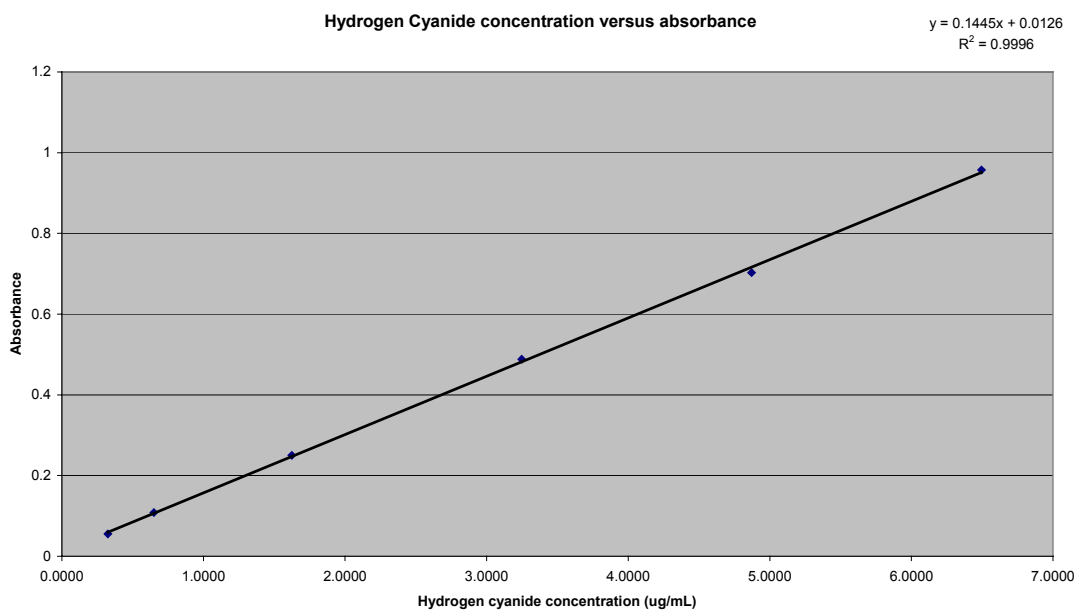
The following is a summary of the validation data obtained when validating the hydrogen cyanide method.

1. Overview

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 eluted from the pad & 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. Calibration

Six standards are used to calibrate the instrument. They are run at the beginning of the run. A standard is also run at the end to check that the calibration has not drifted. A typical calibration curve is shown below.



The intercept and slope were found to be reasonably consistent over time, i.e. calibrations carried out using 'new' standards on different days.

As a check on the calibration, a blank sample is run with every batch of samples.

3. Limit of detection/quantitation/reporting

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 $0.10 \mu\text{g mL}^{-1}$. With 2 cigarettes and 150 mL volume, the reporting limit was set at $<8 \mu\text{g cig}^{-1}$.

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 hydrogen cyanide. Brands C – G were smoked to demonstrate the robustness of the method when handling a range of brands.

Brand	No of determinations	Mean Hydrogen Cyanide 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)
Brand A	4	129 ± 13.6	Same day (125 – 183)*
Brand B	4	63.5 ± 12.5	Same day (47 – 106)*
Brand C	2	91.5 ± 11.9	Plain cigarette (UK blend – high tar)
Brand D	2	114 ± 4.10	Filter cigarette (dark air cured tobacco)
Brand E	2	39.9 ± 0.18	Filter cigarette (menthol UK blend medium/low tar)
Brand F	2	114 ± 9.44	Filter cigarette (USA blend – high tar)
Brand G	2	15.1 ± 4.99	Filter cigarette (UK blend – low tar)

* Results from inter comparison exercise used different smoking regimes and analytical methods.

5. Stability

It was found that there was a significant decrease in hydrogen cyanide concentration in the sample solutions after 3 - 4 hours. Therefore, all solutions were analysed within 2 hours of smoking. The coloured complex developed in the test tube is unstable and so should be analysed within 5 –to 15 minutes of the pyridine-barbituric reagent being added.

6. Silica gel & extraction volume

Experiments showed that the silica gel was very efficient at holding on to the hydrogen cyanide. This meant that to ensure that >90% of the hydrogen cyanide is extracted into the sodium hydroxide solution only a small quantity of silica gel should be used in the trap (4 g of particle size 2.5 – 6 mm) and a large volume of extraction solvent (NaOH) is required (150 mL).

The disadvantage of this is that the sample solution absorbances are at the lower end of the calibration range.

7. Good technique

There are a few steps in the method which are dependent on good technique.

1. Care needs to be taken to ensure the final solution containing the coloured complex is thoroughly mixed. Inverting the test tube a couple of times every few minutes, in particular just before the measurement is made does this.
2. Only a small quantity of silica gel is used to trap the hydrogen cyanide. Therefore, it is important to check that it is packed 'securely' into the glass tube to ensure efficient trapping (i.e. no gaps).
3. There are several pipetting operations required for each sample plus a set length of time for the colour to develop. Therefore, it is easier to analyse the sample solutions in small batches to maintain consistency with the timing of adding reagents and subsequent colour development.