High-Pressure Liquid Chromatographic Determination of Adrenergic and Antihistaminic Compounds in Pharmaceutical Preparations

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Abstract □ The application of high-pressure liquid chromatography to the separation and quantitative analysis of certain combinations of adrenergic and antihistaminic compounds in pharmaceutical dosage forms is demonstrated. The analysis of: *(a)* phenylpropanolamine hydrochloride and chlorpheniramine maleate, *(b)* pseudoephedrine hydrochloride and chlorpheniramine maleate, and *(c)* phenylpropanolamine hydrochloride, pheniramine maleate, and pyrilamine maleate, each with an internal standard, from pharmaceutical dosage forms was carried out using high-pressure liquid chromatography. The chromatographic conditions described were chosen to optimize the resolution and total elution time of these amine compounds.

Keyphrases □ Adrenergic-antihistaminic tablets and syrups—high-pressure liquid chromatographic analysis □ Antihistaminic-adrenergic tablets and syrups—high-pressure liquid chromatographic analysis □ Phenylpropanolamine with chlorpheniramine or pheniramine/pyrilamine dosage forms—high-pressure liquid chromatographic analysis □ Pseudoephedrine with chlorpheniramine dosage forms—high-pressure liquid chromatographic analysis

With the broad use of certain combinations of vasoconstrictors and antihistamines in pharmaceutical preparations, rapid separation and quantitative analysis of these combinations have become important. Other investigators have reported several approaches to the rapid analysis of cough–cold preparations. An automated, simultaneous determination of dextromethorphan hydrobromide, glyceryl guaiacolate, and phenylpropanolamine hydrochloride was recently developed (1), and these three compounds and chlorpheniramine were determined by GLC. Ek *et al.* (3) developed an automated procedure for the determination of total antihistamines by UV spectrophotometry and of phenylpropanolamine hydrochloride by colorimetry.

With the recent developments in high-pressure liquid chromatographic instrumentation and column packing materials, this technique of rapid quantitative analysis has proved to be quite useful. The separation from complex mixtures and quantitative analysis of several drugs of biomedically important systems have been reported (4–9). Pyrilamine maleate was separated from phenylephrine hydrochloride and naphazoline hydrochloride by high-pressure anion-exchange chromatography (10). Chlorpheniramine maleate and pyrilamine were also separated from acetaminophen, dextromethorphan hydrobromide, ephedrine sulfate, and methapyrilene by high-pressure reverse-phase chromatography (11).

This paper describes the concurrent separation and quantitative analysis of some decongestant–antihistamine combinations by high-pressure liquid chromatography. The preparation of samples is simple and does not require prior extraction of active ingredients from excipients. Either a single tablet or a ground composite sample of a group of tablets may be assayed by the described procedure. A strong cation-exchange column with an eluent of aqueous dibasic ammonium phosphate and an organic modifier, dioxane, was used to effect the separation in a reasonably short time. Slight changes in the concentration of the organic modifier and ionic strength have rather pronounced effects on the retention times and resolution of the chromatographic peaks.

EXPERIMENTAL[1]

Reagents—The mobile phases were prepared from reagent dibasic ammonium phosphate[2], specially purified dioxane[3], and distilled water. All other reagents were of USP (13) or NF (14) quality and were used as received.

Standard Stock Solutions—*Mixture I*—Standard stock solution I was prepared by weighing accurately and combining 600 mg pseudoephedrine hydrochloride and 40 mg chlorpheniramine maleate and then diluting to 100 ml with distilled water.

Mixture II—Standard stock solution II was prepared by weighing accurately and combining 250 mg phenylpropanolamine hydrochloride and 40 mg chlorpheniramine maleate and then diluting to 100 ml with distilled water.

Mixture III—Standard stock solution III was prepared by weighing accurately and combining 250 mg phenylpropanolamine hydrochloride, 125 mg pheniramine maleate, and 125 mg pyrilamine maleate and then diluting to 100 ml with distilled water.

Internal Standard Stock Solutions—*Internal Standard I*—Internal standard stock solution I was prepared by weighing accurately 400 mg of phenylpropanolamine hydrochloride and diluting to 100 ml with 0.2 *N* HCl.

Internal Standard II—Internal standard stock solution II was prepared by weighing accurately 250 mg ephedrine hydrochloride and diluting to 100 ml with 0.2 *N* HCl.

Internal Standard III—Internal standard stock solution III was prepared by weighing accurately 500 mg of ephedrine hydrochloride and diluting to 100 ml with 0.2 *N* HCl.

Preparation of Tablet Samples—A representative sample consisting of 100 tablets of a batch of tablets was weighed to determine the average tablet weight; then 20 tablets were ground to a fine powder. An accurately weighed portion equivalent to one tablet weight of the powdered sample was transferred to a 10-ml volumetric flask. Five milliliters of the appropriate internal standard stock solution and about 2 ml of distilled water were added to the flask. The sample was stirred for 45 min using a small stir bar and a magnetic stirrer. The stir bar was then removed and the sample was diluted to volume with distilled water. After mixing well, the sample was centrifuged and then filtered through a 5-μm filter[4].

Preparation of Syrup Samples—Five milliliters of the syrup

[1] A DuPont model 830 liquid chromatograph equipped with a UV photometer and a gradient elution accessory, and Autolab model System IV integrator, and a Honeywell model 194, 1-mv recorder were used. The analytical column was 0.5-m, 6.35-mm o.d., 2.10-mm i.d. stainless steel (DuPont) packed by the "modified tap fill" technique (12) with DuPont "Zipax" supported strong cation-exchange (SCX) material.

[2] Fisher Scientific Co.

[3] Burdick & Jackson Laboratories.

[4] Millipore Mitex.

Table I—Effect of Mobile Phase Molarity on Retention Time (Seconds from Injection to Chromatographic Peak)

Compound	Concentration of $(NH_4)_2HPO_4$ in 32% Dioxane–Water (1100 psig, 1.1 ml Flow Rate, Ambient Temperature)		
	0.01 *M*	0.02 *M*	0.04 *M*
Phenylpropanolamine	271	172	112
Pseudoephedrine	760	436	219
Chlorpheniramine	1320	781	367

was transferred using a pipet[5] to a 10-ml volumetric flask, and the pipet was rinsed with several small portions of the appropriate internal standard solution. The sample was then diluted to 10 ml with internal standard and mixed well.

Chromatographic Conditions—All data and chromatograms presented here were obtained under isocratic conditions.

The degassed mobile phase was passed through the strong cation-exchange column under a pressure of about 1100 psig, to obtain a flow rate of 1.0–1.1 ml/min at room temperature. The photometer was operated at an attenuation of 0.01 absorbance unit full-scale with peak attenuation of from ×1 to ×4 made at the integrator channel module. After an initial period of column and detector stabilization, the detector noise was nominally 1% or better at maximum sensitivity.

Eight-microliter injections of sample and standard solutions were made with a syringe[6]. The chromatographic peaks were electronically integrated to determine the areas relative to that of the internal standard.

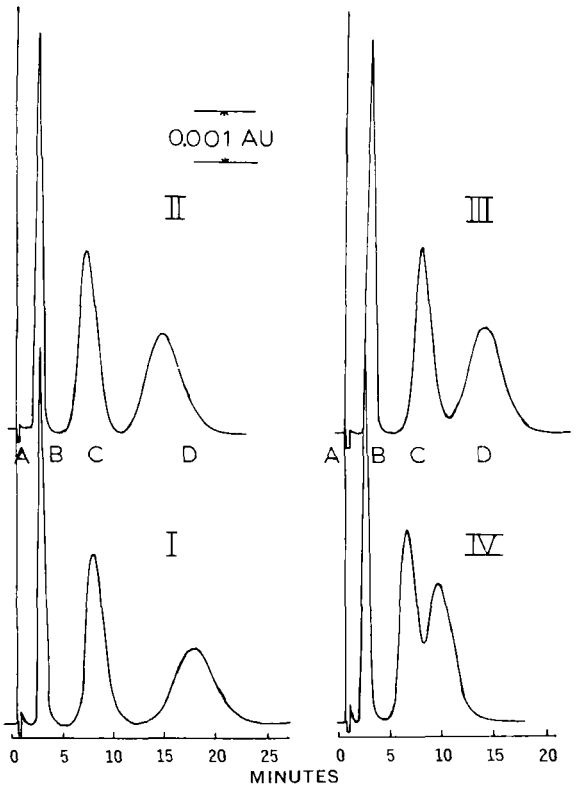

Figure 1—*Elution of Mixture I and Internal Standard I. Peak identification: A, solvent and unretained species (e.g., maleate and excipients); B, phenylpropanolamine; C, pseudoephedrine; and D, chlorpheniramine. Mobile phase was 0.02 M $(NH_4)_2HPO_4$ in 28% (I), 30% (II), 32% (III), and 36% (IV) dioxane–water (v/v) (apparent pH 8.2; 1100 psig; flow rate 1.1 ml/min; ambient temperature).*

[5] Ostwald to-contain.
[6] Hamilton 701N.

Table II—Response Factors for Standard Solution I

Compound	Area Response Factor[a]	Relative Standard Deviation[b]	Area Response Factor/ Concentration[c]	Linear Correlation Coefficient
Pseudoephedrine	1.378	1.11%	0.454	0.9992
Chlorpheniramine	1.403	3.24%	7.323	0.9733

[a] Average of six injections of Standard Solution I with Internal Standard I. Integrated areas of each amine peak divided by area of internal standard peak. [b] Relative standard deviation of the respective area response factors; indicates precision obtained from repetitive injections of the same standard solution. [c] Linearity of integrated area response, with concentration in milligrams per milliliter, is shown for five standard solutions with concentrations of 90, 95, 100, 105, and 110% of Standard Solution I.

RESULTS AND DISCUSSION

High-pressure ion-exchange chromatography of an 8-μl injection of 5 ml of Mixture I diluted with 5 ml of Internal Standard I is shown in Fig. 1. The effect of percent dioxane on the retention and resolution of this mixture is also shown in Fig. 1. As the organic modifier percentage is increased, the retention of the three amines is decreased. As in many other applications of ion-exchange chromatography, changes in ionic strength and pH are most often used to obtain the desired resolution or retention times. The effect of changes in the molar concentration of dibasic ammonium phosphate on retention time is illustrated in Table I.

Relative area precision data (Table II) for the standard solution were obtained after determining the chromatographic conditions that would optimize the resolution of the amines in a reasonably short time (Fig. 1, II). After determining linearity of area response factors (the ratio of each amine peak to the area of the internal standard) with concentration for several concentration levels, the active amine content of several different pharmaceutical preparations was determined (Table III). Response factors used for the data shown in Table III were calculated from a standard injection prior to and following the duplicate sample injections to minimize the changes in response factors caused by changes in chromatographic conditions.

The chromatograms of injections of syrups are changed slightly in the early stages of the elution. The excipients of the syrups that are unretained or very slightly retained by the strong cation-exchange column cause some broadening of the solvent peak. For the syrups sampled, baseline resolution was attained prior to the onset of the internal standard peak. If some excipients of a syrup are slightly retained and interfere with the baseline resolution of the internal standard, norpseudoephedrine hydrochloride, a diastereomer of phenylpropanolamine, may be substituted as an internal standard (Fig. 2). The peak retention time for norpseudoephedrine was about 80 sec longer than for phenylpropanolamine under the conditions described in Fig. 2. This internal standard reduces slightly the baseline resolution prior to the onset of the pseudoephedrine peak; precise analytical area measurements may, however, still be made.

The optimum chromatographic conditions and calibration data

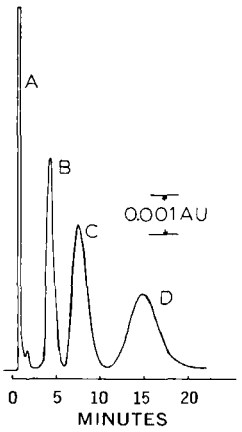

Figure 2—*Chromatogram of a syrup sample using norpseudoephedrine hydrochloride as internal standard (4 mg/ml). Mobile phase was 0.02 M $(NH_4)_2HPO_4$ in 30% dioxane–water (1100 psig; flow rate 1.1 ml/min; ambient temperature). Peak identification: B, norpseudoephedrine; and other peaks same as in Fig. 1.*

Table III—Concentration of Amines in Tablets and Syrups

Sample	Phenylpropanolamine Hydrochloride		Pseudoephedrine Hydrochloride		Chlorpheniramine Maleate		Pheniramine Maleate		Pyrilamine Maleate	
	T.C.[a]	%[b]	T.C.	%	T.C.	%	T.C.	%	T.C.	%
Tablet A	—	—	30.0	100.5	2.00	103.7	—	—	—	—
Tablet B	—	—	30.0	99.9	2.00	104.0	—	—	—	—
Tablet C	—	—	30.0	95.7	2.00	100.6	—	—	—	—
Syrup A	—	—	30.0	101.6	2.00	100.3	—	—	—	—
Syrup B	—	—	30.0	97.9	2.00	98.1	—	—	—	—
Tablet D	12.5	97.5	—	—	2.00	103.0	—	—	—	—
Tablet E[c]	25.0	100.8	—	—	4.00	103.5	—	—	—	—
Syrup C	12.5	98.1	—	—	2.00	99.5	—	—	—	—
Syrup D	12.5	98.3	—	—	2.00	101.8	—	—	—	—
Tablet F[c]	25.0	98.0	—	—	—	—	12.5	100.7	12.5	97.8
Tablet G[c]	25.0	99.8	—	—	—	—	12.5	95.1	12.5	100.5
Syrup E	12.5	101.8	—	—	—	—	6.25	101.6	6.25	99.4
Syrup F	12.5	99.9	—	—	—	—	6.25	100.8	6.25	101.2

[a] Theoretical content: milligrams per tablet or milligrams per 5 ml syrup. [b] Content of sample found by this procedure; percent of theoretical content. [c] One-half tablet weight used in sample preparation.

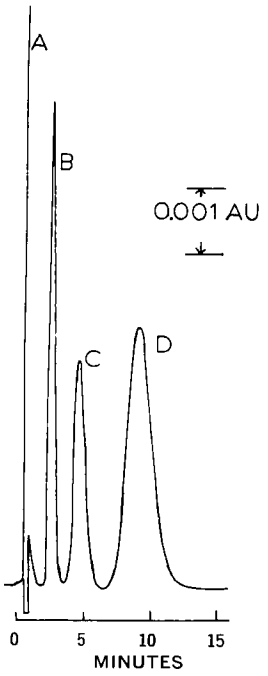

Figure 3—*Chromatogram of Tablet D sample preparation with Internal Standard II. Peak identification: A, solvent and unretained species; B, phenylpropanolamine; C, ephedrine; and D, chlorpheniramine. Mobile phase was 0.02 M $(NH_4)_2HPO_4$ in 36% dioxane–water (apparent pH 8.2; 1100 psig; flow rate 1.1 ml/min; ambient temperature).*

for the other mixtures were determined in a similar manner. Changes in the dioxane content of the mobile phase were most useful since large increases in the ionic strength reduce the resolution between sample excipients and the initial peak. An example of the chromatographic elution of a tablet sample preparation with an active ingredient composition (Tablet D, Table III) similar to Mixture II is shown in Fig. 3. An example of the chromatographic elution of a syrup sample preparation with an active ingredient composition (Syrup E, Table III) similar to Mixture III is shown in Fig. 4.

Representative analytical data obtained from several lots of tablets and syrup formulations are presented in Table III. These data demonstrate the utility of high-pressure liquid chromatography for the analysis of certain combinations of adrenergic and antihistaminic compounds in pharmaceutical dosage forms.

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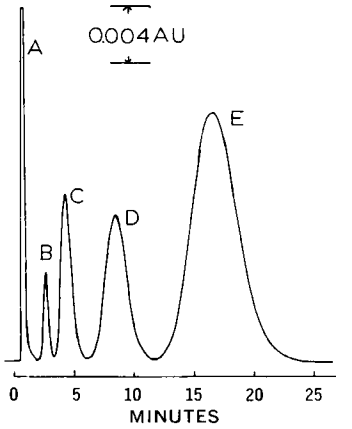

Figure 4—*Chromatogram of Syrup E sample preparation with Internal Standard III. Peak identification: A, solvent and excipients; B, phenylpropanolamine; C, ephedrine; D, pheniramine; and E, pyrilamine. Mobile phase and conditions were the same as in Fig. 3.*

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