Beiträge zur Tabakforschung · Band 6 · Heft 4 · August 1972 DOI: 10.2478/cttr-2013-0287

An Automated Procedure for the Determination of Ammonia in Tobacco*

by P. F. Collins, W. W. Lawrence and J. F. Williams

Research Department, Liggett and Myers Incorporated, Durham, North Carolina, USA

INTRODUCTION

The determination of ammonia in tobacco is usually carried out by first volatilizing the ammonia from a mildly alkaline suspension of the finely ground tobacco or tobacco extract followed by measurement of the ammonia by acidimetry or colorimetry. Although automated techniques have been utilized for the colorimetric determination of ammonia after manual separation from tobacco (1, 6), apparently no automated procedures have been described which have been evaluated for the direct analysis of tobacco extracts. Inasmuch as a rapid method was desired for the routine determination of ammonia in tobacco, an investigation was undertaken in an attempt to develop a completely automated procedure which would accurately measure the ammonia in extracts of tobacco.

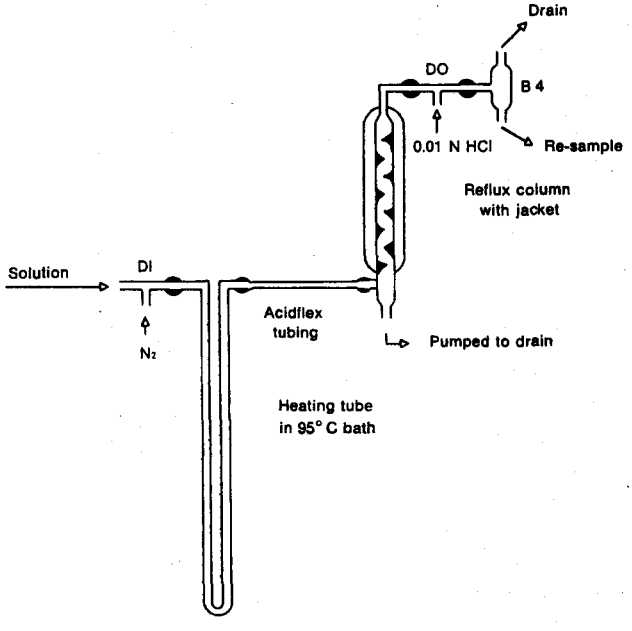
The reaction of ammonia with phenol and hypochlorite to form a blue-colored, indophenol type compound has been utilized in many automated procedures for ammonia. Initial experiments indicated that the procedure is subject to interference by various tobacco components including amino acids and nicotine, so separation of the ammonia through automated dialysis utilizing standard and Type C membranes was examined but found inadequate. A special automated distillation unit was then assembled and evaluated for its ability to adequately separate ammonia from interferences prior to colorimetry using the phenate-hypochlorite reaction. This automated distillation and colorimetric system employs a Technicon AutoAnalyzer and, as described in the following sections, was found to be directly applicable to aqueous tobacco extracts to provide a reasonably accurate determination of ammonia in tobacco.

METHODS AND MATERIALS

Apparatus

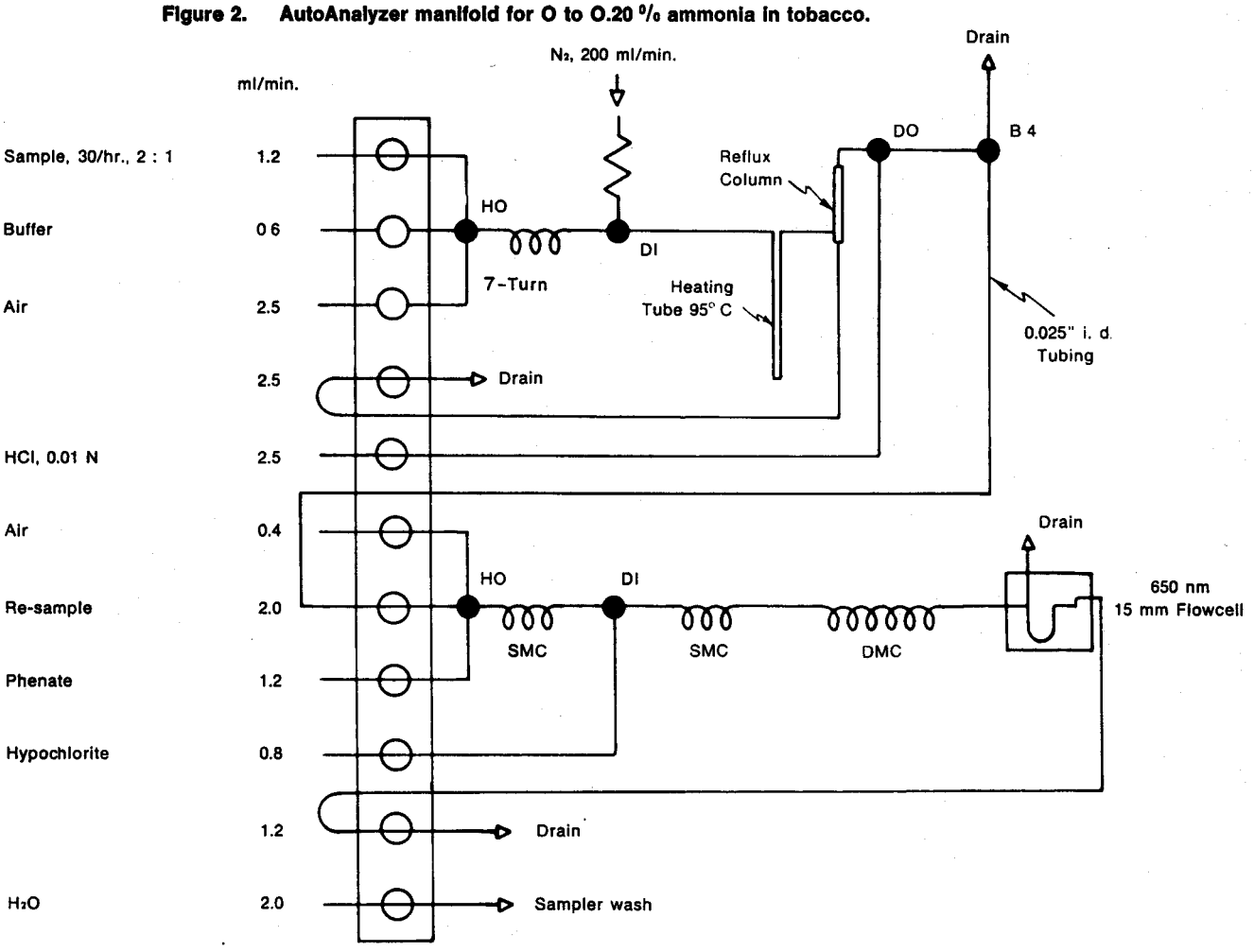
The Technicon AutoAnalyzer used in this study consisted of the special distillation unit in addition to standard modules including a Sampler II with a 30 per hour, 2:1 cam, a Pump I, a 95° C heating bath and a colorimeter-recorder unit equipped with a 15 mm tubular flow cell and 650 nm filters. The distillation unit is illustrated in Figure 1 and is composed of a heating tube, reflux column and several AutoAnalyzer fittings interconnected with Acidflex tubing. The heating tube is fabricated from a 44 cm length of 4 mm o.d. standard wall borosilicate tubing bent sharply into a 19 cm long U-tube and is inserted into the heating bath through the port normally used for the thermometer. The glass reflux column is 1 cm in diameter and 10 cm in length with pointed indentations, and is partially jacketed by another glass tube. Nitrogen is introduced from a regulator equipped cylinder into the unit at the rate of 200 ml per minute as measured by a Brooks "Sho-Rate" flowmeter, Tube No. 3—15—4, through a pulse suppressor consisting of a 2-inch length of 0.010 inch i. d. tubing. The complete manifold as used for samples containing 0 to 0.2 % ammonia is shown in Figure 2. Most samples of flue-cured and cigarette-filler tobacco will fall in this range but burley

Figure 1. Automated distillation unit.



* Received for publication: 22nd November, 1971.

Figure 2. AutoAnalyzer manifold for 0 to 0.20 % ammonia in tobacco.



samples often contain a higher concentration of ammonia. If only occasional samples containing above 0.20 % ammonia are encountered, the extracts may be simply diluted with water. If many such samples are to be analyzed, the procedure may be modified to cover the range of 0 to 1.0 % ammonia by changing the sample and buffer pump tubes to 0.025 (orange-white) and 0.073 (green) in. i.d., respectively, and using the proper buffer solutions and standards as described below.

Reagents

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

1. *Sodium Hydroxide,* 23.2 %: Dissolve 232 g of sodium hydroxide in water, cool and dilute to one liter with water.
2. *Alkaline Phenate Solution:* Place 26 ml of liquid phenol (90 %, Fisher No. A-931) in a 250 ml volumetric flask, add 100 ml of water and 50 ml of 23.2 % sodium hydroxide solution (reagent No. 1). Mix, add 7 ml of acetone and dilute to volume with water. Add 10 drops of Brij-35 solution (Technicon), mix and transfer the solution to a brown bottle. Prepare this solution on the day it is to be used.
3. *Sodium Hypochlorite Solution:* Dilute 65 ml of 5.25 % sodium hypochlorite (Chlorox bleach has been found satisfactory) to 250 ml with water.
4. *Buffer,* pH 9: To prepare the buffer solution for use with the illustrated manifold for the range of 0 to 0.20 % NH_3, dissolve 31.0 g of boric acid and 8.5 g of sodium hydroxide in water to make one liter of solution. For the modified manifold covering the range of 0 to 1.0 % NH_3, prepare the buffer by dissolving 11.6 g of boric acid and 3.2 g of sodium hydroxide in water to make one liter of solution.
5. *Hydrochloric Acid,* 0.01 N: Dilute 1.67 ml of concentrated hydrochloric acid to 2 liters with water.
6. *Standard Ammonia Solutions:* Prepare a 1000 ppm NH_3 solution by dissolving 0.388 g of dried ammonium sulfate in water and diluting to 100 ml. Prepare working standards by appropriate dilution of this 1000 ppm solution with water. For the range of 0 to 0.20 % NH_3, prepare standards containing 0, 2, 4, 6, 8, 10, 15, and 20 ppm NH_3. For the range of 0 to 1.0 % NH_3, prepare standards containing 0, 20, 40, 60, 80, and 100 ppm NH_3.

Procedure

Extraction: Place 1.000 g of the ground tobacco sample in a 125 ml Erlenmeyer flask, add 100 ml of water and shake for 30 minutes on a Burrell "Wrist-Action" shaker. Filter the mixture, using S and S No. 588 folded filter paper, and reserve the filtrate for analysis.

Analysis: The AutoAnalyzer is operated in the normal manner at a sampling rate of 30 per hour with a 2:1

sample to wash ratio. Sufficient time should be allowed for the system to become stable with the reagents being pumped and the nitrogen flow adjusted to 200 ml per minute before sampling is begun. Run duplicate cups of each sample extract with standards before and after the samples. At the completion of the run, construct a calibration curve relating ppm of NH_3 to average peak height with the data obtained from the standards. Read ppm of NH_3 for each extract and calculate % NH_3 in the tobacco sample:

$$\% NH_3 = \text{ppm } NH_3/(100 \times \text{g of sample}).$$

RESULTS AND DISCUSSION

The automated system used in this procedure employs a distillation unit which is somewhat similar in concept to that described by *Keay* and *Menage* (4). Modifications were made, however, in order to obtain a distillation unit that would give adequate volatilization of ammonia from the tobacco extract with minimum interference from other tobacco components, principally glutamine and nicotine. Glutamine can interfere positively in the distillation of ammonia through hydrolysis to form ammonia especially as the basicity of the solution, temperature and length of heating are increased. Nicotine interferes in the colorimetric phenate-hypochlorite reaction, as reported by *Frankenburg* (2). Thus, in this system the tobacco extract, buffered at pH 9, is passed rapidly through a short tube immersed in the 95° C heating bath to minimize both the hydrolysis of amides such as glutamine and the volatilization of nicotine. Nitrogen is used as the carrier gas for the volatilized ammonia because it is readily available from a cylinder in a sufficiently pure form and at a constant pressure which simplifies obtaining a constant flow rate. A suitably regulated compressed air supply with scrubbers to remove ammonia would probably also be satisfactory. A constant flow rate of the gas is necessary as the extent of ammonia volatilization is somewhat dependent on flow rate.

Following separation of the gas stream from the solution, the ammonia is absorbed in dilute acid and a portion of the stream resampled for colorimetric measurement using the phenate-hypochlorite reaction. This part of the automated system is based on the work of *Gehrke* et al. (3) with some modification to minimize interference due to nicotine which is not completely eliminated in the distillation. Typical AutoAnalyzer recordings are shown in Figures 3 and 4.

The extent of interference caused by nicotine in this system was investigated by analyzing aqueous solutions containing various concentrations of ammonia and nicotine using the manifold for the range of 0 to 0.20% NH_3. Nicotine alone gives no response but its presence decreases to some extent the response obtained for a given amount of ammonia. The data presented in Table 1 show the severity of this interference for solutions containing 2 to 20 ppm of ammonia and 100 to 1000 ppm of nicotine which are equivalent to 0.02 to 0.20% ammonia and 1 to 10% nicotine for a 1 g

Figure 3. Typical AutoAnalyzer recording of standards, 0 to 0.2 % NH_3.

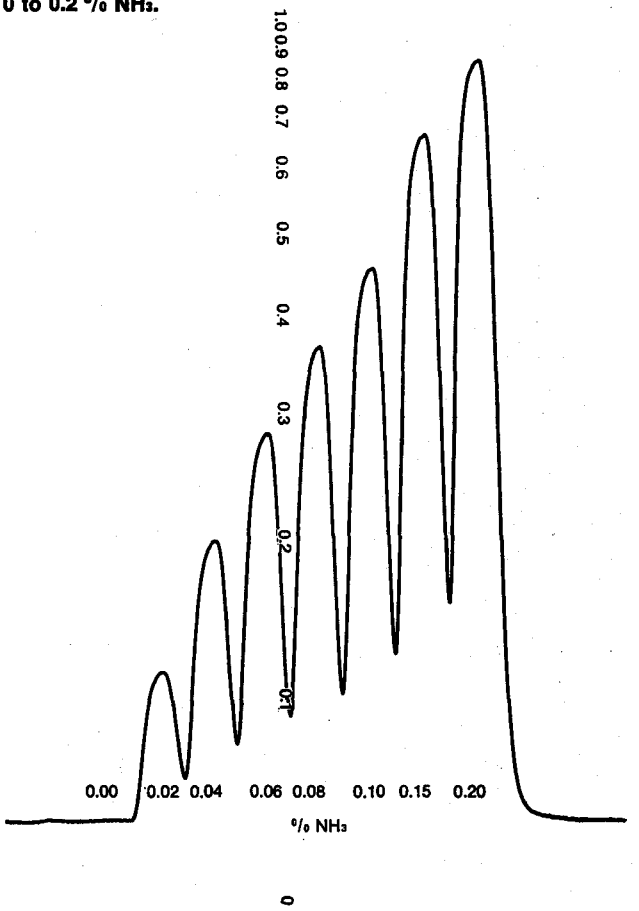


Figure 4. Typical AutoAnalyzer recording of standards, 0 to 1.0 % NH_3.

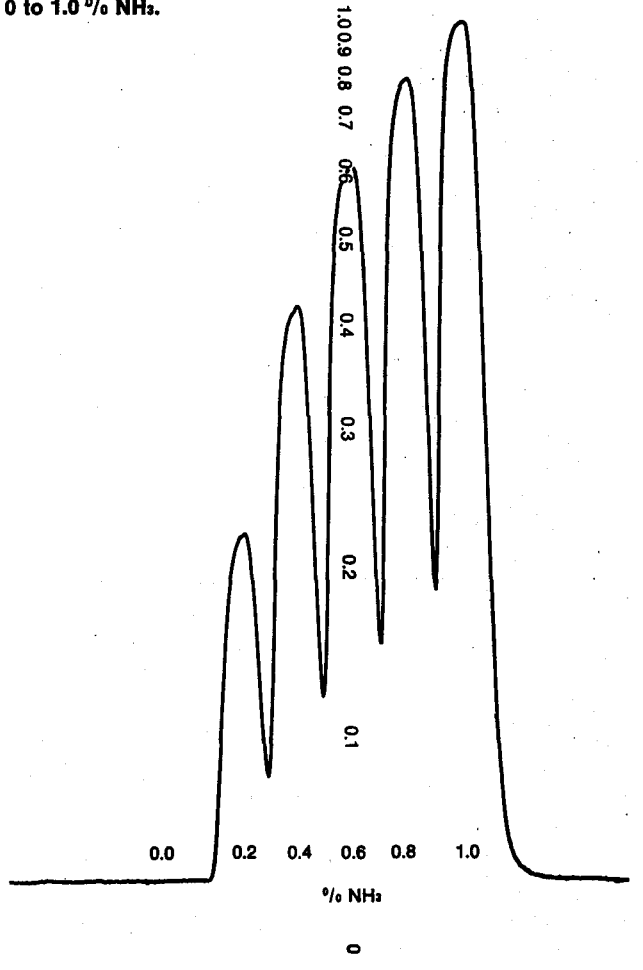


Table 1. Interference of nicotine in the automated procedure.

% Nicotine[a]	% NH_3[a]	% Recovery of ammonia
1	0.020	100
1	0.050	99
1	0.100	99
1	0.200	98
5	0.020	96
5	0.050	95
5	0.100	95
5	0.200	100
10	0.020	94
10	0.050	93
10	0.100	94
10	0.200	98

a: Concentrations of nicotine and ammonia are on the basis of 1 g of sample per 100 ml.

sample in 100 ml of water. These data indicate that, on the average, 1% nicotine will cause the result for ammonia to be low by 1% while 5 and 10% nicotine will cause results to be low by 3.5 and 5.2%, respectively.

The possible interference of various amino acids was studied by determining if they would affect the recovery of ammonia or decompose to form ammonia during the distillation. Aqueous solutions containing only the individual amino acid at a concentration of 0.005 M, corresponding to 500 μmoles per gram of sample, were analyzed using the manifold designed for the range of 0 to 0.20% ammonia. The amino acids tested were alanine, 4-aminobutyric acid, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, and threonine. Of these, only lysine, glycine and glutamine gave any response and, on the basis of one g of sample per 100 ml, the responses correspond to 0.001, 0.002 and 0.003% ammonia. These responses may arise from ammonia as an impurity in the samples of amino acids used and, at any rate, are considered negligible. In the case of glutamine, if the ammonia detected originated from hydrolysis of the amide group then 0.4% of the amide nitrogen was hydrolyzed. The effect of the individual amino acids on recovery of ammonia was determined using solutions 0.004 M in the amino acid and containing 2 ppm of added ammonia; the recoveries of added ammonia ranged from 96 to 103%. It thus appears that amino acids do not interfere significantly in this procedure.

The ability of water to adequately extract ammonia from tobacco was evaluated by comparing the ammonia found in tobacco and in tobacco extracts prepared as described above, using for the analysis the manual procedure of *Pucher* et al. (5) which could be applied directly to ground tobacco or to tobacco extracts. The ammonia in the distillates was determined by automated ninhydrin colorimetry which had been found applicable for this use by *Williams* and *Hunt* (6). The average results obtained for various tobacco samples are shown in Table 2 along with the percent extraction obtained. For these samples the extraction found ranged from 92 to 111% with an average of 99%, indicating that water is generally adequate for the extraction of ammonia from tobacco. Other extractants including 1% aqueous hydrochloric acid and 2% hydrochloric acid + 20% methanol in water were tried as well as elution of the tobacco with water but none of these appeared to offer any advantage over simple extraction with water.

Table 2. Comparison of ammonia found by analyzing tobacco versus extracts of tobacco by a manual distillation procedure.

Tobacco type	Average % NH_3 found		Percentage extracted
	Tobacco	Extract	
Cigarette filler	0.099	0.094	94.9
	0.124	0.133	107.3
	0.067	0.065	97.0
	0.135	0.133	98.5
	0.441	0.431	97.7
	0.076	0.080	105.3
	0.077	0.071	92.2
	0.366	0.366	100.0
	0.079	0.079	100.0
	0.174	0.171	98.3
	0.094	0.091	96.8
	0.169	0.158	93.5
Flue-cured	0.022	0.022	100.0
	0.040	0.040	100.0
	0.046	0.051	110.9
Burley	0.272	0.277	101.8
Reconstituted	0.138	0.129	93.5
	0.130	0.125	96.2
Average	0.142	0.140	99.1

Recovery tests were carried out with three samples of tobacco by adding ammonium sulfate solutions to the tobaccos at the beginning of the extraction. The manifold for the range of 0 to 0.20% ammonia was used with the flue-cured sample and the manifold for 0 to 1.0% ammonia was used with the samples of burley and cigarette-filler tobaccos. Recovery of added ammonia ranged from 96 to 98% as shown in Table 3 and is considered satisfactory.

Table 3. Recovery of ammonia added to tobacco by the automated procedure.

Tobacco type	% NH_3			% Recovery of added NH_3
	Initial	Added	Found	
Burley	0.318	0.150	0.462	96
	0.318	0.300	0.608	97
Cigarette-filler	0.078	0.050	0.126	96
	0.078	0.100	0.176	98
Flue-cured	0.025	0.030	0.054	97
	0.025	0.050	0.073	96

Table 4. Ammonia found in various tobaccos by the automated procedure and by a manual distillation method.

Tobacco type	Average % NH_3 found		% Relative difference
	Automated	Manual	
Cigarette filler	0.094	0.099	— 5.1
	0.134	0.124	8.1
	0.063	0.067	— 6.0
	0.133	0.135	— 1.5
	0.440	0.441	— 0.2
	0.080	0.076	5.3
	0.064	0.077	— 16.9
	0.362	0.366	— 1.1
	0.076	0.079	— 3.8
	0.171	0.174	— 1.7
	0.095	0.094	1.1
	0.163	0.169	— 3.6
Flue-cured	0.026	0.022	18.2
	0.039	0.038	2.6
	0.044	0.046	— 4.3
Burley	0.292	0.272	7.4
Reconstituted	0.123	0.126	— 2.4
	0.124	0.130	— 4.6
Average	0.140	0.141	— 0.5

The accuracy of the automated procedure was further evaluated by analyzing a number of tobacco samples using the manifold for the range of 0 to 0.20% ammonia with manual dilution of the extracts where necessary. These results were compared with those obtained by the manual distillation method of *Pucher* et al. (5) applied directly to the ground tobacco samples, again using automated ninhydrin colorimetry for measurement of the ammonia in the manually obtained distillates. The average results obtained for each sample are shown in Table 4 along with the percent relative difference of the average by the automated method compared to the average by the manual distillation method. These relative differences range from −16.9 to +18.2% for the various samples. For each sample, the individual results by the two methods were compared using the t-test and for none of the samples was the difference between methods found to be statistically significant at the 95% level of significance. Considering the overall group of samples, it is seen that the average ammonia values obtained by the two methods are in very good agreement. The results obtained on these samples by the automated procedure were also used to estimate the precision of the method. Each tobacco was analyzed in duplicate or triplicate and the pooled standard deviation calculated from the replicates was 0.0032% ammonia, corresponding to an overall relative standard deviation of 2.3%. To evaluate the precision of the modified procedure for the range of 0 to 1% NH_3, a series of 65 burley tobaccos were analyzed in duplicate, with an overall average of 0.253% ammonia and a pooled standard deviation of 0.0044% ammonia, corresponding to a relative standard deviation of 1.7%. All replicate analyses were on separate extracts.

SUMMARY

A procedure for the automated determination of ammonia in tobacco has been developed. Ammonia is extracted from the ground tobacco sample with water and is determined with a Technicon AutoAnalyzer system which employs separation of the ammonia through volatilization followed by colorimetry using the phenate-hypochlorite reaction. The procedure has been applied to a variety of tobaccos containing from 0.02 to 0.5% ammonia with an overall relative standard deviation of 2%. The accuracy of the procedure as judged by recovery tests and by comparison to a manual distillation method is considered adequate.

ZUSAMMENFASSUNG

Die Autoren entwickelten ein Verfahren für die automatische Bestimmung von Ammoniak im Tabak. Ammoniak wird mit Wasser aus Tabakpulver extrahiert und mit Hilfe eines Technicon-AutoAnalyzers bestimmt, bei dem die Abtrennung des Ammoniaks durch Verdampfung erfolgt; anschließend wird unter Benutzung der Phenolat-Hypochlorit-Reaktion kolorimetriert. Die Methode wurde bei verschiedenen Tabakarten angewendet, die 0,02 bis 0,5% Ammoniak enthielten (Variationskoeffizient insgesamt 2%). Gemessen an der Wiedergewinnung (recovery) und im Vergleich mit einer manuellen Destillationsmethode wird die Genauigkeit des Verfahrens als hinreichend angesehen.

RESUME

On a mis une méthode au point pour la détermination automatisée de l'ammoniaque dans le tabac. On extrait l'ammoniaque de l'échantillon de tabac moulu au moyen d'eau et on le détermine au moyen d'un Technicon-AutoAnalyzer, qui sépare l'ammoniaque par évaporisation et le passe au colorimètre en employant la réaction au phénate d'hypochlorite. Ce procédé a été appliqué à différents tabacs contenant de 0,02 à 0,5% d'ammoniaque et dans l'ensemble il n'a montré une déviation standard relative que de 2% seulement. La précision de la méthode a été considérée comme adéquate, si on juge d'après les tests de récupération et en comparant à une méthode de distillation manuelle.

REFERENCES

1. Ayers, C. W.: The determination of nitrogen and ammonia in tobacco and cigarette smoke; Proc. Soc. Analyt. Chem. 8 (1971) 17—19.
2. Frankenburg, W. G., Gottscho, A. M., Kissinger, S., Bender, D., and Ehrlich, M.: Analytical studies on harvested tobacco leaves; Anal. Chem. 25 (1953) 1784—1798.

3. Gehrke, C. W., Kaiser, F. E., and Ussary, J. P.: Automated spectrophotometric method for nitrogen in fertilizers; J. Ass. Offic. Anal. Chem. 51 (1968) 200–211.
4. Keay, J., and Menage, P. M. A.: Automated distillation procedure for the determination of nitrogen; Analyst 94 (1969) 895–899.
5. Pucher, G. W., Vickery, H. B., and Leavenworth, C. S.: Determination of ammonia and of amide nitrogen in plant tissue; Ind. Eng. Chem., Anal. Ed., 7 (1935) 152–156.
6. Williams, J. F., and Hunt, G. F.: Ammonia in mainstream and side-stream cigarette smoke; 21st Tobacco Chemists' Research Conference, Durham, N. C., Oct. 19–20, 1967.

The authors' address:

Liggett and Myers Incorporated, Research Department, Durham, North Carolina, 27702, USA.