

# An Automated Procedure for the Determination of Total Alkaloids in Cigarette Smoke Using On-line Cyanogen Chloride Generation from Low Hazard Starting Materials\*

by R. G. Lidzey and G. P. Savage

Laboratory of the Government Chemist, Department of Trade and Industry, London, England

## SUMMARY

An automated procedure is described for the determination of alkaloids in cigarette smoke using low toxicity reagents. Cyanogen chloride is formed on line from potassium thiocyanate and sodium hypochlorite. The procedure is compared with the previously used cyanogen bromide method which also uses AutoAnalyzer II methodology.

## ZUSAMMENFASSUNG

Eine automatische Methode wird beschrieben, mit der Alkaloide im Zigarettenrauch unter Verwendung von Reagenzien geringer Toxizität bestimmt werden können. Chlorcyan entsteht direkt aus Kaliumthiocyanat und Natriumhypochlorit. Das neue Verfahren und die bisher verwendete Cyanbromid-Methode werden miteinander verglichen. In beiden Verfahren wird der AutoAnalyzer AA II eingesetzt.

## RESUME

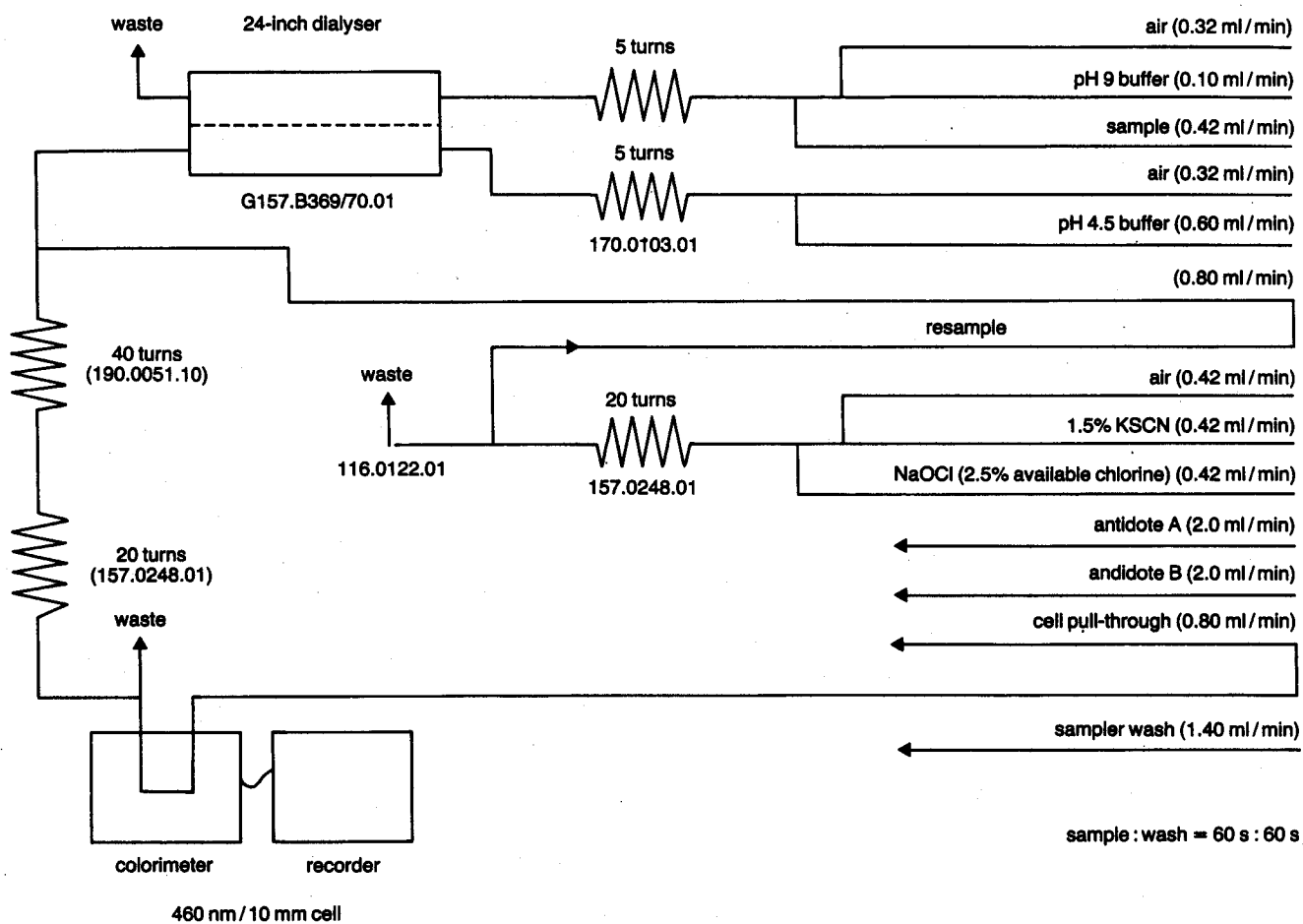
Ce travail présente une méthode automatique permettant de doser les alcaloïdes dans la fumée de cigarette au moyen de réactifs de faible toxicité. Le chlorure de cyanogène se forme directement par action du thiocyanate de potassium sur l'hypochlorite de sodium. L'étude compare ensuite ce nouveau procédé avec la méthode employée jusqu'alors utilisant le bromure de cyanogène. On utilise l'«AutoAnalyzer AA II» pour les deux procédés.

## INTRODUCTION

The automated analysis of nicotine and its analogues in smoke has been carried out in this Laboratory for the last eight years by the "T.R.C. standard method" (1) which employs cyanogen bromide in a colorimetric assay. Cyanogen bromide has lately become decreasingly available as a commercial reagent and its toxic properties, combined with its volatility, have made it necessary to find an alternative. Cyanogen chloride can be formed by oxidising potassium cyanide with chloramine-T on-line, which effectively removes the volatile cyanide hazard (2). However, for our purposes potassium cyanide was considered to be too poisonous to be used as a starting material in an on-line preparation. It is noted that cyanogen bromide is formed from bromine and the thiocyanate radical in an analytical test for thiocyanates in water (3). Investigation showed that cyanogen chloride is formed by mixing appropriate strengths of sodium hypochlorite and potassium thiocyanate solutions. We have confirmed its presence in the mixture by mass spectrometry. The spectrum was obtained from the heated vapour of two microlitres of the liquid mixture which was diffused into the source of a Kratos MS 30 mass spectrometer using a standard leak. Two peaks were found in the mass spectrum at 61 and 63, corresponding to the molecular weight of CNCl, one for each chlorine isotope. The quantities found, as shown by their peak heights, were in the normal proportions for chlorine isotopes. A reduction in intensity of both peaks was observed 15 to 20 minutes after mixing, indicating that the cyanogen product decays slowly. The spectra of potassium thiocyanate and sodium hypochlorite showed no peaks at these mass numbers. For total alkaloid determination, colour development takes place in the presence of an amine (4), e.g. aniline or sulphanilic acid. If the pre-formed cyanogen

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**Figure 1.**  
**Technicon AutoAnalyzer II (AA II) manifold for nicotine and analogues in cigarette smoke.**



chloride is added to a mixture of the Cambridge filter extract and the amine, a colour develops with the same peak maximum but with less absorbance than the cyanogen bromide complex.

## MATERIALS AND METHODS

### Apparatus

- Technicon AutoAnalyzer II (AA II) pump II with air bar.
- ChemLab sampler (model No. CS40): sample/wash ratio 1:1.
- Vitatron UPM colorimeter, fitted with a 460 nm interference filter and a 10 mm flow cell.
- Technicon AA II dialyser fitted with a "C" type membrane.

### Reagents

1. *Sodium Hypochlorite* (2.5% available chlorine): As the commercial product varies in composition, titrate 5 ml of a solution of 10% to 14% nominal chlorine content by the method described by VOGEL (5) for bleaching powder and adjust the strength with deionised water to 2.5% available chlorine. Prepare 100 ml daily. Discard commercial solutions where the chlorine content has fallen below 10%. Some hypochlorite solutions have been found by titration (6) to contain no excess alkali and therefore give poor colour development. In such cases add sodium hydroxide to achieve a free hydroxide content of about 0.05 M.
2. *Potassium Thiocyanate* (1.5%): Weigh 3.0 g of potassium thiocyanate (GPR). Add 4 drops of the wetting agent Brij® 35 (33% (w/v)) and dilute to 200 ml with deionised water.
3. *Buffer Solution* (pH 9): Weigh 30 g of disodium tetraborate decahydrate (AnalaR). Add ten drops of Brij® 35 (33% (w/v)) and dilute to 1000 ml with deionised water.
4. *Buffer Solution* (pH 4.5): Weigh 40 g of sodium dihydrogen orthophosphate dihydrate (AnalaR) and dissolve with 2 ml of aniline in water, add two drops of Brij® 35 (33% (w/v)) and dilute to 1000 ml with deionised water.
5. *Antidote A*: Dissolve 1 g of citric acid and 10 g of ferrous sulphate in deionised water and dilute to 1000 ml.
6. *Antidote B*: Dissolve 10 g of sodium carbonate in deionised water and dilute to 1000 ml.
7. *Nicotine Standards*: Weigh out  $(100 \times 0.7681/Z)$  g of (+)-nicotine hydrogen tartrate, where Z is the percentage purity of the tartrate based on the nicotine analysis, and transfer to a 200 ml volumetric flask. Dissolve and

make up to volume with sulphuric acid (0.02 M). Pipette 1 ml of this solution into a 50 ml volumetric flask, add 10 ml of propan-2-ol and dilute to 50 ml with sulphuric acid (0.02 M). This gives a standard solution containing the equivalent of 0.5 mg of nicotine per cigarette. For 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg per cigarette, pipette respectively 2, 3, 4, 5, 6 and 7 ml of solution into 50 ml flasks, add 10 ml of propan-2-ol and dilute to 50 ml with sulphuric acid (0.02 M). The purity of the (+)-nicotine hydrogen tartrate can be determined by gravimetric assay (7).

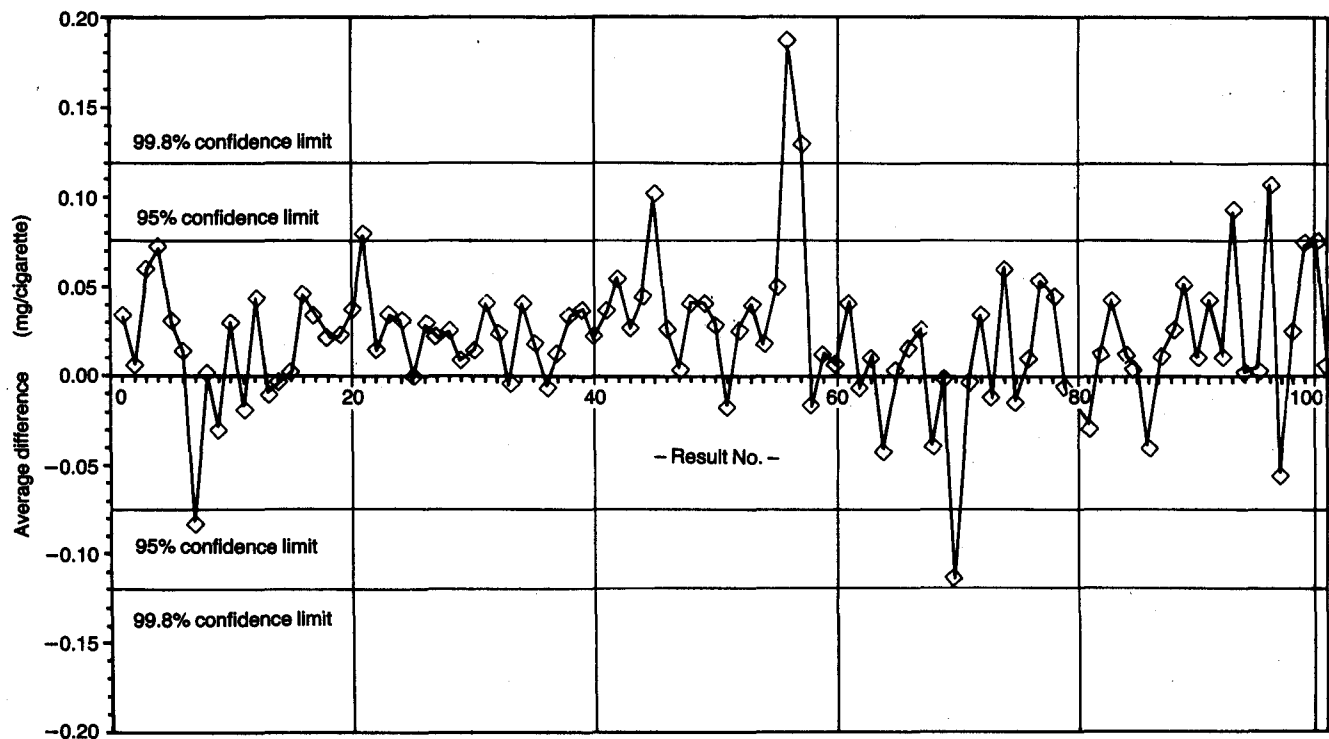
## ANALYTICAL PROCEDURE

The Cambridge filter pad from the smoking machine, which contains the particulate matter from 5 cigarettes, is extracted with 20 ml of propan-2-ol in a 150 ml conical flask for 20 minutes. After removal of 250 µl for water analysis by GLC, 80 ml of sulphuric acid (0.02 M) are added to the flask and extraction is continued for a further 20 minutes. A portion is transferred to a 2 ml sample cup after centrifuging or filtering and placed in the sampler tray between two sets of standard solutions. The manifold arrangement of AutoAnalyzer II is illustrated in Figure 1, and a trace of the recorder output in Figure 3. Peak heights are measured automatically and are corrected for baseline drift. The sample nicotine concentrations are calculated by a microcomputer which interpolates unknown peak heights on a regression line produced from the standard peak heights. A description of the data collection and analysis is published elsewhere (8). Cell waste is added to the mixed antidotes before disposal.

## EXPERIMENTAL DESIGN

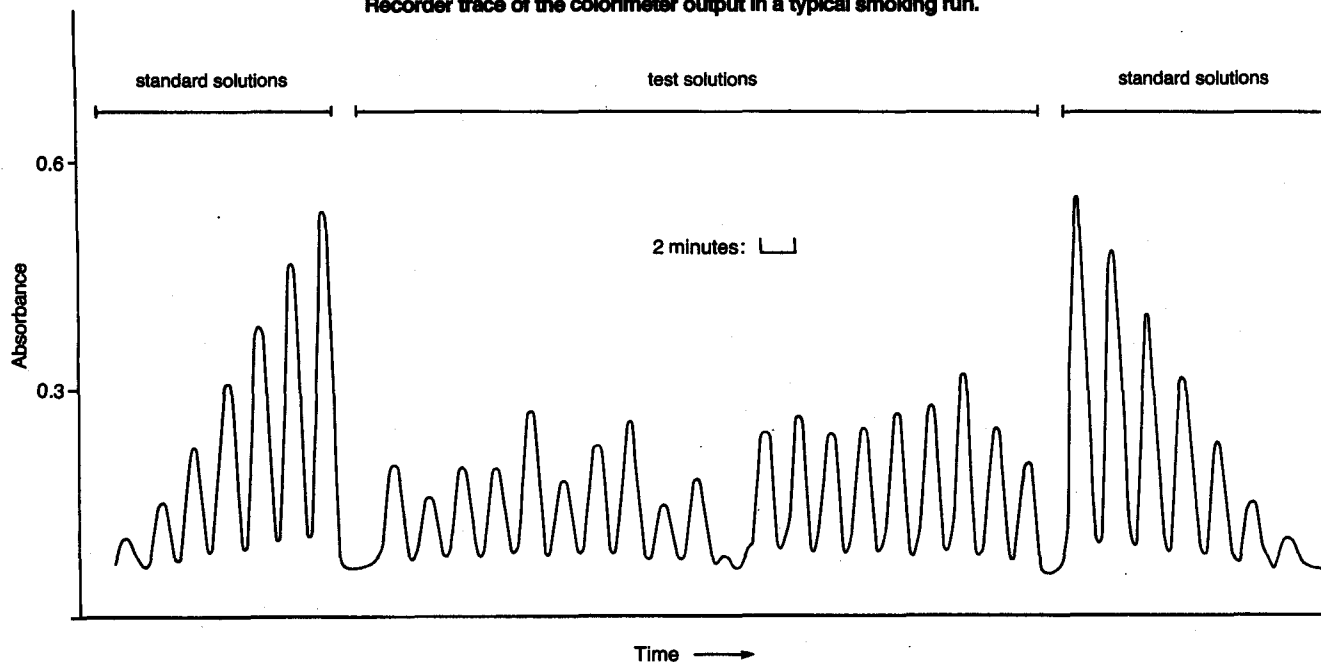
When sodium hypochlorite and potassium thiocyanate solutions are mixed, a small amount of chlorine is generated in the form of bubbles which would break up the evenly segmented flow unless they were removed. This is accomplished on the manifold by debubbling the mixture before adding it to the receptor stream. A 24-inch dialyser is used in the manifold instead of the 12-inch version used in the cyanogen bromide method (1) to compensate for the reduction in the intensity of the colour formed. This allows 15% to 20% more nicotine to be transferred from the donor stream without a concomitant loss of discrimination between adjacent peaks. Improved sensitivity was also obtained by using sodium dihydrogen phosphate as a buffer at pH 4.5, slightly different from that used originally (1). The ratio of sodium hypochlorite to potassium thiocyanate was also optimised for maximum absorbance. Chloramine-T, although ionising to hypochlorous acid in aqueous solution, was found not to be an acceptable

**Figure 2.**  
**Comparison, over 100 smoking runs, of the cyanogen bromide method and the new cyanogen chloride method (mean total alkaloid difference).**



nicotine: average difference per run  
 new method - current method (mean value = 0)

**Figure 3.**  
**Recorder trace of the colorimeter output in a typical smoking run.**



alternative to sodium hypochlorite. Much lower sensitivities were given with chloramine-T and the commercial product slowly deteriorated. Greater concentrations caused precipitation when mixed with potassium thiocyanate. It is necessary to delay feeding the sodium hypochlorite into the manifold until the potassium thiocyanate has been introduced to prevent reaction with the aniline.

## RESULTS

Over a period of three months, 15 samples of each of 147 brands of cigarette were analysed concurrently by both the chloride and bromide methods. More than 100 smoking runs, each of 20 samples, were carried out on a Filtrona 300 smoking machine and the mean difference (CNCl - CNBr) per run was found to be  $\pm 0.019$  mg/cigarette (see Figure 2).

A comparison of precision was made by carrying out an analysis of variance on 40 brands randomly selected from the 147 tested. The difference in the precisions of the cyanogen chloride and bromide methods, 0.100 and 0.088 mg/cigarette respectively, was also found to be quite small. The chloride method has now replaced the bromide method for routine analysis and Figure 3 illustrates a recorder trace from a typical smoking run. The system is now operating without the protection of a fume hood as any cyanogen chloride formed is confined to the pipe work and is destroyed by the antidote before it emerges.

## REFERENCES

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### *Authors' address:*

*Laboratory of the Government Chemist,  
Department of Trade and Industry,  
Cornwall House,  
Waterloo Road,  
London, SE1 8XY, England.*