(3) M. I. Blake and L. B. Shumaker, *J. Ass. Offic. Anal. Chem.*, **56**, 653(1973).
(4) G. B. Barlin and D. D. Perrin, *Quart. Rev.*, **20**, 75(1966).
(5) T. D. Edmonson and J. E. Goyan, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 810(1958).
(6) R. H. Cundiff and P. C. Markunas, *Anal. Chem.*, **28**, 792(1956).
(7) M. I. Blake and D. A. Nona, *J. Pharm. Sci.*, **53**, 570(1964).
(8) M. I. Blake, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 287(1957).
(9) S. L. Lin and M. I. Blake, *J. Pharm. Sci.*, **55**, 781(1966).
(10) *Ibid.*, **56**, 43(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 2, 1973, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication July 27, 1973.

The authors gratefully acknowledge the Arnar-Stone Laboratories, Mt. Prospect, Ill., for supplying samples of dosage forms.

* Present address: Pharmacy Service, Hines Veterans Administration Hospital, Hines, IL 60141

x To whom inquiries should be directed.

# Analysis of Acetaminophen, Phenylephrine Hydrochloride, Diphenhydramine Hydrochloride, and Ascorbic Acid in a Capsule Preparation

FABRIZIO De FABRIZIO

**Abstract** □ A column chromatographic procedure was developed for separating acetaminophen, phenylephrine hydrochloride, and diphenhydramine hydrochloride in combination with ascorbic acid. While acetaminophen, which passes through an alginic acid column with the washings, is determined spectrophotometrically at 257 nm, phenylephrine hydrochloride and diphenhydramine hydrochloride are both eluted from the column with 0.01 *N* hydrochloric acid and determined simultaneously at two different wavelengths. Ascorbic acid is analyzed by a visual titration method, employing 2,6-dichloroindophenol as the titrant.

**Keyphrases** □ Acetaminophen—method for separation and analysis in combination with phenylephrine hydrochloride, diphenhydramine hydrochloride, and ascorbic acid □ Phenylephrine hydrochloride—method for separation and analysis in combination with acetaminophen, diphenhydramine hydrochloride, and ascorbic acid □ Diphenhydramine hydrochloride—method for separation and analysis in combination with acetaminophen, phenylephrine hydrochloride, and ascorbic acid □ Ascorbic acid—method for separation and analysis in combination with acetaminophen, phenylephrine hydrochloride, and diphenhydramine hydrochloride

Acetaminophen, phenylephrine hydrochloride, diphenhydramine hydrochloride, and ascorbic acid are used alone and in combination with other drugs in various pharmaceutical preparations. Several methods are available (1–7) for the analysis of these therapeutically active ingredients as pure substances and in combination. Smith (8) recently described a method for the separation and determination of sympathomimetic amines, antihistamines, and phenothiazines in various mixtures using polystyrene cation and quaternary ammonium anion resins packed into two assembled columns. Although this method might have served as the basis for the present study, a previously reported analytical procedure (9), which employed an alginic acid column with UV spectrophotometry and which was adequate for the separation and determination of acetaminophen, phenylephrine hydrochloride, codeine phosphate, and pyrilamine maleate in tablets or powder, was chosen. Therefore, the present report concerns the feasibility of utilizing alginic acid (10) for cation exchange in the analysis of pharmaceutical products. This investigation found that acetaminophen and ascorbic acid were not retained by the alginic acid but passed through the prepared column; phenylephrine hydrochloride and diphenhydramine hydrochloride were both eluted with 0.01 *N* hydrochloric acid and were simultaneously determined at 236 and 273 nm. Finally, ascorbic acid was analyzed by a visual titration method in an aliquot of the original solution using 2,6-dichloroindophenol.

**Table I**—Recovery of Phenylephrine Hydrochloride and Diphenhydramine Hydrochloride Mixed Standard in 50% Ethanol in 0.5% (v/v) Acetic Acid

| | Phenylephrine Hydrochloride | | Diphenhydramine Hydrochloride | |
|---|---|---|---|---|
| Mixture | Amount Taken, mg | Recovery, % | Amount Taken, mg | Recovery, % |
| 1 | 7.5 | 98.3 | 15.0 | 98.3 |
| 2 | 7.5 | 101.8 | 15.0 | 99.5 |
| 3 | 15.0 | 99.4 | 30.0 | 101.4 |
| 4 | 15.0 | 99.1 | 30.0 | 98.2 |
| 5 | 7.5 | 98.7 | 15.0 | 98.3 |
| 6 | 15.0 | 100.5 | 30.0 | 101.2 |
| 7 | 7.5 | 101.3 | 15.0 | 100.7 |
| 8 | 7.5 | 101.4 | 15.0 | 98.6 |
| 9 | 15.0 | 99.6 | 30.0 | 98.3 |
| 10 | 7.5 | 99.4 | 15.0 | 98.8 |
| Average, % | — | 99.9 | — | 99.8 |
| *SD*, % | | ±1.23 | | ±1.29 |

## EXPERIMENTAL

**Apparatus**—A recording spectrophotometer[1] with matched UV

[1] Beckman DB.

**Table II**—Recovery of Acetaminophen, Phenylephrine Hydrochloride, Diphenhydramine Hydrochloride, and Ascorbic Acid from Synthetic Mixtures in the Presence of the Excipient

| Mixture | Acetaminophen | | Phenylephrine Hydrochloride | | Diphenhydramine Hydrochloride | | Ascorbic Acid | |
|---|---|---|---|---|---|---|---|---|
| | Added, mg | Recovery, % | Added, mg | Recovery, % | Added, mg | Recovery, % | Added, mg | Recovery, % |
| A | 590.6 | 98.6 | 8.8 | 99.2 | 15.7 | 98.3 | 74.7 | 98.9 |
| B | 600.0 | 99.0 | 7.9 | 101.3 | 15.4 | 99.5 | 75.3 | 98.3 |
| C | 584.3 | 98.9 | 8.0 | 98.0 | 15.8 | 101.4 | 75.3 | 99.2 |
| D | 599.1 | 99.8 | 7.3 | 100.5 | 14.7 | 98.2 | 75.4 | 97.9 |
| E | 600.0 | 100.3 | 7.9 | 99.7 | 15.2 | 98.3 | 74.9 | 100.9 |
| F | 579.9 | 98.7 | 7.5 | 101.3 | 14.9 | 101.2 | 75.1 | 99.0 |
| G | 589.3 | 99.8 | 7.4 | 98.7 | 15.8 | 100.7 | 75.8 | 98.3 |
| H | 578.4 | 98.2 | 8.7 | 101.4 | 15.3 | 98.7 | 75.4 | 98.6 |
| I | 595.5 | 98.9 | 8.1 | 99.0 | 15.6 | 98.3 | 74.3 | 99.1 |
| J | 577.9 | 101.0 | 8.4 | 100.0 | 14.8 | 98.8 | 75.1 | 98.8 |
| Average, % | | 99.3 | | 99.9 | | 99.3 | | 98.8 |
| *SD*, % | | ±0.87 | | ±1.29 | | ±1.29 | | ±0.80 |

1- and 4-cm cells was used. The glass column, 30 × 1.8 cm with a 5-cm stem, was fitted with a buret key.

**Reagents**—The following were used: alginic acid[2] cation-exchange resin, 40–100 mesh; 0.01 *N* hydrochloric acid in water; 2 *N* hydrochloric acid in water; 0.1 *N* sodium hydroxide in water; 50% ethanol in 0.5% (v/v) acetic acid in water; and 0.5% 2,6-dichloroindophenol in water.

**Standard Solutions**—The following were used: acetaminophen, 0.006 mg/ml in 0.01 *N* sodium hydroxide; phenylephrine hydrochloride, 0.015 mg/ml in 0.01 *N* hydrochloric acid; diphenhydramine hydrochloride, 0.03 mg/ml in 0.01 *N* hydrochloric acid; and ascorbic acid, 1.5 mg/ml in 50% ethanol in 0.05% (v/v) acetic acid.

**Column Preparation**—About 4 g alginic acid was slurried in water and allowed to soak 4 hr. The slurry was poured into a glass column and allowed to settle. The column was washed with 2 *N* hydrochloric acid until the absorbance of the eluate (path length 4 cm) was less than 0.005 at 235 and 273 nm, and it was then washed with distilled water until the eluate was neutral to litmus solution. Finally, 15 ml of 50% ethanol was passed through the column.

**Sample Treatment**—The average weight of 20 capsules was determined, and an accurately weighed portion of powder, equivalent to about 1.5 capsules[3], was transferred to a 50-ml volumetric flask. Then 50% ethanol–acetic acid solution was added to the mark. The mixture was stirred for 3 min and then centrifuged for 2 min.

**Determination**—*Ascorbic Acid*—Dilute 1 ml of standard ascorbic acid solution (containing 1.5 mg ascorbic acid) in a small erlenmeyer flask with 10 ml of 50% ethanol–acetic acid solution. Titrate with the dye solution to a pink color, which persists for 15 sec. Since this volume of dye represents 1.5 mg ascorbic acid, the ascorbic acid equivalent of 1 ml of dye solution is equal to 1.5 divided by the volume in milliliters of the dye solution in this titration.

Dilute a 1-ml aliquot of the centrifuged sample solution in a small erlenmeyer flask with 10 ml of solvent and titrate immediately with the standard dye solution to a faint pink end-point, which persists for 15 sec.

*Acetaminophen*—Pipet a 10-ml aliquot of the centrifuged sample solution onto the prepared alginic acid column. Place a 500-ml volumetric flask beneath the column and start collecting the eluate at a rate of 1 ml/min. Then add 200 ml water divided into four portions, allowing each portion to sink into the resin. Remove the flask and dilute to volume with water. Dilute further with 0.01 *N* sodium hydroxide to give a concentration of acetaminophen equal to about 0.006 mg/ml. Scan the sample and standard solutions between 360 and 220 nm in 1-cm cells against 0.01 *N* sodium hydroxide as the reference. Determine the absorptivity at the maximum (about 257 nm) for acetaminophen standard and calculate the concentration of acetaminophen in the sample solution.

*Phenylephrine Hydrochloride and Diphenhydramine Hydrochloride*—Elute the column with 0.01 *N* hydrochloric acid at a rate of 2 ml/min. Discard the first 10 ml of eluate, collecting the rest in a 100-ml volumetric flask to volume. This solution contains phenylephrine and diphenhydramine. Determine the absorbances of the sample solution at 236 and 273 nm in 4-cm cells against 0.01 *N* hydrochloric acid as reference. Determine the absorptivities at the same wavelengths for both phenylephrine hydrochloride and diphenhydramine hydrochloride standard and calculate their concentrations in the sample solution.

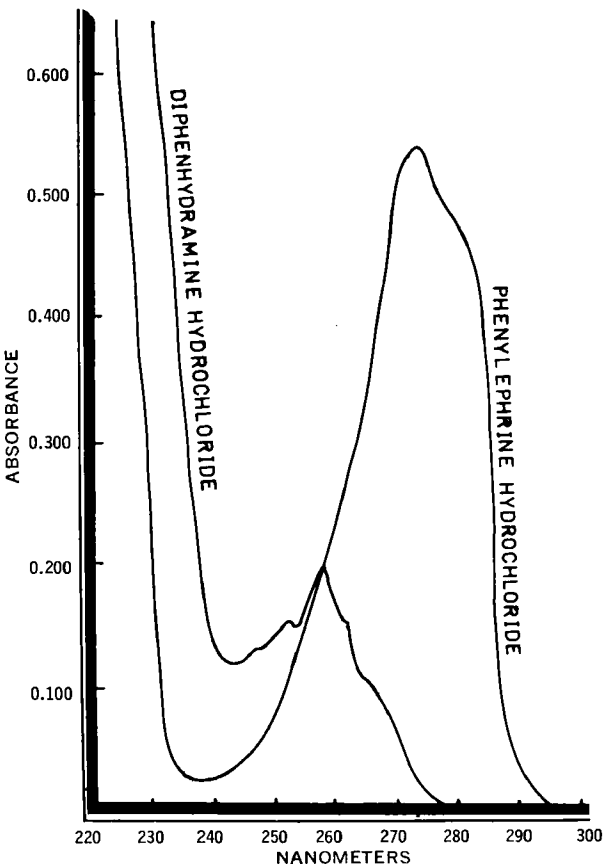


**Figure 1**—*Spectra of 0.015 mg/ml of phenylephrine hydrochloride and of 0.03 mg/ml of diphenhydramine hydrochloride in 0.01* N *HCl.*

[2] British Drug Houses.

[3] The analysis was applied to capsules containing 400 mg acetaminophen, 5 mg phenylephrine hydrochloride, 10 mg diphenhydramine hydrochloride, and 50 mg ascorbic acid; these capsules were marketed as "Flustop."

## RESULTS AND DISCUSSION

The presented method reaffirms that ion-exchange chromatography is an excellent general procedure for quantitatively separating alkaloid bases and organic acids. Initially, the choice of the most suitable solvent for readily dissolving all of the active ingredients as well as providing a solution with pH 3–4 (the optimum pH range for alginic acid absorption) was important. An equivalent mixture of 0.5% (v/v) acetic acid in water and ethanol was the most appropriate. Although this study did not present particular difficulties because a previous method served as its basis, attention was focused on the possibility of finding two different wavelengths where the interferences of phenylephrine hydrochloride and diphenhydramine hydrochloride, contained in the same eluate, were as low as possible. Several standard solutions, containing these two active ingredients in the same weight ratio as in the commercial formulation, were subjected to a spectrophotometric test at 285 nm (peak for diphenhydramine hydrochloride), 273 nm (peak for phenylephrine hydrochloride), 252, 243, and 236 nm. It was found that the most favorable results were obtained by using wavelengths at 273 and 236 nm, which correspond to the maximum and the minimum absorbances for phenylephrine hydrochloride, respectively (Fig. 1). The results of 10 determinations of mixtures containing phenylephrine hydrochloride are shown in Table I.

In the determination of acetaminophen, the effects of ascorbic acid, starch[4], and the alcoholic-acetic acid solvent which passed through the alginic acid column together with acetaminophen were investigated. Standard mixtures of these three ingredients in an alcoholic-acetic solution were subjected to the described procedure. It was found that they did not affect the results at 257 nm because they were in such high dilution. The accuracy of the proposed method was based upon results of 10 synthetic mixtures containing all ingredients and prepared in a manner similar to commercial formulations (Table II).

In the quantitative evaluation of ascorbic acid, the use of 2,6-dichloroindophenol yielded reasonable results. This titrant did not interfere with the other components present in this formulation. The possibility of utilizing other methods for the determination was not investigated.

This method provides a simple and rapid means by which the concentration of each component of this preparation is quantitatively determined.

[4] Starch was used as the excipient.

## REFERENCES


(1) "The British Pharmacopoeia," The Pharmaceutical Press, London, England, 1968, p. 708.

(2) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, pp. 589, 590.

(3) C. F. Hiskey and N. Levin, *J. Pharm. Sci.*, **50**, 393(1961).

(4) E. Stahl, "Thin Layer Chromatography," Springer-Verlag, Berlin, Germany, 1969, p. 524.

(5) K. T. Kosby, *J. Pharm. Sci.*, **53**, 1280(1964).

(6) N. Shane and M. Kowblansky, *ibid.*, **57**, 1218(1968).

(7) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 208.

(8) D. J. Smith, *J. Ass. Offic. Anal. Chem.*, **55**, 596(1972).

(9) J. S. Foster and J. W. Murfin, *Analyst*, **85**, 462(1961).

(10) F. De Fabrizio, *J. Pharm. Sci.*, **57**, 644(1968).


## ACKNOWLEDGMENTS AND ADDRESSES


Received June 29, 1973, from *Abbott Laboratories South Africa, P.O. Box 1616, Johannesburg, South Africa.*

Accepted for publication August 28, 1973.

The method was developed in the Control Pharmaceutical Laboratory of Caps Industries (S.A.) (Pty.) Ltd., Johannesburg, South Africa.


# Quantitative Determination of Resorcinol and Phenol in Resorcinol–Phenol–Boric Acid Solution


**V. DAS GUPTA[x] and LINDLEY A. CATES**



**Abstract** □ The quantitative determination of resorcinol and phenol in resorcinol–phenol–boric acid solution is reported. They are assayed by combining a bromination technique with NMR spectroscopy. There is no interference from other ingredients of the solution (acetone, alcohol, boric acid, and water).



**Keyphrases** □ Resorcinol and phenol in resorcinol–phenol–boric acid solution—analysis, bromination technique and NMR spectroscopy □ Phenol and resorcinol in resorcinol–phenol–boric acid solution—analysis, bromination technique and NMR spectroscopy □ NMR spectroscopy—analysis, phenol and resorcinol in resorcinol–phenol–boric acid solution


A colorless resorcinol–phenol–boric acid solution is used externally as an antifungal preparation. No method for analyzing resorcinol and phenol quantitatively in each other's presence has been reported. Most available methods (1, 2) such as the bromination technique and UV spectroscopy are useful for the combined determination of both ingredients. Two GLC procedures (3, 4) for the quantitative determination of phenolic compounds have also been reported. A method was suggested for the quantitative determination of resorcinol in Castellani's paint by condensing the former with 4-dimethylaminobenzaldehyde and measuring the intense violet color in acetone (5). Recently, the quantitative determination of resorcinol monoacetate in creams and lotions by GLC was reported (6).

This paper reports the quantitative determinations of resorcinol and phenol in resorcinol–phenol–boric acid solution. The method is based on the bromination technique for combined results. The individual quantities of resorcinol and phenol are then computed from the bromination value and the molar ratio obtained from the NMR spectroscopic analysis.

## EXPERIMENTAL

**Chemicals and Reagents**—All chemicals and reagents were USP, NF, or ACS grade.

**Preparation of Resorcinol–Phenol–Boric Acid**—The solution