The values shown in Table I are per cent recovery. These results appear to confirm that the proposed method is both accurate and precise. In addition to its greater precision, it is also much faster than the official method; about 8 min. compared with near 40 min. in the latter.

Thin-layer chromatographic examination of official dosage forms of pyrvinium pamoate and a U.S.P. reference standard indicated that the drug consists of a number of components. Pyrvinium pamoate contained in official preparations separated into two major red spots at R_f 0.33 and 0.44 and a minor red spot at R_f 0.50. Upon spraying, these spots stained intense blue, while pamoic acid, previously visible only by fluorescence at R_f 0.50, stained light blue. U.S.P. reference standard pyrvinium pamoate was found to contain all of the above components as well as another minor red constituent, staining intense blue, at R_f 0.15. Pamoic acid could be separated from the red material also appearing at R_f 0.50 by using 0.1 *N* NaOH instead of 0.5 *N* NaOH in preparing the chromatographic plates (new R_f of pamoic acid: 0.69).

The occurrence of a greater number of red spots than expected, on the basis of two isomeric configurations of the compound, should not be attributed to the degradative effect of sodium hydroxide in the aqueous system since, for example, chromatography with chloroform–ethanol (7:3) on neutral Silica Gel G (Merck), although giving very poor separation, clearly indicated three red constituents in commercial and four such components in U.S.P. reference standard pyrvinium pamoate. These compositional characteristics of the drug will be examined further.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 548–551.
(2) "First Interim Revision Announcement," U.S.P. XVII, August 1965, p. 3.
(3) Alves, M. A. P., *Rev. Port. Farm.*, **14**, 13(1964); through *Chem. Abstr.*, **61**, 9357*c*(1964).
(4) Stahl, E., "Thin-Layer Chromatography," Academic Press Inc., New York, N.Y., 1965, p. 5 *ff*.

Analysis of Combinations Containing Phenylephrine in Liquid Dosage Forms

By K. O. MONTGOMERY, P. V. JENNINGS, and M. H. WEINSWIG

A method is presented for the isolation, separation, and determination of phenylephrine, an antitussive, and an antihistamine in commercial products containing the combination. All three compounds are extracted with strong cation exchange resin AG 50W-X4. The phenylephrine is eluted from the resin bed with 8.0 *N* phosphoric acid. The antitussive is then eluted with 1.0 *N* hydrochloric acid in 60 per cent methanol in water. Finally the antihistamine is eluted with 3.5 *N* hydrochloric acid in 40 per cent methanol in water. The compounds are determined by subjecting the eluates to ultraviolet spectrophotometry. The method is used successfully on several commercial products.

THE ASSAY of complex formulations containing several active ingredients is usually a difficult and time-consuming problem. This is especially true if the active ingredients are similar in their chemical and physical properties. Ion-exchange chromatography was selected as the method of choice for this assay because of its ability to isolate completely the cationic active ingredients from the inactive ingredients, and separate these active ingredients by selective elution. Each ingredient was then quantitatively eluted in a form suitable for determination by ultraviolet spectrophotometry.

Ion-exchange chromatography has been used extensively to accomplish difficult separations of inorganic ions. Hofer (1) used an ion-exchange resin[1] to absorb calcium and magnesium from tissue extracts. Magnesium was then eluted with 2 *N* hydrochloric acid and calcium was eluted with 3 *N* hydrochloric acid. Grasselly (2) used anion-exchange resins to separate iron, aluminum, manganese, calcium, and magnesium. Pollard (3) separated magnesium, strontium, barium, and calcium on the ion-exchange resin[1] utilizing a gradient elution technique. Pitstick and associates (4) separated magnesium, cobalt, zinc, copper, and iron with anion-exchange resins.

Most of the references to organic separations utilize a simple adsorption–elution technique to separate anions or cations from neutral components. Wang and Hunter (5) assayed 11 different alkaloids separately by adsorbing them on an ion-exchange resin,[2] eluting with glacial acetic acid, and titrating the eluate with perchloric acid.

Morphine has been separated from the nonphenolic alkaloids of opium utilizing ion exchange (6). Blake and associates (7, 8) applied tech-

Received October 18, 1966, from the College of Pharmacy, Butler University, Indianapolis, IN 46207
Accepted for publication November 29, 1966.
[1] Marketed as Dowex 50-X8 by the Dow Chemical Co., Midland, Mich.
[2] Marketed as Amberlite IRC-50 by Rohm & Haas, Philadelphia, Pa.

niques similar to those used in inorganic separation to determine active ingredients in pharmaceuticals.

The combinations of compounds considered in this paper have been studied by several groups. Schriftman and Shultz (9) used paper electrophoresis for their separation and measured spot size on a densitometer to determine the active ingredients. Thin-layer chromatography has also been used with spot size measurement (10). Hyatt (11, 12) has proposed the use of partition columns to separate the active ingredients. Three columns are used and the separated compounds determined spectrophotometrically.

EXPERIMENTAL

Apparatus—Glass column 20 cm. × 1 cm. with a stopcock made of Teflon and containing a built-in needle valve for control of flow rate; the column is also fitted with a reservoir with a capacity of 250 ml.

A suitable recording ultraviolet spectrophotometer such as a Beckman DK-2A or Spectronic 505 which records in absorbance units.

Reagents—Cationic exchange resin AG 50W-X4, 100–200 mesh, hydrogen form, available from Bio-Rad Laboratories, Richmond, Calif. Enough resin, about 3 Gm., is added in the form of an aqueous slurry to the glass column; the resin bed is rinsed with water.

Phosphoric acid, 8.0 *N* in water; hydrochloric acid, 0.05 *N* in 50% methanol in water; hydrochloric acid, 1.0 *N* in 60% methanol in water; hydrochloric acid, 3.5 *N* in 40% methanol in water.

Standard Solutions—Prepare the following standard solutions using U.S.P., N.F., or other suitable reference standards. (*a*) Phenylephrine hydrochloride 10 mg./250 ml.; 8.0 *N* phosphoric acid in water. (*b*) Phenylephrine hydrochloride, 20 mg./250 ml.; 8.0 *N* phosphoric acid in water. (*c*) Codeine phosphate, 20 mg./200 ml.; 1.0 *N* hydrochloric acid in 60% methanol in water. (*d*) Dextromethorphan hydrobromide, 30 mg. in 200 ml.; 1.0 *N* hydrochloric acid in 60% methanol in water. (*e*) Chlorpheniramine maleate, 4 mg./200 ml.; 3.5 *N* hydrochloric acid in 40% methanol in water. (*f*) Promethazine hydrochloride, 10 mg./200 ml.; 3.5 *N* hydrochloric acid in 40% methanol in water.

Sample Treatment—Use 10-ml. samples of preparations *A*,[3] *B*,[4] and *C*.[5] For preparations *D*,[6] *E*,[7] and *F*[8] use 20-ml. samples. Pipet the sample into the reservoir, rinse the pipet with distilled water, and add to the reservoir.

Add distilled water to the sample to make the volume approximately 100 ml. and mix well. Allow this sample solution to flow through the resin bed at the rate of 2–3 ml./min. Wash the column by adding 100 ml. of distilled water to the reservoir and allow it to flow through the resin at the rate of 5 ml./min.

Traces of aromatic amines from flavors or coloring agents are removed by allowing 50 ml. of 0.05 *N* hydrochloric acid in 50% methanol in water to flow through the column at 5 ml./min.

Position a 250-ml. volumetric flask under the column and add 240 ml. of 8.0 *N* phosphoric acid to the reservoir. Allow this to flow through the column at the rate of 2 ml./min. The eluate volume is adjusted with 8.0 *N* phosphoric acid. This eluate contains the phenylephrine.

Position a 200-ml. volumetric flask under the column and add 190 ml. of 1.0 *N* hydrochloric acid in 60% methanol in water to the reservoir. Allow this to flow through the resin at 3 ml./min. Adjust the eluate volume with 1.0 *N* hydrochloric acid in 60% methanol in water. This eluate contains the antitussive.

Position another 200-ml. volumetric flask under the column and add 190 ml. 3.5 *N* hydrochloric acid in 50% methanol in water to the reservoir. Allow this to flow through the resin at the rate of 4 ml./min. Adjust the eluate volume with 3.5 *N* hydrochloric acid in 40% methanol in water. This eluate contains the antihistamine.

For re-use the column is washed with 100 ml. of distilled water.

Determinations—Record the ultraviolet spectra of the sample eluates and the standard solutions using a suitable spectrophotometer. Absorbance units should be used. Using the base line technique, determine the absorbances for each sample and standard at the particular maximum. Calculate the amounts of each compound present in each sample from the values obtained for the standard solutions.

DISCUSSION AND RESULTS

Standard solutions, when subjected to the above procedures, gave the results shown in Table I. These results are derived from ten determinations for each compound. These data indicate that the method is accurate to within 1% of the true values and is reproducible to ±1.5% at 95% confidence limits. The data for the analyses of marketed products are shown in Table II.

A slightly raised base line was noted with some products where a small portion of the coloring agent was held and eluted with the sample. This did not cause any problem since the base line technique was used in the calculations.

When the maleate salt of a compound is used for a standard, there is a small difference in the base line caused by ultraviolet absorbance of the maleic acid radical. This also is overcome for the most part by the use of the base line technique.

One deviation from the described procedures was necessary for preparation *E*. Since terpin hydrate is precipitated on dilution with water, the sample dilution prior to column application and the washing of the column were performed using 50% methanol in water instead of distilled water.

In products containing acetaminophen, a small amount of *p*-aminophenol is usually present which could interfere with the analysis of phenylephrine. This is effectively removed during the rinse using

[3] Marketed as Phenergan VC Expectorant with Codeine by Wyeth Laboratories, Philadelphia, Pa.
[4] Marketed as Tussquellin by Circle Pharmaceuticals.
[5] Marketed as Novahistine Expectorant by Pitman-Moore Division of the Dow Chemical Co., Indianapolis, Ind.
[6] Marketed as Pediacof by Winthrop Laboratories, New York, N. Y.
[7] Marketed as Adulton by Bristol-Myers Co., Syracuse, N. Y.
[8] Marketed as Coryban D by J. B. Roerig and Co., New York, N. Y.

TABLE I—ACCURACY, REPRODUCIBILITY, AND PRECISION ANALYSIS DATA

Std. Material	Concn., mg./10 ml.	Means ($\bar{x}$) of Results of 10 Determinations in % of Theoretical	S.D. of 10 Determinations in % of Theoretical
Phenylephrine HCl	20	99.42	±0.61
Codeine phosphate	20	99.94	±0.64
Chlorpheniramine maleate	4	100.38	±0.44
Dextromethorphan hydrobromide	15	99.04	±0.52

TABLE II—ANALYSIS OF PRODUCTS CONTAINING THREE AMINE COMPONENTS

Prepn.	Ingredients	Label Claim, mg./5 ml.	Found % of Label Claim
D	Phenylephrine HCl	2.5	99.9
	Codeine phosphate	5	100.2
	Chlorpheniramine maleate	0.75	99.6
A	Phenylephrine HCl	5	98.4
	Codeine phosphate	10	97.9
	Promethazine HCl	5	99.0
E	Phenylephrine HCl	5	101.1
	Dextromethorphan HBr	15	100.3
	Chlorpheniramine maleate	1	98.6
F	Phenylephrine HCl	5	102.8
	Dextromethorphan HBr	7.5	99.5
	Chlorpheniramine maleate	1	100.2
B	Phenylephrine HCl	5	98.8
	Dextromethorphan HBr	15	98.1
	Chlorpheniramine maleate	2	100.2
C	Phenylephrine HCl	10	100.85
	Codeine phosphate	10	100.82
	Chlorpheniramine maleate	2	101.65

0.05 *N* hydrochloric acid in 50% methanol in water.

One product, preparation *B*, actually contained four amines as active ingredients: phenylephrine, dextromethorphan, chlorpheniramine, and phenylpropanolamine. The last compound does not have a significant ultraviolet absorbance and therefore was outside the scope of this study. However, because of its lack of ultraviolet absorbance character, it did not interfere with the assay for the other active ingredients.

To demonstrate the characteristics of this method of assay, a mixture of phenylephrine, codeine, and chlorpheniramine standards was sorbed onto a column and eluted according to the previously described scheme. The eluate from the column was monitored utilizing a flow-through cell in an ultraviolet spectrophotometer. A strip chart recorder was connected to the spectrophotometer to record absorbance *versus* volume of eluate. (Fig. 1.) This chart shows a complete and concise separation of the three compounds.

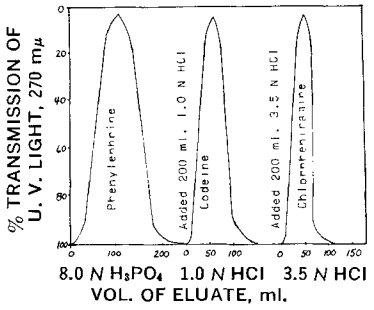

Fig. 1—Graph of per cent transmission of column eluate vs. *volume.*

SUMMARY

A method of assay for liquid dosage forms containing the combination of phenylephrine, an antitussive, and an antihistamine in a single product has been presented. The procedures have been used successfully on commonly available products with accurate and reproducible results.

REFERENCES

(1) Hofer, M., *Experientia*, **19** (7), 367(1963).
(2) Grasselly, G., *Acta Univ. Szeged., Acta Mineral.-Petrog.*, **15**, 7(1962); through *Chem. Abstr.*, **59**, 3309*h*(1963).
(3) Pollard, F. H., Nickless, G., and Spencer, D., *J. Chromatog.*, **11** (4), 542(1963).
(4) Pitstick, G., Sweet, T., and Morie, G., *Anal. Chem.*, **35**, 995(1963).
(5) Wang, S. M., and Hunter, R. W., *J. Pharm. Sci.*, **50**, 265(1961).
(6) Brochmann-Hanssen, E., and Svendsen, A., *ibid.*, **52**, 1134(1963).
(7) Blake, M. I., and Nona, D. A., *ibid.*, **52**, 945(1963).
(8) Blake, M. I., and Siegel, F. P., *ibid.*, **51**, 944(1962).
(9) Schriftman, H., and Shultz, R. C., *ibid.*, **50**, 332 (1961).
(10) Morrison, J. C., and Chatten, L. G., *ibid.*, **53**, 1205 (1964).
(11) Hyatt, R., *J. Assoc. Offic. Agr. Chemists*, **47**, 475 (1964).
(12) *Ibid.*, **48**, 594(1965).