Simultaneous Semiautomated Assay of Pyrrobutamine Phosphate, Cyclopentamine Hydrochloride, and Methapyrilene Hydrochloride in Pharmaceutical Mixtures

J. L. HALE

Abstract □ Antihistamine preparations containing methapyrilene hydrochloride, pyrrobutamine phosphate, and cyclopentamine hydrochloride were assayed by introducing an aqueous sample solution into the appropriate automated system. Methapyrilene hydrochloride was determined by UV spectrophotometry. Pyrrobutamine phosphate was extracted as an ion-pair and quantitated colorimetrically by forming the bromcresol purple acid–dye complex. Cyclopentamine hydrochloride was determined colorimetrically by using the copper dithiocarbamate reaction for secondary amines.

Keyphrases □ Pyrrobutamine phosphate—semiautomated colorimetric analysis, pharmaceutical mixtures □ Cyclopentamine hydrochloride—semiautomated colorimetric analysis, pharmaceutical mixtures □ Methapyrilene hydrochloride—semiautomated UV spectrophotometric analysis, pharmaceutical mixtures □ Colorimetry—semiautomated analyses, pyrrobutamine phosphate and cyclopentamine hydrochloride, pharmaceutical mixtures □ UV spectrophotometry—semiautomated analysis, methapyrilene hydrochloride, pharmaceutical mixtures □ Automated analyses—pyrrobutamine phosphate, cyclopentamine hydrochloride, and methapyrilene hydrochloride, pharmaceutical mixtures □ Antihistamine preparations—pyrrobutamine phosphate, cyclopentamine hydrochloride, and methapyrilene hydrochloride, semiautomated analyses

Pharmaceutical mixtures of pyrrobutamine phosphate (I), cyclopentamine hydrochloride (II), and methapyrilene hydrochloride (III) are assayed by manual spectrophotometric methods and by temperature-programmed GLC[1]. These methods are time consuming and do not lend themselves to control work where numerous samples are involved. An automated method was developed to determine the content uniformity of capsules[2] containing these antihistamines.

A modification of the bromcresol purple acid–dye method for tertiary amines (1) was used to assay pyrrobutamine phosphate. A manual method for secondary amines (2) was modified and adapted to the automated system for the colorimetric determination of cyclopentamine hydrochloride. Methapyrilene hydrochloride was determined using UV spectrophotometry by basically the same method as reported by Fernandez *et al.* (3).

EXPERIMENTAL

Equipment—The analytical train consisted of a liquid sampler[3], a proportioning pump[4], spectrophotometers[5] equipped with 10-mm flowcells, and suitable recorders.

Reagents—*Pyrrobutamine Phosphate*—The wash solution was 0.02 *N* hydrochloric acid and was the same for the three systems. The bromcresol purple reagent was prepared by dissolving 350 mg of bromcresol purple (5′,5″-dibromo-*o*-cresolsulfonphthalein sodium salt[6]) in about 200 ml of purified water contained in a 1-liter flask, adding 250 ml of 0.2 *M* potassium biphthalate and 22.6 ml of 1 *N* sodium hydroxide, and diluting to volume with purified water (pH should be 5.0 ± 0.1). The hydrochloric acid solution was 1% (v/v). Chloroform (analytical reagent) was used as the displaced organic solvent.

Cyclopentamine Hydrochloride—The displaced organic solvent was prepared by mixing 50 ml of carbon disulfide with 950 ml of chloroform.

Copper reagent was prepared by dissolving 800 mg of copper sulfate pentahydrate and 10 g of ammonium acetate in about 500 ml of purified water, adding 100 ml of 28% ammonia solution, and diluting to 1 liter with purified water.

Table I—Precision Determined by Assaying Common Solutions and Powders

Formulation	Cyclopentamine Hydrochloride, %	Pyrrobutamine Phosphate, %	Methapyrilene Hydrochloride, %
1	1.11	0.68	0.50 (solution)
	0.76	0.99	— (powder)
2	0.70	0.83	0.58 (solution)
	0.90	0.66	— (powder)

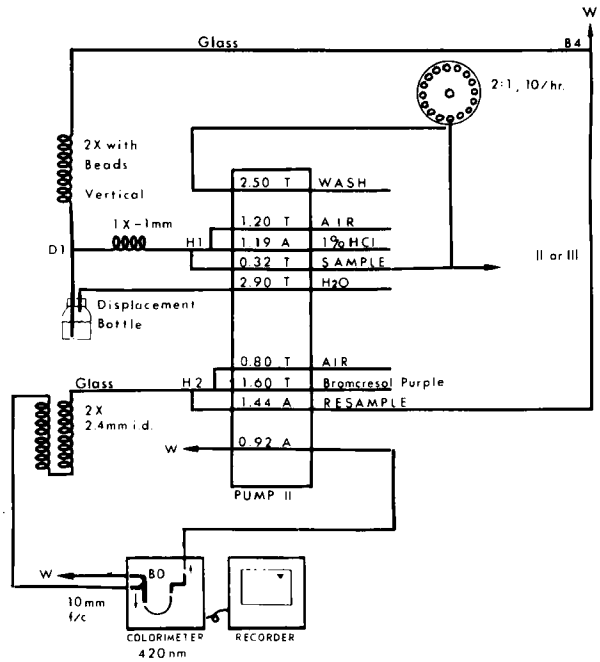

Figure 1—*Components for semiautomated analysis of pyrrobutamine phosphate.*

[1] Unpublished methods, Eli Lilly and Co.
[2] Co-Pyronil, Eli Lilly and Co., Indianapolis, IN 46206
[3] Technicon Liquid Sampler II.
[4] Technicon Proportioning Pump II.
[5] Perkin-Elmer model 55 or 124D.
[6] Eastman No. 6266.

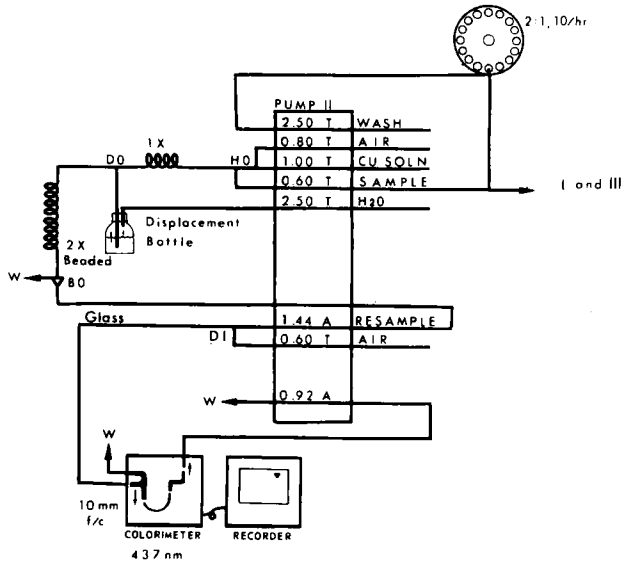

Figure 2—*Components for semiautomated analysis of cyclopentamine hydrochloride.*

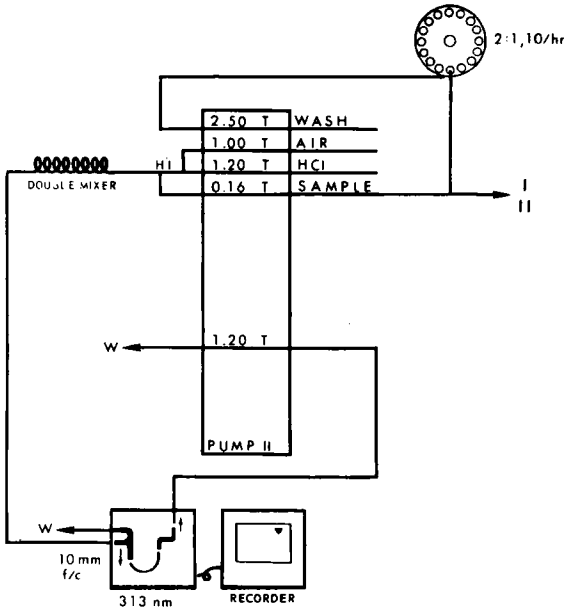

Figure 3—*Components for semiautomated analysis of methapyrilene hydrochloride.*

Methapyrilene Hydrochloride—One percent hydrochloric acid (v/v) was used.

Standards—For the standards, 25.0 mg of methapyrilene hydrochloride, 15.0 mg of pyrrobutamine phosphate, and 12.5 mg of cyclopentamine hydrochloride were dissolved in about 100 ml of purified water. Then the solution was diluted to 200.0 ml with purified water. The standard solution is stable for at least 2 weeks.

Procedure—Dissolve the contents of each capsule in sufficient purified water to achieve a concentration of about 125 μg/ml for methapyrilene hydrochloride, 75 μg/ml for pyrrobutamine phosphate, and 62.5 μg/ml for cyclopentamine hydrochloride. If the formulation contains magnesium carbonate, silicone fluid, or mineral oil, use 0.02 *M* hydrochloric acid as the sample diluent and heat the solution gently on a steam bath for 15 min with intermittent swirling. Allow the solution to stand until the insolubles settle or, alternatively, centrifuge and use the supernate as the assay solution.

Place the sample solution in 8.5-ml sample cups and place on the liquid sampler. The automated modules should be arranged as shown in Figs. 1–3. A common sampler may be used with a stream splitter. The usual practice in this laboratory is to place two components on one pump and use another pump for the third component, if present. The usual sampling pattern is three standards, five samples, one standard, five samples, and one standard.

RESULTS AND DISCUSSION

The manual assay involves a chloroform extraction of the acidified sample solution wherein the extractable ion-pair formed by pyrrobutamine is quantitatively separated from the other two components. The chloroform extract is evaporated to dryness, the residue is taken up in methanol, and the UV absorbance at 226 nm is determined. Methapyrilene hydrochloride is determined by UV spectrophotometry using an acidified, aqueous solution of the sample. Cyclopentamine hydrochloride is determined colorimetrically by forming the extractable copper dithiocarbamate complex and determining the absorbance in methanol–chloroform solution at 437 nm.

The manual procedure for methapyrilene hydrochloride was easily adapted to automation. The pyrrobutamine phosphate assay was more difficult because of the chloroform evaporation step. This portion of the assay was avoided by employing the bromcresol purple reaction for tertiary amines. The acid–dye complex is ordinarily formed by placing the analyte and the bromcresol purple in an acidic aqueous solution and then extracting into an immiscible organic solvent. It was found that the extracted ion-pair reacted quantitatively with an aqueous bromcresol purple solution buffered at pH 5.0. The reaction was linear throughout the concentration range of interest (0–100 μg/ml). A 100-μg/ml pyrrobutamine phosphate solution gave an absorbance of about 0.450.

Cyclopentamine hydrochloride was adapted to automation by making several changes in the reported manual methods (2, 4, 5). Attempts to incorporate chloroform-miscible solvents such as methanol, propanol, and pyridine were made, but little or no advantage was gained so they were eliminated from the system. A sludge buildup in the beaded extraction coil was largely eliminated by using a large excess of ammonium hydroxide. Use of the displacement bottle helped prevent particles of acid-flex tubing from entering the system. The reaction obeys Beers law from 0 to 75 μg/ml with an absorbance of about 0.490 for a 75-μg/ml solution.

The precision of the assay was determined by performing 10 replicate assays from a common solution and from a common powder. Table I shows the relative standard deviation values obtained. Accuracy was determined by preparing authentic mixtures of the various formulations encountered (Table II). The excipient materials encountered were silica gel, starch, talc, magnesium stearate, silicone fluid, and mineral oil. Magnesium carbonate, when present, reduced pyrrobutamine phosphate recovery by as much as 50%. Silicone fluid and mineral oil reduced pyrrobutamine phosphate recovery by 5–8%. Dissolution of the sample with 0.02 *M* hydrochloric acid followed by mild heating eliminated these interferences. Satisfactory recoveries

Table II—Accuracy Determined by Assaying Authentic Mixtures

Cyclopentamine Hydrochloride			Pyrrobutamine Phosphate			Methapyrilene Hydrochloride		
Milligrams Added	Milligrams Found	Recovery, %	Milligrams Added	Milligrams Found	Recovery, %	Milligrams Added	Milligrams Found	Recovery, %
10.73	10.80	100.6	12.81	12.64	98.6	21.7	21.3	98.2
12.52	12.62	100.6	15.09	14.82	98.2	25.2	24.8	98.4
14.31	14.32	100.1	17.19	16.87	98.1	29.2	28.7	98.1
5.39	5.44	100.9	6.43	6.47	100.6	10.69	10.64	99.5
6.32	6.35	100.4	7.52	7.44	98.9	12.68	12.49	98.4
7.17	7.21	100.5	8.68	8.62	93.3	14.54	12.28	98.2

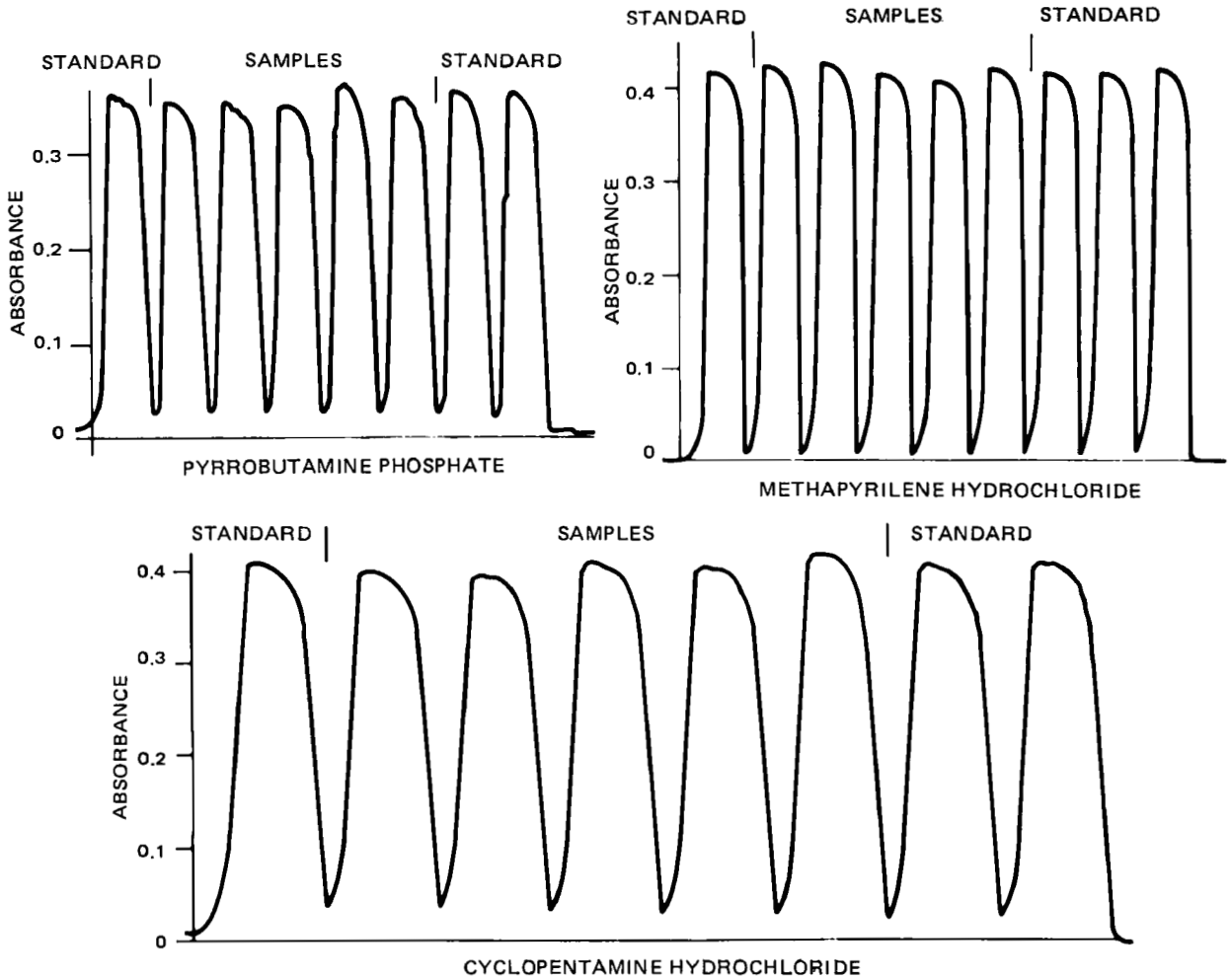

Figure 4—*Recorder traces for semiautomated assay of methapyrilene hydrochloride, pyrrobutamine phosphate, and cyclopentamine hydrochloride.*

were exhibited for the three components at 85, 100, and 115% of theoretical amounts. Typical curves are shown in Fig. 4.

The system has been in use for more than a year in this laboratory for content uniformity assays on capsules, bulk powder analyses, new formulation development, and homogeneity studies. The method is accurate and precise and offers a substantial time saving over manual spectrophotometric and GLC assays.

REFERENCES

(1) N. R. Kuzel, "Automation in Analytical Chemistry, Technicon Symposia 1966," vol. 1, Mediad, White Plains, N.Y., 1967, pp. 218–221.

(2) G. R. Umbreit, *Anal. Chem.*, **33**, 1572(1961).

(3) J. Fernandez, E. Laszlo, and L. C. Leeper, "Automation in Analytical Chemistry, Technicon Symposia 1967," vol. 1, Mediad, White Plains, N.Y., 1968, pp. 471–482.

(4) F. E. Critchfield and J. B. Johnson, *Anal. Chem.*, **28**, 430 (1956).

(5) S. Siggia, "Quantitative Organic Analysis via Functional Groups," 3rd ed., Wiley, New York, N.Y., 1963, p. 497.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 22, 1975, from *Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication January 8, 1976.

The author thanks W. G. Richardson, A. D. Garner, and R. W. Roller for their guidance and assistance in preparing the paper.