Automated Analysis of Tertiary Amines

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An automated method for the analysis of tertiary amines has been developed using a basic automatic analyzer arrangement. The method is based on a modification of the familiar dye complex extraction method for amines. The method is simple and is not subject to adsorption of the dye on components of the analytical train. A new technique for automated extraction is also described as part of the method. The specificity of the method can be highly controlled by suggested changes in the dye reagent formulation enabling certain amines to be analyzed in the presence of other amines. The method has been designed for the analysis of solid samples.

A LARGE NUMBER of commercially useful compounds are tertiary amines. These compounds traditionally involve difficult and exacting assay procedures. A simple, general automated method for these compounds would be very desirable. It would be of even greater value if a controllable degree of specificity could be incorporated into the automated method since many of these compounds are produced or formulated as mixtures with other amines. The work described in this paper in large measure achieves these objectives.

The majority of published analytical methods for tertiary amines (1–13) are based on some modification of a dye complex formation with a halogenated acid dye as first observed by LaMer and Downes (14) and later supplemented by Griffiths (15). Prudhomme (11) was the first to utilize this principle as the basis of his method for the determination of quinine with eosin. Lehman and Aitken (8) used bromothymol blue to form their amine complex which was extracted into benzene and then reextracted into aqueous alkali. Brodie and Udenfriend (2) used methyl orange, and Cronheim and Ware (3) were the first to use bromocresol purple.

EXPERIMENTAL

Apparatus—The analytical system consisted of the following modules[1]: (*a*) automatic solid sampler; (*b*) proportioning pump; (*c*) photoelectric colorimeter (15-mm., tubular flow cell); (*d*) linearized recorder.

Preliminary Investigations—Since bromothymol blue is most often used in dye complex methods for tertiary amines, it was investigated for its suitability in an automated system. A basic automatic analyzer[2] with the previously mentioned modules was set up for evaluation. The analytical train was equipped with a Technicon glass-bead extractor and Acidflex tubing for all tubes exposed to chloroform. Bromothymol blue reagent was used at a pH of 7.8 and the reagent was extracted in the glass-bead extractor with chloroform. The reagent was decolorized in the system followed by a heavy build-up of yellow dye in the glass-bead extractor. The concentration of dye was increased until some blue color remained, but very high and variable blanks were observed. Other extraction solvents such as methylene chloride, ethylene dichloride, carbon tetrachloride, chlorobenzene, and mixtures of these with methanol, ethanol, propanol, butanol, acetonitrile, dioxane, and tetrahydrofuran were evaluated with very little improvement in performance. Coating the extractor with silicone offered only slight improvement. Mixtures of chloroform with ethanol showed the best response, but results continued to be variable and a pronounced base line drift still occurred.

Methyl orange at a pH of 5.0 and 8.0 was substituted for bromothymol blue with very little improvement. During these studies it was observed that as the dye solutions were made more acidic, dye retention decreased. Several halogenated sulfonphthalein dyes such as bromocresol green, bromophenol blue, bromochlorophenol blue, chlorophenol red, cresol red, iodophenol blue, metacresol purple, phenol red, and thymol blue were evaluated for their performance in acid solutions, and bromocresol purple was selected as the dye of choice. It was shown to have a high sensitivity to certain tertiary amines even in highly acid solutions and, because of its sensitivity, reextraction into alkali was considered unnecessary for most applications. Bromocresol purple also showed very little retention in the analytical train and the base line was only slightly elevated over that observed without reagent.

Reagents—(*a*) Bromocresol purple[3]; (*b*) dye solution—200 mg. of bromocresol purple dissolved in 1,000 ml. of 2% v/v glacial acetic acid; (*c*) Dow Antifoam B added to the wash solution pumped into the automatic sampler.[4]

Procedure—Fig. 1 shows a flow diagram of the

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[1] Technicon Instruments Corp., Chancey, N. Y.

[2] AutoAnalyzer, Technicon Corp.

[3] Eastman Corp., New York, N. Y.

[4] Solidprep Sampler, Technicon Corp.

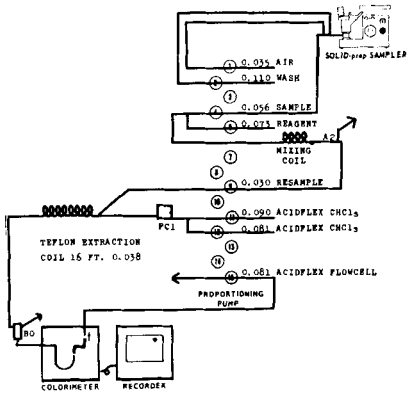

Fig. 1—Flow diagram of automated system for tertiary amine analysis.

analytical train including all tubing sizes. The automatic sampler was programmed to operate at 20 samples per hr. A 0.5 ml./min. sample is pumped from the automatic sampler and segmented with air. The sample stream is mixed with reagent and about 15% of the mixed stream is resampled and pumped back through the pump. Chloroform is pumped through Acidflex tubing into a PCI pulse chamber. The two phases are joined in a Teflon coil extractor and the aqueous dye complex is extracted with the chloroform. The phases are separated in a BO phase separator, and a portion of the chloroform extract is drawn through the flow cell by pumping from the cell. The extract is measured in the colorimeter equipped with 420-mμ filters and the absorbance recorded on a linearized recorder. A 10-roller chain is used on the proportioning pump to provide smooth flow of the pumped solutions. A small glass wool filter is placed in the chloroform line as it emerges from the pump to filter out shreds of Acidflex tubing that are produced in the proportioning pump. The system is started by pumping ethanol through the Acidflex tubing before any aqueous solutions are pumped. The ethanol is replaced by chloroform and after all the ethanol has been purged, the aqueous solutions are started. This technique has provided excellent base line stability and has prevented contamination of the flow cell with aqueous solutions. Dow Antifoam B is used in the wash solution to provide uniform air segmentation.

RESULTS AND DISCUSSION

The method was evaluated primarily for its application to the analysis of propoxyphene hydrochloride (16) (dextro)-4-dimethylamine-1,2-di-phenyl-3-methyl-2-propionoxybutane hydrochloride.

The response follows Beer's law over an absorbance range of 0–0.9 and relative standard deviations of an average of five observations are typically 0.5–1.2%. Figure 2 shows typical recordings of the response of the system to five replicate standards of propoxyphene hydrochloride. Inspection of the curve shows a stable base line, good reproducibility, symmetrical peaks, and no sample interaction. Comparative results between the automatic method and a manual nonaqueous titration method are shown in Table I. The results by the automatic method represent an average of

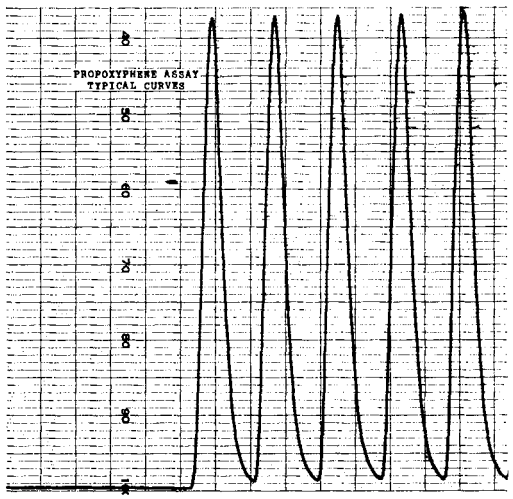

Fig. 2—Response of replicate standards of propoxyphene hydrochloride.

Table I—Comparative Data on Propoxyphene Hydrochloride Samples Between Manual and Automated Methods

Lot	Manual mg./dose	Automated mg./dose	
1	66.2	66.3	(20)
2	65.0	64.4	(20)
3	65.0	64.9	(20)
4	33.6	32.6	(10)
5	32.8	32.8	(10)
6	33.2	32.9	(10)

the indicated number of single-dose assays, while the manual method represents the result obtained on a composite sample of 10 doses. Considering that the same doses were not assayed by both methods, the correlation is very good. This data was obtained on multicomponent mixtures in both capsule and tablet formulations.

The method is shown using an automatic sampler since the majority of the tertiary amines analyzed in our laboratory are assayed as solid samples, primarily as multicomponent tablets or capsules. Tablets are placed directly in the cups of the automatic sampler and capsules are emptied into the cups and the empty capsules are discarded. Standard solutions of the amine are normally dissolved in the appropriate solvent and aliquots of these solutions are pipeted into the sample cups. A correction is made in the absorbance of the standard to account for the volume change resulting from the use of liquid standards. Sample size differences are accommodated by changing the amount of solvent delivered by the automatic sampler or by changing to an alternate size pump tube. Alternate pump tubes are placed in the proportionating pump manifold and tube connections are changed without opening the pump platen.

The concentration of the dye required in the reagent was determined by adjusting the concentration until linearity was obtained throughout the desired concentration range. An additional 50% excess of dye was used since large increases in dye concentration increase the reagent blank slightly. The acidity was adjusted for highest sensitivity to

propoxyphene hydrochloride. The acid concentration can be varied from 1.5 to 3% glacial acetic acid without significant change in response. Eastman bromocresol purple provides a much higher response to the amines tested than some of the other brands, but the reason for this difference in response was not investigated.

The automatic sampler was evaluated for its performance characteristics by analysis of potassium permanganate tablets. These tablets were selected since they provide a relatively stable, highly colored sample which can be traced throughout the system and their tablet weight is essentially equivalent to their content of potassium permanganate. Use of these tablets vividly emphasizes any localized problems in sampling, mixing, sample interaction, and wash characteristics. Hydroxylamine hydrochloride is run through the system occasionally to clean out any traces of manganese dioxide formed in the analytical train. This technique pointed up two serious problems in the use of the automatic sampler. Sample interaction was significant and solvent delivery was erratic. The source of the eratic solvent delivery was found to be due to faulty check valves in the sampler, thus allowing variable amounts of air to be delivered along with the solvent. This problem was solved by replacing the valves with check valves[5] which provide a very positive action and result in precise dispensing of solvent in the sampler. The conventional system of withdrawing a sample from the automatic sampler resulted in too large a sample interaction for precise work and a new sampling device was designed. This consisted of a small rectangular Lucite block with a 0.89-mm. (0.35-in.) hole drilled through its long axis and 2 side ports. The device was placed in the sample line adjacent to the blender compartment of the sampler. The side port nearest the sampler was connected to the wash pump tube. This tube is selected to provide at least twice as much wash volume as is withdrawn by the sample pump tube. During sampling the wash tube is bypassed and has no effect on the system. During the rinse part of the cycle the wash solution divides so that a portion flows into the sample pump tube and the remainder flows into the blender compartment of the sampler, effectively blocking off any leakage of sample from the blender. The other side port is connected to the air pump tube and all air segmentation occurs at this point during both sample and rinse cycles. With this arrangement a uniformly segmented stream, originating near the blender, is always pumped. Positive and precise sampling is obtained with no evidence of sample interaction.

The glass-bead extractor was found to provide inconsistent and erratic extraction in the described system. Base line drift was encountered and significant sample interaction was observed. Several other extractor designs were evaluated with only moderate improvement in results. One of the critical factors in automatic extractions is the method of introduction of the two phases. If one phase is allowed to lag or lead the other or if one phase is allowed to pool in the extractor, erratic results are obtained. An excellent, yet simple extractor was designed to eliminate these problems. It consisted of a coil of 0.96-mm. (0.38-in.) i.d. Teflon spaghetti tubing wound around a 2.54-cm. (1-in.) diameter cylinder. One end of the Teflon coil is simply connected to the pulse chamber containing chloroform by a short length of Acidflex tubing. A piece of 0.8-mm. (0.030-in.) o.d. stainless steel tubing is inserted into the side of the Acidflex tubing so that one end of the stainless steel tube enters the end of the Teflon coil extractor in a coaxial arrangement. The other end of the extractor coil is connected to a small volume phase separator by another short piece of Acidflex tubing. The aqueous phase is introduced through the stainless steel tube and is swept into the extraction coil by the chloroform. Because of the small diameter of the Teflon extraction tubing and the high velocities of flow, the aqueous phase is separated into small discrete segments and remains segmented throughout the entire extraction coil resulting in excellent sample separation. The aqueous stream is debubbled prior to introduction into the extractor and the aqueous phase in effect replaces the air bubble for segmentation. Since this extractor is free of violent agitation, essentially no emulsion formation has been observed. This allows the use of small volume phase separators, and clean phase separations are obtained as a result of the uniform floating off of the very small segments of the lighter phase as it exits from the extraction coil. Because of the segmentation, the length of the extraction coil can be adjusted for optimum extraction efficiency without affecting sample interaction.

SPECIFICITY

A limited investigation was made into the specificity of the method as described. About 100 amines and related compounds were evaluated for their response in the system. Some of the results are shown in Table II. As can be seen, the method in

TABLE II—RESPONSE OF COMPOUNDS TO AUTOMATED METHOD

Compound	Strong Response	Weak Response	No Response
Acetanilide	...	...	×
p-Aminobenzoic acid	...	...	×
4-Aminosalicylic acid	...	...	×
Amphetamine hydrochloride	...	×	...
Aniline	...	...	×
Atropine	×	...	...
Benzalkonium chloride	...	...	×
Benzamide	...	...	×
Benzylamine	...	×	...
n-Butylamine	...	×	...
Cetylpyridinium chloride	...	...	×
Codeine phosphate	×	...	...
Dimethylamino-benzaldehyde	...	...	×
Diphenylamine	...	...	×
Erythromycin	×	...	...
Ethylenediamine	...	...	×
Glycine	...	...	×
Procaine hydrochloride	×	...	...
Pyridine	×	...	...
Quinine hydrochloride	×	...	...
Sulfathiazole	...	...	×

[5] Circle Seal, 230T1—2pp Circle Seal Products Co., Inc. Pasadena, Calif.

TABLE III—MOLAR EXCITATION VALUES OBTAINED BY THE AUTOMATED METHOD IN RESPONSE TO VARIATIONS IN THE DYE REAGENT FORMULATION

Compound	pH 5.0 Citrate Buffer	Acetic Acid, 2%	Sulfuric Acid, 1%
Propoxyphene hydrochloride	10,600	15,600	10,900
Levopropoxyphene hydrochloride	10,700	15,700	10,800
Piperocaine hydrochloride	17,200	16,800	13,500
Quinine hydrochloride	12,400	16,400	190
Erythromycin base	11,600	15,200	7,800
Methapyrilene hydrochloride	12,000	12,750	neg.
Cyclomethycaine hydrochloride	1,970	8,950	4,180
Procaine hydrochloride	8,200	6,400	neg.
Amphetamine hydrochloride	1,070	760	17
2-Diethylaminoethanol	1,070	375	neg.
Benzylamine	730	200	neg.
n-Butylamine	170	60	neg.
Benzalkonium chloride	neg.	neg.	neg.

general responds to aromatic tertiary amines while showing very little or no response to primary and secondary amines or to amides. This response is quite different from that reported by Woods *et al.* (13) in their work with bromocresol purple which did not depend on extraction of the dye complex from acid solutions. The lack of response to quaternary ammonium salts was probably due to adsorption in the analytical train since a positive response was seen in a manual extraction procedure. Special techniques for prevention of adsorption in the analytical train might allow the method to be used for analysis of certain quaternary amines.

Some of the compounds which did show response were further evaluated for their response to changes in dye reagent formulation. Reagents were made up at a pH of 5.0 with citrate-phosphate buffer, pH 2.2 with 2% glacial acetic acid, and pH 1.2 with 1% sulfuric acid. Table III shows some of the results. The results are expressed as molar extinction values calculated from sample size, dilution in the automated system, and the absorbance obtained. Molar extinction values reported here are about 30% lower than those obtained by manual extraction and measurement in a suitable spectrophotometer. A very interesting spectrum of response was observed and considerable change in specificity was observed with changes in reagent.

ANALYTICAL POTENTIAL

The described method should prove very useful in the precise analysis of large numbers of tertiary amine samples such as might be encountered in single-dose analysis profiles of production lots of pharmaceutical formulations. Appropriate process samples, both solid and liquid, could also be analyzed in large numbers. Modifications of the dye reagent such as those indicated might allow analysis of the desired amine in the presence of other amines while still retaining satisfactory sensitivity. The described specificity could also be effectively utilized in manual adaptations of this method. The new extractor design provides the possibility of repetitive extractions or scrubbing with immiscible solvents without significant loss of sample separation or increase of sample interaction. Decreased emulsion problems could lead to an increased sensitivity in repetitive extraction procedures since this would allow a larger volume of the desired phase to be retained for the next step of analysis. All of these advantages could be utilized in the application of this method to analysis of natural products, feeds, or fermentation samples.

REFERENCES

(1) Amundson, M. E., Johnson, M. L., and Manthey, J. A., *J. Pharm. Sci.*, **54,** 684(1965).
(2) Brodie, B. B., and Udenfriend, S., *J. Biol. Chem.*, **158,** 705(1945).
(3) Cronheim, G., and Ware, P. A., *J. Pharmacol. Exptl. Therap.*, **92,** 98(1948).
(4) Dill, W. A., and Glazko, A. J., *J. Biol. Chem.*, **179,** 395(1949).
(5) Durick, F., King, J. S. Ware, P. A., and Cronheim, G., *J. Am. Pharm. Assoc., Sci. Ed.*, **39,** 680(1950).
(6) Fogh, J., Rasmussen, P. O., and Skadhauge, K., *Anal. Chem.*, **26,** 392(1954).
(7) Gettler, A. O., and Sunshine, I., *ibid.*, **23,** 779(1951).
(8) Lehman, R. A., and Aitken, T., *J. Lab. Clin. Med.*, **28,** 787(1943).
(9) Pearce, A. S., *Chem. Ind.*, **1961,** 825.
(10) Porter, C. C., and Silber, R. H., *J. Biol. Chem.*, **182,** 109(1950).
(11) Prudhomme, R. O., *J. Pharm. Chim.*, **9,** 8(1940).
(12) Scott, C. C., and Chen, K. K., *J. Pharmacol. Exptl. Therap.*, **87,** 63(1946).
(13) Woods, L. A., Cochin, J., Fornefeld, E. J., McMahon, F. G., and Seevers, M. H., *ibid.*, **101,** 188(1951).
(14) LeMer, V. K., and Downes, H. C., *J. Am. Chem. Soc.*, **55,** 1840(1933).
(15) Griffiths, D. C., *J. Chem. Soc.*, **1938,** 815.
(16) Pohland A., and Sullivan, H. R., *J. Am. Chem. Soc.*, **75,** 4458(1953).

Keyphrases

Tertiary amines—automated analysis
Automated analysis—dye complex extraction
Bromocresol purple—dye reagent
Diagram—automated analyzer
Colorimetric analysis