AN AUTOMATED METHOD FOR DETERMINATION OF HYDROGEN CYANIDE IN CIGARETTE SMOKE[1]

BY P. F. COLLINS, N. M. SARJI and J. F. WILLIAMS,
Research Department, Liggett & Myers, Inc., Durham, N. C., U.S.A.

A procedure for the determination of HCN in cigarette smoke is described which enables relatively large numbers of cigarettes to be smoked per sample. A multiple-port smoking machine is utilized and the HCN is trapped from whole smoke by Ascarite contained in a small glass tube mounted immediately behind the cigarette. This method of trapping was found to circumvent losses of HCN on Cambridge pads normally used for separation of particulate and gas phases of smoke. Five cigarettes are smoked through each trap. After smoking, the Ascarite is extracted with water and the extract analyzed for cyanide by a colorimetric pyridine-pyrazolone procedure which has been automated through use of a Technicon AutoAnalyzer. This procedure employs the reaction of cyanide with chloramine-T in a buffered solution to form cyanogen chloride which in turn reacts with pyridine and pyrazolone to form a colored product. The method has been applied to various types of cigarettes with typical values in micrograms of HCN per puff for unfiltered smoke of 28 to 39; for cellulose acetate filtered smoke, 24 to 30; and for carbon + cellulose acetate filtered smoke, 14. The standard deviation for a port of 5 cigarettes usually ranges from 1 to 3 micrograms of HCN per puff with a sample obtained from a single carton. The colorimetric procedure as described detects HCN free or combined as cyanohydrin.

INTRODUCTION

If one desires to obtain analyses for smoke which are representative of a particular cigarette brand or type, it is necessary to average the values obtained from a sufficiently large sample. This has been discussed at some length by Bates, *et al.* (1) with regard to the analysis of cigarette brands for "tar" and nicotine deliveries. In order to accomplish this with reasonable effort using standard smoking techniques, it becomes almost imperative that some type of multiport smoking apparatus be utilized. Most of the available multiport smoking devices incorporate the use of a Cambridge filter to trap out particulate smoke which also prevents fouling of the pumping mechanism. Use of the Cambridge filter is customary in separating the particulate from the gas phase of cigarette smoke.

Several procedures have been described for the analysis of HCN in the gas phase of cigarette smoke. All of these utilize smoke passing through the Cambridge filter for the analytical sample. The method of Knoop and Rosene (5) utilizes a tube of activated carbon positioned between the Cambridge filter and the puffing device to trap the HCN from the smoke. Determination of the HCN is accomplished by elution and automated alkaline picrate colorimetric analysis. Five cigarettes are smoked through each Cambridge pad.

Urbanic, *et al.* (9) utilized 2 per cent sodium hydroxide to trap HCN from the gas phase of smoke and determined the cyanide by a manual pyridine-pyrazolone colorimetric procedure. The type of trapping did not appear readily adaptable to multiport smoking devices.

A procedure has been described by Norman, *et al.* (8) in which a special four port smoking device is utilized with sodium hydroxide solution for trapping of HCN from the gas phase. These investigators recognized that HCN is partially absorbed when it passes a Cambridge pad and, for this reason, limited smoking to one cigarette per pad to minimize this loss. This procedure also was not readily adaptable for large numbers of cigarettes.

The method described here utilizes the desirable features of a simple tube trap and automated analysis of the cyanide but differs from that of Knoop and Rosene in respect to the material used to absorb the HCN, the placement of the trap, and the colorimetric procedure used in the automated analysis of the cyanide. Ascarite contained in a glass tube positioned between the cigarette and the Cambridge filter is used to absorb the HCN from the whole smoke. Following smoking of five cigarettes, the Ascarite is extracted with water and cyanide is determined in an aliquot of the extract by the pyridine-pyrazolone colorimetric method which is automated through the use of a Technicon AutoAnalyzer.

The trapping of HCN from whole smoke was found to be 98-99 per cent efficient. For such efficient trapping to be obtained in this manner, it is concluded that the HCN in whole smoke is either in the gas phase or rapidly exchanged with the gas phase.

MATERIALS AND METHODS

Apparatus

Smoking System. The smoking machine which was used in this work is that described by Keith and Newsome (4) and was adjusted for a 35-ml puff of 2 seconds duration with 58 seconds between consecutive puffs. A Cambridge filter pad holder with a 44-mm Cambridge CM-113 filter pad (available from Phipps and Bird, Inc., Richmond, Va.) is attached to each port of the smoking machine. The HCN trap consists of an

[1] *Presented at the 23rd Tobacco Chemists' Research Conference, Philadelphia, Pa, October 22-24, 1969.*

8.5 cm length of 9 mm i.d. glass tubing fitted with a female 14/10 standard taper glass joint. The plain end of this tube is connected to the Cambridge filter holder with a sleeve of rubber tubing sufficiently rigid to hold the tube in a horizontal position. The cigarette is attached to the trap with a Teflon cigarette holder and thin latex sleeve, also described by Keith and Newsome (4). The trapping assembly is shown in **Figure 1.** Other equivalent smoking devices such as the Philip Morris Smoking Machine could be used provided proper consideration is given to those factors affected by the extension of the cigarette by the trap, such as draft, simultaneous lighting of cigarettes and automatic cut-off.

Technicon AutoAnalyzer. The AutoAnalyzer consists of standard components including a Sampler II, a proportioning pump and a colorimeter-recorder unit with a 15-mm tubular flowcell and 544 millimicron filters. The manifold is shown in **Figure 2.**

Reagents

Prepare all solutions with distilled water and reagent grade chemicals except as noted.

Ascarite. Use 8 to 20 mesh Ascarite (A. H. Thomas Company).

Chloramine-T, 0.4%. Dissolve 2.0 g of chloramine-T (Eastman 1022) in 500 ml of water. Prepare fresh each week.

Pyrazolone Solution, Saturated. Stir 5 g of 3-methyl-1-phenyl-2-pyrazolin-5-one (Eastman 1397) with 1 liter of water for several hours. Store in a brown bottle.

Pyridine-Pyrazolone Solution. Dissolve 0.080 g of "bispyrazolone" (Eastman 6969) in 80 ml of pyridine contained in a brown bottle. About 30 minutes is required for dissolution and the bottle should be occasionally shaken during this time. When complete solution is obtained, add 400 ml of filtered saturated pyrazolone solution and mix.

Buffer Solution. Dissolve 13.6 g of KH_2PO_4 and 0.28 g $Na_2H\ PO_4$ in water and dilute to 1 liter. Add 0.5 ml of Brij-35 (Technicon).

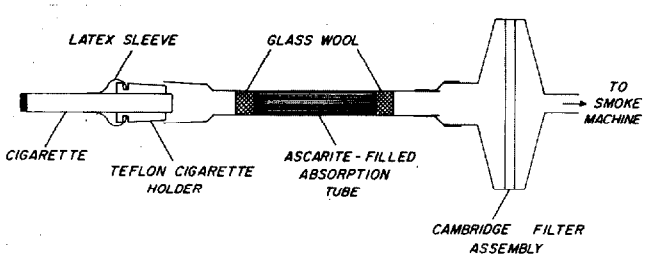

Figure 1—HCN trapping assembly.

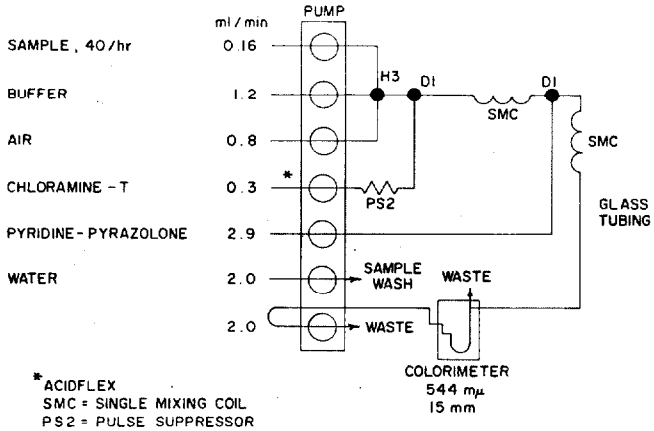

Figure 2—AutoAnalyzer manifold for HCN in smoke.

Sodium Hydroxide Solution, 2.5N. Dissolve 100 g of NaOH in 800 ml of water, cool to room temperature and dilute to 1 liter.

Cyanide Standards. Dissolve 0.602 g of potassium cyanide in water and dilute to 500 ml to obtain a solution equivalent to 500 ppm of HCN. Dilute 0.5, 1, 2, 3, 4 and 5 ml aliquots of this solution with 25 ml of 2.5 N NaOH and water to 500 ml to obtain working standards equivalent to 0.5 to 5 ppm in HCN and 0.125 N in NaOH. Also dilute 25 ml of 2.5 N NaOH to 500 ml with water for a blank standard. Standards should be prepared fresh each two weeks.

PROCEDURE

Smoking of Cigarettes. Cigarettes should be conditioned at 74°F and 60% relative humidity for 48 hours before smoking. Mark the cigarettes for the desired butt length; in this work, a butt length of 30 mm or tipping paper plus 3 mm, whichever was greater, was used. For cigarettes smoked without filter, the specified butt length included the length of the removed filter.

Pack each absorption tube with 3.0 g of Ascarite, using small loose plugs of glass wool to retain the Ascarite in the narrow portion of the tube. Store the packed tubes in a closed bottle until used.

Assemble the trapping system as shown in **Figure 1,** and smoke 5 cigarettes through the trap. Take a clearing puff after extinguishing each cigarette but do not include these clearing puffs in counting the total number of puffs smoked through the trap. Extract the trap within three hours.

Extraction of Ascarite. Place the trap, plain end down, in the neck of a 500-ml volumetric flask containing about 300 ml of water. Push the contents of the trap down into the water, using a 6-7 mm glass rod. Wash the rod and tube with water and remove from the flask. Stopper and swirl the flask vigorously. Allow to stand for 15 minutes, mixing occasionally, then dilute to volume and mix well. Analyze this solution within one hour.

Automated Analysis of Extracts. With the AutoAnalyzer manifold assembled as shown in **Figure 2,** turn on the colorimeter and recorder and pump water through the lines for at least 15 minutes. If convenient, centrifuge portions of the Ascarite extracts for filling the AutoAnalyzer cups. Otherwise, fill each cup by pouring the extract through a small piece of glass wool placed on the lip of the flask. Place standards before and after the samples. Pump reagents and when a stable baseline is obtained, check the zero and adjust the baseline to 95% T in the normal manner. Begin sampling at the rate of 40 per hour with a 2:1 sample to wash ratio. At the completion of the run, construct a calibration curve relating ppm of HCN to peak height with the data obtained from the standards. Read ppm HCN for each sample and calculate micrograms per puff:

$$\text{Micrograms HCN/Puff} = \frac{\text{ppm HCN} \times 500}{\text{Number of Puffs}}$$

RESULTS AND DISCUSSION

The ability of Ascarite to absorb HCN from whole cigarette smoke was evaluated by smoking five non-filter cigarettes through the described trapping system using 3 g of Ascarite. The puffs after passage through the Ascarite trap and Cambridge pad were collected in a smoke collection flask described by Newsome, *et al.* (7) with dilute NaOH as the absorbing solution. After each puff was taken into the flask, the solution was

shaken vigorously for 30 seconds, a time which had been found sufficient for absorption of the HCN. Following smoking, the Ascarite was extracted with water and the Cambridge pad with dilute NaOH solution. These extracts and the absorbing solution were analyzed by the automated procedure and of the total HCN found, 98.6% had been trapped by the Ascarite, 0.9% retained by the Cambridge pad and 0.5% was in the vapor phase. Analysis of many other Cambridge pads used in this procedure indicated equally satisfactory trapping of HCN by Ascarite.

The use of 3 g of Ascarite for smoking of 5 cigarettes provides a large excess of sodium hydroxide as shown by the alkalinity of the extracts. The excess is equivalent to over 2 g of sodium hydroxide as determined by titration to the phenolphthalein endpoint with standard hydrochloric acid. That this is actually an excess for trapping HCN from cigarette smoke was shown by experiments in which 5 cigarettes were smoked through traps containing 1.5 and 3.0 g of Ascarite. The results, shown in **Table 1**, are essentially the same for the two levels of Ascarite and analysis of the Cambridge pads following the traps indicated equally complete HCN trapping by the two levels of Ascarite.

The automated pyridine-pyrazolone procedure is based on the manual methods described by Epstein (3) and Boxer and Rickards (2). Cyanide is reacted with chloramine-T to form cyanogen chloride which, in turn, reacts with pyridine and pyrazolone to give a colored product. The initially formed pink color is measured instead of allowing time for development of the relatively stable blue color as in the manual procedures. A phosphate buffer is used to adjust for the alkalinity of the extracts which has been found to range between 0.10 and 0.14 N in phenolpthalein alkalinity. Standards are prepared to be 0.125 N in sodium hydroxide and over the range of 0.10 to 0.15 N, the concentration of sodium hydroxide has a negligible effect on the sensitivity of the procedure. A typical AutoAnalyzer recording of the cyanide standards is shown in **Figure 3.**

Hydrogen sulfide and aldehydes were considered by Boxer and Rickards (2) to possibly interfere in the pyridine-pyrazolone procedure, and as these are present in cigarette smoke, their interference was examined. Acetaldehyde, present at a concentration level of 39 ppm, caused the color response given by 4 ppm of HCN in 0.1 N sodium hydroxide to be low by 1%. Hydrogen sulfide, 0.6 ppm, with 3 ppm HCN in 0.1 N NaOH caused the response to be low by 3%. These levels of acetaldehyde and hydrogen sulfide are higher than would be expected in the Ascarite extracts even if completely trapped from smoke, and thus these components are not considered to cause severe interference.

Recovery tests were carried out in which potassium cyanide was added to various extracts of Ascarite, prepared from fresh Ascarite and from that used in trapping smoke from five cigarettes. In each case the extracts contained 3 g of Ascarite per 500 ml. The results are summarized in **Table 2.** Recovery of cyanide found with fresh Ascarite ranged from 94 to 96%, and with extracts of used Ascarite, the recovery was somewhat higher, ranging from 97 to 100%. While these recovery tests do not duplicate the conditions employed in smoking where the HCN and other components are collected on Ascarite from the smoke stream, they do tend to indicate the noninterference of the other trapped smoke components in the determination of cyanide by the automated procedure.

The stability of the HCN absorbed from smoke on Ascarite was investigated by smoking five non-filter cigarettes through each of 12 traps. Four of the traps were analyzed immediately, with an average of 33.5 micrograms of HCN per puff, four after standing one hour gave an average of 36.2, and four after 3.5 hours gave 36.4. The differences are not statistically significant at 95 percent confidence limits. The stability of the extracts of Ascarite was determined by analyzing nine extracts immediately and then after standing 4 and 20 hours. An average decrease of 2.5% was found after 4 hours, and a 5% decrease after 20 hours. Thus, it is

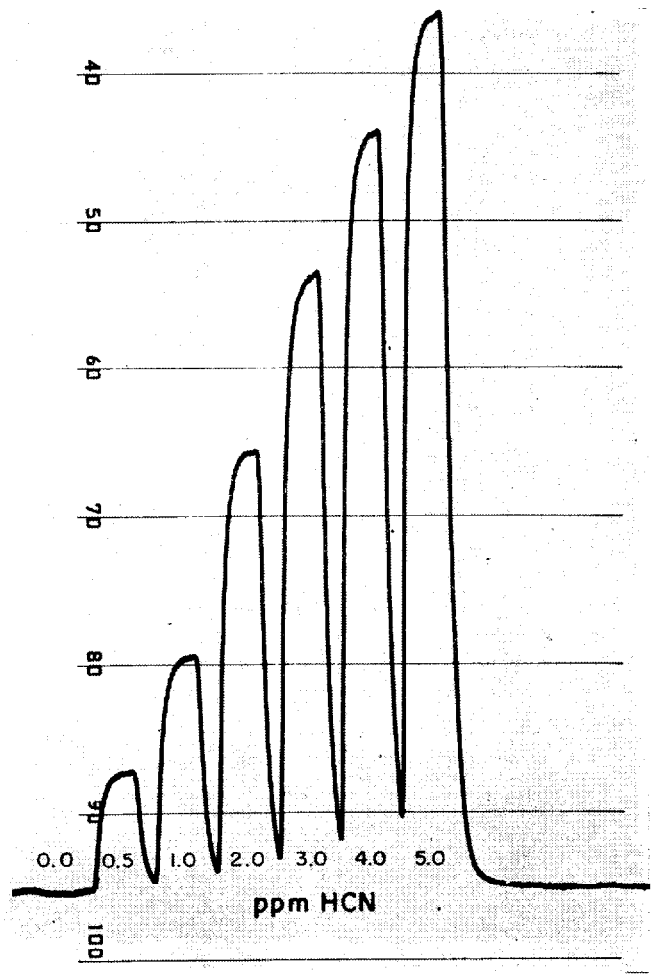

Figure 3—Typical AutoAnalyzer recording of the cyanide standards.

Table 1. HCN in smoke using two levels of Ascarite for trapping.

Cigarette	Grams of Ascarite	Avg. ug HCN/Puff[1]
A	1.5	14.9 ± 2.2
A	3.0	15.9 ± 0.5
A without filter	1.5	35.7 ± 1.8
A without filter	3.0	35.8 ± 0.5

[1] ± *Values are 2 x the standard error of mean*

Table 2. Recovery of cyanide added to Ascarite extracts.

Cigarette Type Smoked	Micrograms HCN Initial	Added	Found	% Recovery of Total Expected
None	0	500	482	96
None	0	1500	1405	94
None	0	2500	2380	95
Filter	970	500	1465	100
Filter	1310	500	1750	97
Non-Filter	1530	500	2010	99
Non-Filter	1495	500	1980	99

recommended that the Ascarite traps be extracted within three hours after smoking and the extracts be analyzed within one hour.

One of the objectives of this work was to develop a procedure which would determine total HCN in smoke, avoiding the loss due to retention of HCN on the Cambridge pad when only the vapor phase is analyzed. This loss may be considerable as was shown by an experiment in which varying numbers of cigarettes (85 mm with cellulose acetate filters) were smoked through Cambridge pads with Ascarite traps behind the pads to collect the HCN in the vapor phase. Analysis of the Cambridge pads and Ascarite traps gave the results shown in **Table 3**, indicating that 36 to 49% of the total HCN is retained on the pad when 1 to 5 cigarettes are smoked. Thus, in determining total HCN the necessity of analyzing whole smoke, or both phases separated by a Cambridge pad, is evident.

Although the particulate phase collected on a Cambridge pad contains considerable HCN, the particulate phase after passage through an Ascarite trap contains only about 1% of the total HCN in whole smoke. The Ascarite trap has been found to retain about half of the particulate phase, based on measurements of the dry smoke solids and nicotine passing through the trap. Therefore, the essentially complete trapping of HCN by the Ascarite indicates that the HCN in mainstream smoke, as it issues from the cigarette, must be present in the gas phase or easily exchanged with the gas phase, and not combined in compounds of relatively low volatility such as cyanohydrins. However, once the smoke is collected, complexed forms of HCN may be present as reported by Nall (6) and by Norman, *et al* (8) with the predominant species probably being acetaldehyde cyanohydrin. The possibility that the HCN collected on Ascarite is complexed has not been studied, but it has been found that the automated procedure will determine 97% of the contained HCN in acetaldehyde cyanohydrin added to an Ascarite extract.

This procedure has been applied to the analysis of many types of cigarettes with some typical results shown in **Table 4** for several cigarette brands smoked with and without filter, 5 cigarettes per Ascarite trap. The cigarettes for each brand were obtained from one or two cartons and were smoked to a butt length of 30 mm or tipping paper + 3 mm, whichever was greater. No selection other than discarding defective cigarettes was exercised. The standard deviation shown is typical, usually being in the range of 1 to 3 micrograms of HCN per puff for a sample from a single carton.

Table 3. Retention of HCN from whole smoke on Cambridge filter pads.

Number of Cigarettes per Pad	Micrograms of HCN/Puff On Pad	In Trap	Total[1]
1	9.5	16.6	26.1
3	11.8	13.7	25.5
5	12.5	12.9	25.4

[1] *Normal Ascarite trapping procedure gave 24.3 micrograms of HCN per puff for whole smoke.*

SUMMARY

A method for the determination of HCN in cigarette smoke has been developed which employs a multiple-port smoking machine. The HCN is trapped by 3 g of Ascarite contained in a small glass tube mounted immediately behind the cigarette during smoking. Five cigarettes are smoked through each trap. After smoking, the Ascarite is extracted with water and the extract analyzed for cyanide by a colorimetric pyridine-pyrazolone method which has been automated through use of a Technicon AutoAnalyzer. The procedure has been applied to various brands of cigarettes, and the precision in terms of standard deviation for a sample from a single carton is usually between 1 and 3 micrograms of HCN per puff.

LITERATURE CITED

1. Bates, W. W., R. B. Griffith, E. S. Harlow, M. Senkus and H. Wakeham, Determination and Reporting of Total Particulate Matter, Water in Total Particulate Matter, and Nicotine in Cigarette Smoke. *Virginia J. Sci. 18:* 130-135. 1967. Reprinted in *Tobacco Sci. 12:* 192-196. 1968.

2. Boxer, G. E. and J. C. Rickards, Chemical Determination of Vitamin B_{12}. II. The Quantitative Isolation and Colorimetric Determination of Millimicrogram Quantities of Cyanide. *Arch. Biochem. 30:* 372-381. 1951.

3. Epstein, J., Estimation of Microquantities of Cyanide *Anal. Chem. 19:* 272-274. 1947.

4. Keith, C. H. and J. R. Newsome, Quantitative Studies on Cigarette Smoke. I. An Automated Smoking Machine. *Tobacco Sci. 1:* 51-57. 1957.

5. Knoop, J. L. and C. J. Rosene, Automated Analysis of Cigarette Smoke. *18th* Tobacco Chemists' Research Conference, Raleigh, N. C., October 20-22, 1964.

6. Nall, J. F., Complexed Cyanide in Collected Cigarette Smoke. *20th* Tobacco Chemists' Research Conference, Winston-Salem, N. C. November 1-3, 1966.

7. Newsome, J. R., V. Norman and C. H. Keith, Vapor Phase Analysis of Tobacco Smoke. *Tobacco Sci. 9:* 102-110. 1965.

8. Norman, V., J. R. Newsome and C. H. Keith, Smoking Machines for the Analysis of the Vapor Phase of Cigarette Smoke. *Tobacco Sci. 12:* 216-221. 1968.

9. Urbanic, J. E., R. S. Joyce, J. J. Petty and D. D. Tiggelbeck, The Removal of Hydrogen Cyanide from Cigarette Smoke. *21st* Tobacco Chemists' Research Conference, Durham, N. C., October 19-20, 1967.

Table 4. Typical results for HCN in cigarette smoke.

Cigarette Description	Smoking Condition	Micrograms HCN/Puff[1] Average	Standard Deviation	% Removal of HCN by Filter[2]
Brand A, 85mm with cellulose acetate + granular carbon filter	With Filter	13.5	2.2	57
	Without Filter	31.3	2.3	
Brand B, 85mm with cellulose acetate filter	With Filter	27.3	2.2	18
	Without Filter	33.5	2.0	
Brand C, 85mm with cellulose acetate + paper impregnated with carbon filter	With Filter	26.5	2.7	33
	Without Filter	39.4	1.9	
Brand D, 85mm with cellulose acetate filter	With Filter	30.3	2.1	18
	Without Filter	37.2	1.6	
Brand E, 85mm with cellulose acetate filter	With Filter	25.4	2.1	17
	Without Filter	30.5	1.6	
Brand F, 85mm menthol with cellulose acetate filter	With Filter	23.6	1.8	17
	Without Filter	28.4	1.5	

[1] *The average is for 20 ports and the standard deviation is for a single port.*

[2] $\%\ Removal = \frac{100\ (WOF\text{-}WF)}{WOF}$, *where WF and WOF are micrograms of HCN per puff with and without filter.*