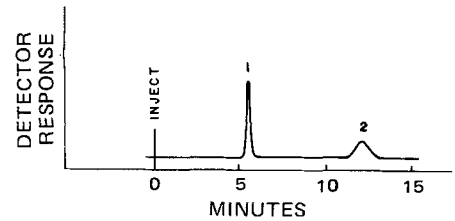

Figure 3—*HPLC analysis of some inactive ingredients used in cough syrups. Peaks 1 and 2 are sodium benzoate and FD&C Yellow No. 5, respectively.*

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-

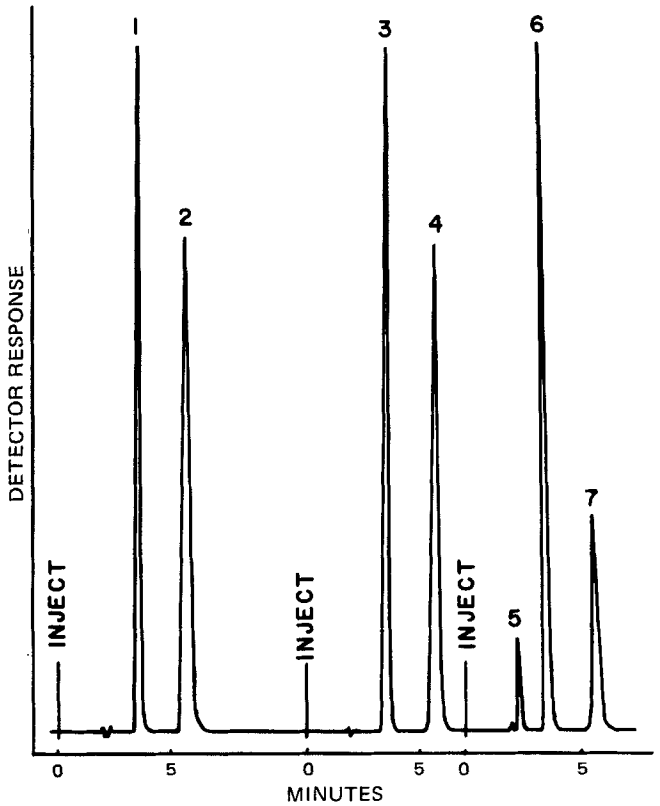

Figure 1—*HPLC analysis of antipyrine and benzocaine in combination (for chromatographic conditions, see text). Peaks 1 and 2 are antipyrine and benzocaine, respectively, from a standard solution; peaks 3 and 4 are antipyrine and benzocaine, respectively, from a commercial sample. Peaks 5, 6, and 7 are from the decomposed standard solution.*

tions of antipyrine and benzocaine in combination in the NF solution (1) using high-pressure liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents 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 0.02 *M* KH_2PO_4 in water containing 40% (v/v) methanol. The temperature was ambient, and the solvent flow rate was 1.6 ml/min (at an inlet pressure of approximately 2200 psig). The chart speed was 30.5 cm/hr, and the absorbance units for full scale deflection were 0.08 for benzocaine and 0.64 for antipyrine.

Standard Solutions—The following standard solutions were prepared in water containing 0.5% (v/v) glycerin: A, 0.027% antipyrine alone; B, 0.007% benzocaine alone; C, 0.027% antipyrine plus 0.007% benzocaine in combination; and D, 0.027% antipyrine, 0.007% benzocaine, and 0.002% oxyquinoline sulfate in combination. Solutions E–G were the same as Solutions A–C, respectively, except that pH 9 borate buffer USP (2) was substituted for water.

[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.

Table I—Assay Results on Antipyrine and Benzocaine

Solution	Assay Results, % of Claim	
	Antipyrine	Benzocaine
Prepared NF solution	99.52[a]	99.43[a]
Universe standard deviation (estimated)	0.68	1.18
Commercial sample	99.23[b]	99.13[b]
NF solution after decomposition	99.42[c]	52.86

[a] Average of four. The statistical data are based on four results. [b] The assay results by the NF method (1) as supplied by the manufacturer were 98.52% for antipyrine and 99.29% for benzocaine. [c] A small difference in the assay result from the undecomposed sample may be an experimental error.

Assay Procedure for NF Solution or Commercial Product—First 0.5 ml of the solution is diluted to 100.0 ml with water and then 10 μl is injected. For comparison, an identical volume of the appropriate standard solution is injected after the assay sample is eluted.

Calculations—Since preliminary investigations indicated that the peak area of each ingredient was directly related to the concentration (range of 1.35–4.05 μg for antipyrine and of 0.35–1.40 μg for benzocaine), the results were calculated by direct comparison of the peak areas:

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

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 assay results are presented in Table I.

Procedure for Decomposition of Solution—For decomposition studies, 25.0 ml of the standard solution containing both antipyrine and benzocaine in pH 9 borate buffer [with 0.5% (v/v) glycerin] was heated on a hot plate until the volume was reduced to approximately 5 ml. The solution was cooled and brought to the original volume (25.0 ml) with water. A 10-μl aliquot of this solution was injected for quantitative determinations of antipyrine and benzocaine as described. Since preliminary investigations indicated that borate buffer does not interfere with the quantitative determinations of the active ingredients, the results were calculated using Eq. 1. The detector's response is presented in Fig. 1, and the results are presented in Table I.

DISCUSSION

The results (Table I) indicate that antipyrine and benzocaine can be easily separated (Fig. 1) and assayed by HPLC. The method developed is simple, accurate, precise, and short (requires only about 30 min *versus* 2–3 hr by the NF method). However, the developed method requires a high-pressure liquid chromatograph, costing about three times more than the equipment required for NF methods. Since the proposed method appears to be stability indicating (Fig. 1), it may be preferred by those who already have a high-pressure liquid chromatograph available.

In the decomposed sample, the assay results on benzocaine were 52.86 *versus* 99.43% in the undecomposed sample. In the process used for the decomposition, benzocaine appears to have hydrolyzed. The hydrolyzed sample showed an additional peak (Fig. 1, peak 5). There was no interference from the preservative, oxyquinoline sulfate.

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