ACKNOWLEDGMENTS

Supported by the Food and Drug Administration under the SARAP Program.

The authors thank Dr. Raymond H. Lindsay, Director of Pharmaceutical Research, Veterans Administration Hospital, Birmingham, Ala., for generously supplying propylthiouracil glucuronide, propylthiouracil sulfinic acid, and propylthiouracil sulfonic acid.

Ion-Pair Reversed-Phase High-Pressure Liquid Chromatography of Cough–Cold Syrups I: Pseudoephedrine Hydrochloride, Brompheniramine Maleate, and Dextromethorphan Hydrobromide

MARCUS K. CHAO ˣ, IRA J. HOLCOMB, and SALVATORE A. FUSARI

Received March 23, 1979, from *Parke-Davis, Division of Warner-Lambert Company, Morris Plains, NJ 07950.* Accepted for publication May 14, 1979.

Abstract □ Pseudoephedrine hydrochloride (I), brompheniramine maleate (II), and dextromethorphan hydrobromide (III) in a cough–cold syrup were separated and determined by ion-pair reversed-phase high-pressure liquid chromatography. The separation was carried out using a μBondapak C_{18} column (30 cm × 3.9 mm i.d.) and a mobile phase of acetonitrile–water–acetic acid (40:60:1) with 0.01 *N* 1-octanesulfonic acid sodium salt and 0.05 *N* potassium nitrate. Detection was accomplished using a UV detector at 265 nm for I and II; III was monitored at 280 nm. Concentration *versus* peak height plots in the ranges of 0.37–1.9 mg/ml for I, 0.025–0.126 mg/ml for II, and 0.125–0.625 mg/ml for III were linear. Ten consecutive injections of a mixture gave a percent relative standard deviation of <1% for all three components. Average recoveries from laboratory-prepared samples were 100.5% for I, 100.9% for II, and 100.1% for III. No precolumn cleanup was necessary, and the chromatogram was complete in 16 min.

Keyphrases □ Cough–cold syrup—analysis, ion-pair reversed-phase high-pressure liquid chromatography, pseudoephedrine hydrochloride, brompheniramine maleate, dextromethorphan hydrobromide □ Pseudoephedrine hydrochloride—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups □ Brompheniramine maleate—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups □ Dextromethorphan hydrobromide—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups

Antihistamines, antitussives, and decongestants are used extensively in cough–cold syrups. Often, two or more of these compounds are combined in a preparation, and an isolation of the desired analyte from the other components is necessary prior to measurement.

Reversed-phase high-pressure liquid chromatography (HPLC) was used to investigate 21 antihistaminic, antitussive, and analgesic drugs in cough–cold mixtures (1), and the separation of four antihistamines was examined by reversed-phase HPLC (2). Ion-pair reversed-phase HPLC is a relatively new and extremely useful technique. General discussions of the method were published (3, 4).

The present study investigated the feasibility of applying ion-pair reversed-phase HPLC to the separation and assay of a mixture of antihistaminic, antitussive, and decongestant drugs in a cough–cold syrup.

EXPERIMENTAL

Apparatus—A piston pump[1], an automatic sampler[2], and two detectors (one set at 280 nm[3] and the other at 265 nm[4]) were used. The chromatograms were recorded on a two-pen recorder[5].

Column—A bonded reversed-phase C_{18} column[6] was used.

Samples—A cough–cold syrup was prepared so that each 5 ml contained 30 mg of pseudoephedrine hydrochloride (I), 2 mg of brompheniramine maleate (II), 10 mg of dextromethorphan hydrobromide (III), and 5% alcohol. The standard solution was prepared by dissolving 120 mg of I[7], 8 mg of II[8], and 40 mg of III[7] in water and diluting to 100.0 ml. The sample solution was prepared by transferring 5 ml of the cough–cold syrup quantitatively to a 25-ml volumetric flask and diluting to volume with water.

Analysis—The chromatographic conditions were: flow rate, 0.8 ml/min (700 psi); mobile phase, acetonitrile[9]–water–acetic acid (40:60:1) with 0.01 *N* 1-octanesulfonic acid sodium salt[10] and 0.05 *N* potassium nitrate[11]; temperature, 25°; and detector scale, 0.064 aufs for 280 nm and 0.1 aufs for 265 nm.

Ten-microliter aliquots of standard and sample solutions were injected in duplicate onto the column using the chromatographic conditions.

Table I—Accuracy Study for HPLC Assay of Pseudoephedrine Hydrochloride (I), Brompheniramine Maleate (II), and Dextromethorphan Hydrobromide (III) from Aqueous Solutions

Percent of Target	Recovery, % I	II	III
85.0	100.6	99.9	102.0
90.0	98.4	97.2	100.3
95.0	102.0	101.8	100.9
97.0	101.4	102.1	99.2
99.0	100.6	98.8	99.8
101.0	100.0	98.8	99.8
103.0	100.0	100.0	100.0
105.0	98.9	98.4	99.1
110.0	100.7	100.9	100.0
115.0	98.3	101.3	100.1
Average	100.1	99.9	100.1
Range	98.3–102.0	97.2–102.1	99.1–102.0
RSD	±1.2	±1.6	±0.84

DISCUSSION

Mobile Phase Selection—Methanol and water or acetonitrile and water mixtures in various proportions were tested with no satisfactory separation of I–III. Sulfonic acid sodium salts of 1-butane, 1-pentane, 1-hexane, and 1-octane and dioctylsulfosuccinate sodium salt[7] (IV) were

[1] Milton Roy.
[2] DuPont 834.
[3] Laboratory Data Control model 1203.
[4] Perkin-Elmer LC 55.
[5] Linear recorder 385.
[6] μBondapak C_{18} column, Waters Associates.
[7] NF reference standard.
[8] USP reference standard.
[9] Burdick & Jackson.
[10] Eastman Organic Chemicals.
[11] Baker Analyzed Reagent.

0022-3549/79/1100-1463$01.00/0

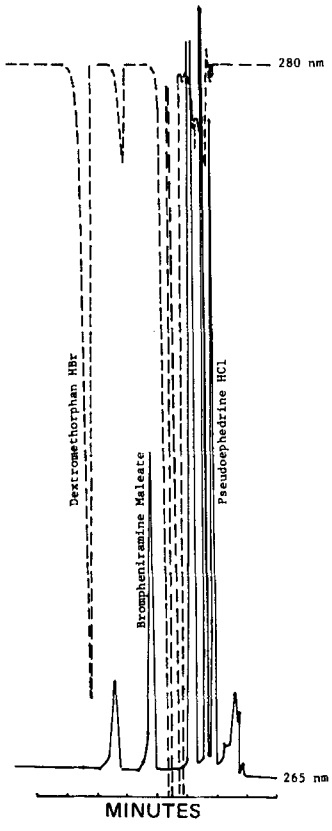

Figure 1—*High-pressure liquid chromatogram for a commercial sample containing pseudoephedrine hydrochloride, brompheniramine maleate, and dextromethorphan hydrobromide.*

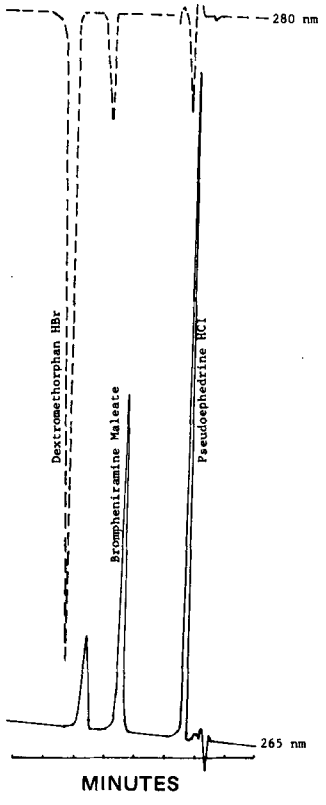

Figure 2—*High-pressure liquid chromatogram for the standard mixture of pseudoephedrine hydrochloride, brompheniramine maleate, and dextromethorphan hydrobromide.*

used as ion-pair reagents. With the reagents having a carbon chain fewer than eight, I was eluted with the solvent front; with IV, III was retained on the column too long. 1-Octanesulfonic acid sodium salt gave an optimum separation of all three analytes.

Potassium nitrate was used to reduce peak tailing. A 0.05 *N* concentration was selected on the basis of the chromatogram and the reproducibility of injections. Excess potassium nitrate (>0.1 *N*) resulted in poor reproducibility of injections.

Stationary Phase Selection—Several microparticle reversed-phase columns including μBondapak CN, μBondapak phenyl, μBondapak C_{18}[6], and Zorbax C_{18}[12] were tested. With μBondapak CN and μBondapak phenyl columns, the separation of II and III was acceptable, but I was eluted with the solvent front. With Zorbax C_{18}, II and III were retained on the column too long. The use of μBondapak C_{18} resulted in a satisfactory separation for I–III. The greater retention with Zorbax C_{18} was probably due to the higher loading of the organic material on the column.

[12] DuPont Instruments.

Table II—Assay of Pseudoephedrine Hydrochloride (I), Brompheniramine Maleate (II), and Dextromethorphan Hydrobromide (III) in Commercial Cough–Cold Syrup Samples

Sample	Recovery, %		
	I	II	III
1	98.8	98.1	99.9
2	99.6	99.5	99.9
3	98.8	99.7	98.1

Detector Wavelength Selection—The most selective analytical absorption wavelengths for I, II, and III were at 257, 265, and 278 nm, respectively. Since II was present in a small amount, 265 nm was selected to obtain the maximum sensitivity for II, and I was also run at this wavelength. Compound III was determined at 280 nm. If desired, I–III can be monitored using one variable-wavelength detector[13] at 265 nm.

RESULTS

With the selected mobile and stationary phases and specified detector wavelengths, the active ingredients (I–III) in the cough–cold syrup could be separated and assayed quantitatively using ion-pair reversed-phase HPLC.

A linearity study showed that the peak height of each ingredient was directly related to the concentration (0.377–1.885 mg of I/ml, 0.025–0.126 mg of II/ml, and 0.125–0.625 mg of III/ml).

No chromatographic interference was encountered with the pharmaceutical ingredients used for the syrup base. The average recoveries of weighed amounts of I–III (85–115% of target) added to the laboratory-prepared syrup vehicle were 100.5% for I, 100.9% for II, and 101.1% for III.

Ten consecutive injections of a standard preparation mixture gave a relative standard deviation of <1% for all three components.

The accuracy of the method was studied based on percent recovery of 10 solutions containing I–III in amounts equivalent to 85, 90, 95, 97, 99, 101, 103, 105, 110, and 115% of target relative to a standard solution at 100% target (Table I). The assay bias for all three components was ±0.1%.

The HPLC method was used to assay I–III in commercial samples, and results are presented in Table II.

The analytical results demonstrate the ability of the ion-pair reversed-phase HPLC procedure to assay the complex drug mixtures in a cough–cold syrup. Typical chromatograms of the sample and standard preparations are shown in Figs. 1 and 2. The drugs can be analyzed in their salt forms, and the sample pretreatment is simple and rapid. The complete separation only requires 16 min.

REFERENCES

(1) I. L. Honigberg, J. T. Stewart, and A. P. Smith, *J. Pharm. Sci.*, **63**, 766 (1974).

(2) A. G. Ghanekar and V. D. Gupta, *Am. J. Hosp. Pharm.*, **34**, 651 (1977).

(3) R. Gloor and E. L. Johnson, *J. Chromatogr. Sci.*, **15**, 413 (1977).

(4) C. Horvath, W. Melander, I. Mulnar, and P. Mulnar, *Anal. Chem.*, **49**, 2295 (1977).

[13] Laboratory Data Control model 1204.