any traces of the derivatizing reagent (10), thus removing the interfering contaminant; however, this washing is not possible with ethyl acetate as the injection solvent (8), since the latter is miscible with the sodium borate solution. Therefore, benzene was selected since the derivatized compounds are freely soluble in it, it is not miscible with aqueous solutions, and no significant loss occurs from this organic layer by washing with the saturated solution of sodium borate.

The sensitivity achieved for salsolinol in the present method is comparable to that for tetrahydropapaveroline using GC–mass spectrometry (5). Sandler *et al.* (4) also used a GC–mass spectrometric method for salsolinol, but they did not report the absolute sensitivity of their method.

Preliminary *in vivo* experiments utilizing this method to investigate the role of salsolinol in the effects of alcohol were reported elsewhere (12).

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Quantitative Determinations of Codeine Phosphate, Guaifenesin, Pheniramine Maleate, Phenylpropanolamine Hydrochloride, and Pyrilamine Maleate in an Expectorant by High-Pressure Liquid Chromatography

V. DAS GUPTA [x] and A. G. GHANEKAR

Abstract □ The quantitative determinations of codeine phosphate, guaifenesin, pheniramine maleate, phenylpropanolamine hydrochloride, and pyrilamine maleate in a liquid dosage form are described. All active and inactive ingredients (sodium benzoate and FD&C Yellow No. 5 dye) can be separated with high-pressure liquid chromatography except the two antihistamines, pheniramine maleate and pyrilamine maleate. Pheniramine maleate is determined colorimetrically, and pyrilamine maleate is determined either by difference or spectrophotometrically. The methods are simple, short, accurate, and precise. The standard deviations are reported.

Keyphrases □ Codeine phosphate—high-pressure liquid chromatographic analysis, cough syrup □ Guaifenesin—high-pressure liquid chromatographic analysis, cough syrup □ Phenylpropanolamine hydrochloride—high-pressure liquid chromatographic analysis, cough syrup □ Pheniramine maleate—colorimetric analysis, cough syrup □ Pyrilamine maleate—spectrophotometric analysis, cough syrup □ High-pressure liquid chromatography—analyses, codeine phosphate, guaifenesin, and phenylpropanolamine hydrochloride, cough syrup □ Colorimetry—analysis, pheniramine maleate, cough syrup □ Spectrophotometry—analysis, pyrilamine maleate, cough syrup □ Antitussives—cough syrup ingredients analyzed by high-pressure liquid chromatography, colorimetry, and spectrophotometry

A commonly used cough syrup contains an antitussive agent, codeine phosphate, an expectorant, guaifenesin, two antihistamines, pheniramine maleate and pyrilamine maleate, and a decongestant, phenylpropanolamine hydrochloride. In addition to active ingredients, most cough syrups contain dye(s), preservative(s), flavor(s), and sweetening agent(s). Due to interferences, no easy methods are available for the quantitative determinations of various ingredients in such a cough syrup. The purpose of these investigations was to develop easy, simple, short, and accurate methods for the quantitative determinations of various ingredients in such a cough syrup by high-pressure liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents including codeine phosphate (I), guaifenesin (II), pheniramine maleate (III), phenylpropanolamine hydrochloride (IV), and pyrilamine maleate (V) were ACS, USP, or NF grade and were used without further purification.

Apparatus—A high-pressure liquid chromatograph[1] equipped with a fixed wavelength (254 nm) detector and recorder[2] was used.

Column—A nonpolar column[3] consisting of a monomolecular layer of octadecyltrichlorosilane permanently bonded to Si–C (30 cm long and 4 mm i.d.) was used.

Chromatographic Conditions—The chromatographic solvent was 0.05 *M* KH_2PO_4 in water containing 13% (v/v) methanol. The temperature was ambient, and the solvent flow rate was 2.0 ml/min (at an inlet

[1] Waters ALC 202 equipped with a U6K universal injector.
[2] Omniscribe 5213-12 equipped with an integrator.
[3] Waters μ-Bondapak/C_{18}, Catalog No. 27324 (prepacked column).

Table I—Assay Results on Various Ingredients Using High-Pressure Liquid Chromatography

Syrup	Percent of Label Claim Found			
	I	II	III + V Combination	IV
Lot A	99.8	101.3	99.2	100.8
SD^a	1.96	1.08	1.24	2.32
Commercial sample	—[b]	106.9	104.3	55.3

[a] Based on four readings. [b] Had no codeine; *i.e.*, the manufacturer did not claim any on the label.

pressure of approximately 2000 psig). The chart speed was 30.5 cm/hr; the attenuation unit for full scale deflection was 0.04, which was changed to 0.08 after 10 min.

Solutions for HPLC—A standard solution containing 20 mg of I, 200 mg of II, 12.5 mg each of III and V, and 25 mg of IV/100 ml of chromatographic solvent was prepared using a simple solution method. Solutions of sodium benzoate (50 μg/ml) and FD&C No. 5 (4 μg/ml) in the chromatographic solvent also were prepared.

Solutions for Colorimetric Analysis of Pheniramine Maleate—A standard solution containing 50 μg/ml of pheniramine maleate in approximately 0.25 *N* HCl was prepared. Cyanogen bromide, 4 g/100 ml of water, also was prepared. A buffer was prepared according to a reported procedure (1).

Solution for Spectrophotometric Analysis of Pyrilamine Maleate—A standard solution containing 20 μg/ml of pyrilamine maleate in approximately 0.1 *N* HCl was prepared. Other solutions were prepared as needed.

Cough Syrup—Lot A cough syrup was prepared containing the following: 200 mg of codeine phosphate, 2 g of guaifenesin, 125 mg of pheniramine maleate, 250 mg of phenylpropanolamine hydrochloride, 125 mg of pyrilamine maleate, 1 ml of dye solution (0.8% FD&C Yellow No. 5 in water), 1 drop of flavor[4], 50 g of sugar, 5 mg of methylparaben, 2 mg of propylparaben, and 5 ml of alcohol.

The flavor was dissolved in alcohol. In 40 ml of water, sugar and guaifenesin were dissolved with the aid of heat and then cooled to room temperature. Then the remaining ingredients were added. The aqueous and alcoholic solutions were mixed and diluted to 100 ml with water.

Two additional lots, B (without pheniramine maleate) and C (without pyrilamine maleate), were prepared.

Assay Procedure for Cough Syrup—First 10.0 ml of the cough syrup (Lot A) was diluted to 100 ml with the chromatographic solvent, and then 10 μl was injected. For purposes of comparison, an identical volume of the standard solution was injected after the assay was eluted.

Since preliminary investigations indicated that the peak area of each ingredient was directly related to the concentration (range for I was 1.4–4 μg, range for II was 10–30 μg, range for III and V in combination was 1.25–5 μg, and range for IV was 1.25–5 μg), the results were calculated by direct comparison of the peak areas as follows:

$$\frac{A_a}{A_s} = \text{percent of label claim found} \qquad \text{(Eq. 1)}$$

where A_a = peak area of the assay sample, and A_s = peak area of the standard solution. The detector's response is presented in Fig. 1, and the results are presented in Table I.

A commercial sample was assayed according to the described procedure. The detector's response is presented in Fig. 2, and the results are presented in Table I.

To identify the additional peaks in the commercial sample, 10 μl each of the dye solution and of the sodium benzoate solution was injected. The detector's response is presented in Fig. 3.

Colorimetric Assay Procedure for Pheniramine Maleate—First 4.0 ml of the cough syrup (Lot A) was diluted to 100 ml with 0.25 *N* HCl and then to 1.0 ml of this dilution were added 7.0 ml of the buffer and 3.0 ml of the cyanogen bromide solution. Immediately after 13 min[5], the absorbance value of the color developed at 480 nm was measured against a blank prepared by substituting 3.0 ml of water for the cyanogen bromide solution. Concurrently, the absorbance value of the standard solution of pheniramine maleate (50 μg/ml) was determined.

Since preliminary investigations indicated that Beer's law was followed

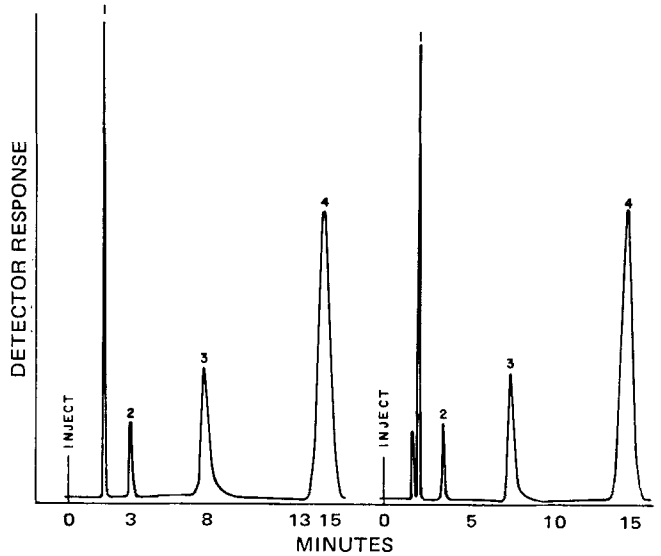

Figure 1—*HPLC analysis of Lot A cough syrup (for chromatographic conditions, see text). Peaks 1, 2, 3, and 4 are two antihistamines in combination (pheniramine maleate and pyrilamine maleate), phenylpropanolamine hydrochloride, codeine phosphate, and guaifenesin, respectively, from a standard solution (first injection) and cough syrup Lot A (second injection).*

(range of 30–80 μg/ml of the standard solution) and that there was no interference from other active and inactive ingredients, the results were calculated by a direct comparison of the absorbance values:

$$\frac{(A_{480})_a}{(A_{480})_s} = \text{percent of label claim} \qquad \text{(Eq. 2)}$$

where $(A_{480})_a$ = absorbance value of the assay sample, and $(A_{480})_s$ = absorbance value of the standard solution.

A commercial sample was also assayed according to the procedure reported (Table II).

Assay Procedure for Pyrilamine Maleate—First 2.0 ml of the syrup (Lot A) was diluted to 100 ml with approximately 0.1 *N* HCl solution and then the absorbance value at 315 nm was determined against 0.1 *N* HCl as the blank. A small interference from the dye may be corrected by either: (*a*) using Lot C syrup (2.0 ml diluted to 100.0 ml with 0.1 *N* HCl solution) as the blank or (*b*) measuring the absorbance values of the diluted Lot C syrup at 315 and 365 nm against 0.1 *N* HCl solution as the blank and determining the correction factor using:

$$\text{factor} = \frac{A_{315}}{A_{365}} \qquad \text{(Eq. 3)}$$

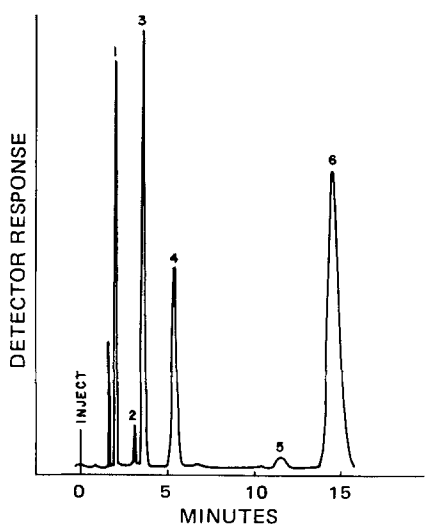

Figure 2—*HPLC analysis of a commercial cough syrup. Peaks 1, 2, and 6 are two antihistamines in combination, phenylpropanolamine hydrochloride, and guaifenesin, respectively. Peak 4 is sodium benzoate, and other peaks are unidentified.*

[4] Dolco Cherry, No. 5225, Dodge & Olcott, Inc., New York, N.Y.

[5] Time required for the maximum sensitivity in the assay procedure as determined in this laboratory.

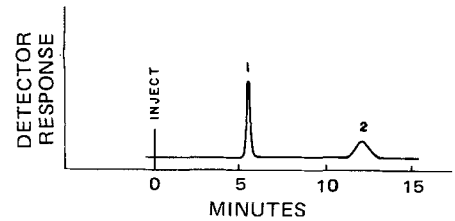

Figure 3—*HPLC analysis of some inactive ingredients used in cough syrups. Peaks 1 and 2 are sodium benzoate and FD&C Yellow No. 5, respectively.*

and:

$$\text{corrected } A_{315} = A_{315} - A_{365} \times \text{factor} \qquad \text{(Eq. 4)}$$

Since preliminary investigations indicated that Beer's law was followed, the results were calculated by direct comparison of the absorbance values at 315 nm:

$$\frac{\text{corrected } A_{315}}{(A_{315})_s} = \text{percent of label claim} \qquad \text{(Eq. 5)}$$

where corrected A_{315} is the absorbance value of the assay sample from Eq. 4, and $(A_{315})_s$ is the absorbance value of the standard solution containing 20 μg/ml of pyrilamine maleate in 0.1 *N* HCl solution against 0.1 *N* HCl as the blank. The results are presented in Table II.

DISCUSSION

The results (Tables I and II) indicate that a complex mixture of five active ingredients and two inactive ingredients (Figs. 2 and 3) can be separated and assayed quantitatively using HPLC. HPLC always has additional advantages such as the identification of the compounds from their retention times and separation of some inactive ingredients. In these investigations, it was possible to separate the preservative (sodium benzoate) and to identify it in the commercial sample (Figs. 2 and 3). Moreover, HPLC is usually short and often stability indicating.

The developed method is easy, accurate, and precise (for standard deviations, see Table I). It was not possible to separate pheniramine maleate from pyrilamine maleate, even by using many other solvents such as: (*a*) 0.05 *M* KH_2PO_4 in water containing 10% (v/v) methanol with the pH adjusted to 3.5 using acetic acid; (*b*) 0.05 *M* KH_2PO_4 in water containing 10, 15, 17, 20, and 45% (v/v) methanol; (*c*) 0.05 *M* KH_2PO_4 in water containing 13% (v/v) methanol with the pH adjusted to 6.5 using 0.2 *N* NaOH; (*d*) 0.05 *M* KH_2PO_4 in water containing 20% (v/v) acetonitrile; (*e*) 0.05 *M* NH_4HCO_3 in water containing 10% (v/v) methanol; and (*f*) 1-heptanesulfonic acid for paired-ion chromatography (2) in water containing 1% (v/v) acetic acid and 13% (v/v) methanol and filtered through a 0.45-μm filter.

Table II—Assay Results on Pheniramine Maleate and Pyrilamine Maleate by Spectrophotometric Methods

	Percent of Label Claim Found		
Syrup	III	V (by Difference)[a]	V (by Spectrophotometry)
Lot A	98.6	99.8	99.5
Commercial sample	105.2	103.4	—[b]

[a] Since preliminary investigations indicated that the combined peak area of III and V was equal to the sum of the separate peak areas of III and V and that, after determining the exact concentration of III by the cyanogen bromide method, it was possible to determine the peak area due to III, the peak area due to V could be determined by difference. This area was directly compared with the peak area of the standard solution of V (125.0 μg/ml in the chromatographic solvent). [b] Not determined since the authors did not know what dyes were used in the commercial sample.

Due to problems in separating III and V, spectrophotometric methods were used to determine the two antihistamines separately (Table II). However, if the concentration of one is known, the concentration of the other may be determined by difference (Table II, footnote *a*).

In these studies, it was possible to determine the concentration of pyrilamine maleate by difference after the quantitative analysis of pheniramine maleate with cyanogen bromide, which opens the pyridine ring (1) and imparts color to the solution. Whenever new prepacked columns are purchased, the percent of methanol in the chromatographic solvent may have to be changed slightly to accomplish the same separation.

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Quantitative Determinations of Antipyrine and Benzocaine in Ear Drops by High-Pressure Liquid Chromatography

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Abstract □ Antipyrine and benzocaine were determined quantitatively in ear drops by high-pressure liquid chromatography on an octadecyltrichlorosilane permanently bonded to a Si–C column, using 0.02 *M* KH_2PO_4 in methanol–water as the mobile phase. Both compounds can be assayed in combination directly without interference from each other or from oxyquinoline sulfate (the preservative). The method is accurate, precise (estimated universe standard deviations of 0.68% for antipyrine and 1.18% for benzocaine), simple, and short (requires 30 min *versus* 2–3 hr by the NF method). The method was tried on a commercial product with excellent results and can be used in the presence of decomposition products.

Keyphrases □ Antipyrine—high-pressure liquid chromatographic analysis, commercial combination with benzocaine □ Benzocaine—high-pressure liquid chromatographic analysis, commercial combination with antipyrine □ High-pressure liquid chromatography—analyses, antipyrine and benzocaine in commercial combination

An antipyrine and benzocaine mixture is used extensively for earache. The NF methods (1) for the quantitative determinations of antipyrine and benzocaine are lengthy and tedious. The purpose of these investigations was to develop a simple, short, accurate, precise, and stability-indicating assay method for the quantitative determina-