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Quantitative Determinations of Two Decongestants and an Antihistamine in Combination Using Paired Ion High-Pressure Liquid Chromatography

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Abstract □ A single method for the quantitative determinations of three active ingredients, phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and brompheniramine maleate, and one inactive ingredient (sodium benzoate) in a commercial product for colds is reported. The method is based on paired ion high-pressure liquid chromatography using 1-heptanesulfonic acid as the counterion. It is accurate and precise. The relative standard deviations based on six readings are reported. This method is sensitive; less than 1 μg of each ingredient can be assayed. The peak area of each ingredient is related to its concentration.

Keyphrases □ Phenylephrine hydrochloride—high-pressure liquid chromatographic analysis in dosage forms □ Phenylpropanolamine hydrochloride—high-pressure liquid chromatographic analysis in dosage forms □ Brompheniramine maleate—high-pressure liquid chromatographic analysis in dosage forms □ High-pressure liquid chromatography—analyses, phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and brompheniramine maleate in dosage forms □ Adrenergics—phenylephrine hydrochloride and phenylpropanolamine hydrochloride, high-pressure liquid chromatographic analyses in dosage forms □ Antihistaminics—brompheniramine maleate, high-pressure liquid chromatographic analysis in dosage forms

One commercial product[1] for colds contains two decongestants, phenylephrine hydrochloride (I) and phenylpropanolamine hydrochloride (II), and an antihistamine, brompheniramine maleate (III). The dosage forms, sustained-release tablets and elixir, also contain excipients, some of which (especially colors and preservatives in the elixir) may interfere with the analysis of the active ingredients. No single method is available to determine the active ingredients quantitatively.

The USP method (1) for the quantitative determination of I is based on column chromatography. This method is quite tedious and time consuming. A GLC method was reported for the quantitative determination of I (2), but it requires derivatization and is quite lengthy. Other methods available for the analysis of I were reviewed (2). A quantitative colorimetric method for II in pharmaceutical dosage forms was reported (3), but other primary and secondary amines interfered. The only method available for the quantitative analysis of III in combination with other drugs apparently is that of Hudanick (4), which requires reaction with cyanogen bromide, a highly toxic and volatile reagent.

The paired ion extraction technique for the quantitative drug analysis is well documented. The theory and some possible uses of this method were reported (5) and reviewed (6). Numerous applications also were reported for thyroid hormones and sulfa drugs (7), dyes (8), niacin and niacinamide (9), and the simultaneous determinations of hydrocortisone and hydrocortisone phosphate (10).

This paper reports the simultaneous quantitative determinations of I–III in commercial dosage forms. The method is based on paired ion high-pressure liquid chromatography (HPLC), which identifies the compounds. Moreover, inactive ingredients also separate out and may be identified or determined quantitatively without additional work.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification. 1-Heptanesulfonic acid sodium salt[2] (IV) was used as received.

Apparatus—A high-pressure liquid chromatograph[3] capable of operating at an inlet pressure up to 6000 psig was used. A multiple wavelength detector[4] was used. For convenience, the wavelength was set at 254 nm (usually found in fixed wavelength detectors) for all ingredients. The detector was attached to a recorder[5] and an integrator[6]. The column[7] (30 cm × 4 mm i.d.) was purchased and used as received.

Chromatographic Solvents—A 13% (v/v) solution of acetonitrile in water containing 1.8% (v/v) acetic acid with or without 0.005 *M* IV was used. The pH of both solvents was 2.6 ± 0.05.

Chromatographic Conditions—The temperature was ambient. The flow rate was 0.6 ml/min (inlet pressure of approximately 300 psig) for the first 12 min and then was 3.6 ml/min (inlet pressure of approximately 3000 psig). The absorbance unit for full-scale deflection was 0.04, and the chart speed was 30.5 cm/hr.

Preparation of Stock Solutions—All stock solutions were prepared in water using a simple solution method. Heating to about 90° for approximately 5 min was required to dissolve III. The concentrations of the

[1] Dimetapp, A. H. Robins, Richmond, Va.

[2] Eastman Kodak Co., Rochester, N.Y.
[3] Waters ALC 202 equipped with a U6K Universal injector, Waters Associates, Milford, Mass.
[4] Spectroflow monitor 770, Schoeffel Instrument Corp., Westwood, N.J.
[5] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[6] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[7] μBondapak CN, Waters Associates, Milford, Mass.

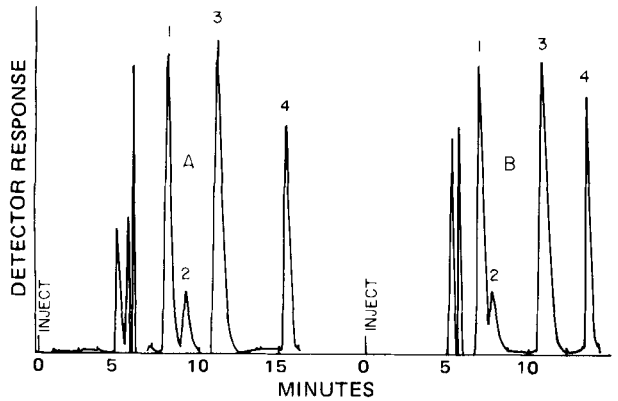

Figure 1—*Sample chromatograms from the standard mixture. Key: A, using mobile phase containing IV; and B, using mobile phase without IV. Peaks 1–4 are from I, II, sodium benzoate, and III, respectively.*

stock solutions were 0.1% each for I, II, and sodium benzoate and 0.08% for III.

Preparation of Standard Solutions—A 10.0-ml quantity of the stock solution was diluted to 100.0 ml with water. A standard mixture containing all active ingredients and sodium benzoate was prepared by mixing 10.0 ml of the stock solution and bringing it to volume (100.0 ml) with water.

Sample Preparation—*Commercial Elixir*—A 5.0-ml quantity of the elixir was diluted to 50.0 ml with water. Based on the label claim, this solution contained the same concentrations of ingredients as the standard mixture.

Sustained-Release Tablets—Ten or 20 tablets were accurately weighed and ground to a fine powder. Enough powder to represent one tablet was weighed accurately and transferred to a 150-ml beaker. Then 40 ml of water was added, and the mixture was heated to about 90° for approximately 5 min and then allowed to cool. The mixture was brought to volume (50.0 ml) with water and filtered. The first 15 ml of filtrate was discarded, and then a portion was collected and diluted 1:3 with water. Based on the label claim, this dilution contained the same concentration of the ingredients as the standard mixture.

All Active Ingredients and Sodium Benzoate—The assay sample (10.0 μl) was injected into the chromatograph using mobile phase with IV. For comparison purposes, an identical volume of the standard mixture was injected after the assay sample was eluted.

Calculations—Since preliminary investigations indicated that the peak area of each ingredient was directly related to concentration (range of 0.5–1.5 μg for I, II, and sodium benzoate and of 0.4–1.2 μg for III), the results were calculated by direct comparison of the peak areas:

$$\frac{A_a}{A_s} \times 100 = \% \text{ of label claim} \qquad \text{(Eq. 1)}$$

where A_a is the peak area of the ingredient in the assay sample and A_s is the peak area of the same ingredient in the standard mixture.

The results are presented in Table I, and sample chromatograms are presented in Figs. 1 and 2. A typical chromatogram using mobile phase without IV is also presented in Fig. 1. The relative standard deviations on all active ingredients and sodium benzoate based on six repeated injections are also presented in Table I.

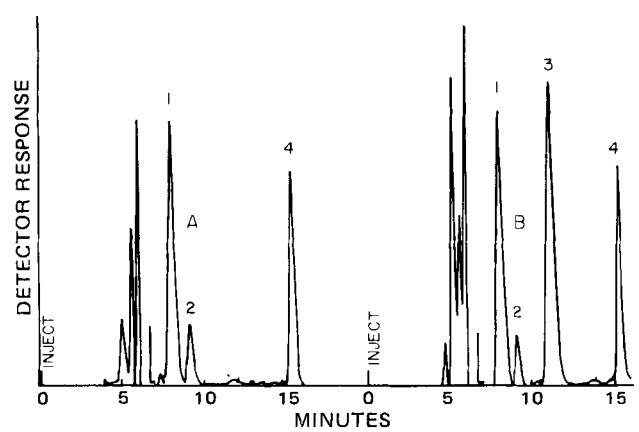

Figure 2—*Sample chromatograms from dosage forms. Key: A, from tablets using mobile phase containing IV; and B, from an elixir using mobile phase containing IV. Peaks 1–4 are from I, II, sodium benzoate, and III, respectively.*

Table I—Assay Results for Various Active Ingredients and Sodium Benzoate

Sample	Assay Results[a], % of Label Claim I	II	III	Sodium Benzoate
Sustained-release tablet	99.6	106.2	101.7	—[b]
Sustained-release tablet[c]	102.9	80.2	65.1	—[b]
Elixir	97.6	92.1	96.9	96.9[d]
Elixir[c]	100.3	118.2[e]	102.6	102.7[d]
RSD[f], %	1.15	1.59	1.78	0.93

[a] Average of three. [b] Tablets did not contain sodium benzoate. [c] Tablets and elixir were from a different manufacturer. [d] There was no label claim. The results are based on 0.1% in the elixir. [e] The manufacturer claimed that the product contained a substantial overage of III for extended shelflife. [f] Based on six injections of the standard mixture.

DISCUSSION

The results (Table I and Fig. 2) indicate that, using a single injection, it is possible to separate all three active ingredients and sodium benzoate present in commercial dosage forms. While assaying active ingredients, sodium benzoate (a preservative present in the elixir) can be assayed without additional time.

The method developed is accurate and precise (Table I). For each ingredient, the area of the peak was directly related to the concentration (range of 0.5–1.5 μg for I, II, and sodium benzoate and of 0.4–1.2 μg for III).

The method is sensitive; less than 1 μg of any ingredient can be assayed. The sensitivity can be improved by decreasing the absorbance units for full-scale deflection and by using the wavelength of maximum absorption instead of 254 nm. For example, the phenylephrine assay is six times more sensitive at 273 nm than at 254 nm. Since sensitivity was not a problem, 254 nm, which is usually found in most fixed wavelength detectors, was used.

The low results on II and III in one commercial dosage form (Table I) cannot be explained easily since these ingredients are supposed to be stable in solid dosage forms. It may be that the manufacturer used some alkaline excipients. The sustained-release tablets may have been made by incorporating part of the active ingredients in the coating, which could cause the problem. The type of excipients used or the processes involved in the manufacturing of these tablets could not be determined.

To use the developed method, it should be determined if the excipients (especially colors and preservatives in liquid dosage forms) interfere.

This report is another example of the application of paired ion HPLC. Without the addition of IV (a counterion), I and II could not be separated (Fig. 1).

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