# Applications of Paired Ion High-Pressure Liquid Chromatography: Quantitative Determination of Potassium Guaiacolsulfonate and Other Ingredients in Cough Syrups

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**Abstract** □ A method for the quantitative determination of potassium guaiacolsulfonate and phenylephrine hydrochloride in commercial dosage forms was developed. The method is based on paired ion high-pressure liquid chromatography with tetrabutylammonium as the counterion. The method not only separates potassium guaiacolsulfonate from phenylephrine hydrochloride but also from some other ingredients: chlorpheniramine maleate, sodium benzoate, colors, and flavors. Furthermore, two isomers of potassium guaiacolsulfonate, potassium salts of 4- and 5-guaiacolsulfonic acid, also separate from each other. The method was tried on five different commercial dosage forms (all with different colors) with excellent results on three. In the other two samples, which also contained codeine, there may have been a stability problem.

**Keyphrases** □ Potassium guaiacolsulfonate—high-pressure liquid chromatographic analysis in pharmaceutical preparations □ Phenylephrine hydrochloride—high-pressure liquid chromatographic analysis in pharmaceutical preparations □ High-pressure liquid chromatography—analyses, potassium guaiacolsulfonate and phenylephrine hydrochloride in pharmaceutical preparations □ Adrenergics—phenylephrine hydrochloride, high-pressure liquid chromatographic analysis in pharmaceutical preparations □ Expectorants—potassium guaiacolsulfonate, high-pressure liquid chromatographic analysis in pharmaceutical preparations

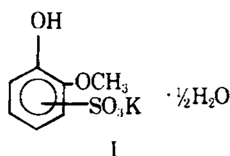
Many cough syrups contain potassium guaiacolsulfonate (I), which is an expectorant, and other active and inactive ingredients. The literature for the quantitative determination of I was reviewed previously (1). A new colorimetric procedure for its quantitative determination, based on the oxidation of I in an alkaline borate buffer solution and subsequent coupling with 4-aminoantipyrine, was developed (1). Since phenylephrine hydrochloride (II) also undergoes this coupling, it interferes with the quantitative determination of I.

## BACKGROUND

Potassium guaiacolsulfonate is a mixture of two isomers (2), potassium salts of 4- and 5-guaiacolsulfonic acid, but the presently available methods (1, 3–5) for the quantitative determination of I do not distinguish between them.

Recently, paired ion high-pressure liquid chromatography (HPLC) has become popular for increasing the retention times of weak acids and bases by the addition of a counterion in the mobile phase (6). Alkyl sulfonates are used for basic compounds, and quaternary ammonium compounds are used for acidic compounds (6).

Paired ion chromatography is an effective means of separating ionic compounds (6). It was used for the separation of thyroid hormones and sulfa drugs (7); and the analysis of FD&C dyes, such as tartrazine, was reported with tertiary or quaternary amines as the counterion in the mobile phase (8). Reversed-phase paired ion HPLC was used for the separation of hydrocortisone from hydrocortisone phosphate (9). This approach also was applied to the simultaneous determination of niacin and niacinamide in multivitamin preparations with dioctyl sulfonate as the counterion (10).




The purpose of this report is to present a method for: (*a*) the quantitative determination of I in the presence of II, (*b*) the separation of the two isomers of I, and (*c*) the quantitative determination of I in the presence of some other active and inactive ingredients present in commercial dosage forms used to relieve the symptoms of coughs and colds. The developed method is based on paired ion HPLC with tetrabutylammonium (III) as the counterion. An identical method in which the ammonium ion was substituted for III also was tried without success.

## EXPERIMENTAL

**Reagents and Chemicals**—All reagents and chemicals were ACS, USP, or NF quality and were used without further purification. Bromdiphenhydramine hydrochloride[1] (IV), chlorpheniramine maleate[2] (V), codeine phosphate[3] (VI), dextromethorphan hydrobromide[4] (VII), diphenhydramine hydrochloride[1] (VIII), potassium guaiacolsulfonate[3], phenylephrine hydrochloride[5], promethazine hydrochloride[6] (IX), and sodium benzoate[3] (X) were used as received.

**Apparatus**—The high-pressure liquid chromatograph[7] was connected to a multiple wavelength detector[8], a recorder[9], and an integrator[10].

**Column**—The column[11] (30 cm × 4 mm i.d.) was of a very nonpolar material, consisting of a monomolecular layer of octadecyltrichlorosilane permanently bonded by silicone–carbon bonds.

**Chromatographic Conditions**—Solvent A consisted of 0.005 *M* dibasic ammonium phosphate in water containing 8.5% (v/v) methanol. The pH was adjusted to 7.75[12] (±0.05) with dilute phosphoric acid (1:100 in water). Solvent B was the same as A except that tetrabutylammonium hydroxide (from a 40% aqueous solution) was substituted for dibasic ammonium phosphate.

The temperature was ambient. The flow rate was 4.0 ml/min except for the first 2 min with Solvent A when it was 2.0 ml/min. The detector was set at a sensitivity of 0.1 (254 nm) for Solvent A and at 0.04 for Solvent B. The chart speed was 30.5 cm/hr.

**Preparation of Solutions**—The stock solutions of all drugs, I, II, and IV–X, were prepared by dissolving 0.250 g of the drug in enough water to make 100.0 ml. The standard solutions were prepared by diluting the stock solution with the appropriate chromatographic solvent as needed. A standard mixture of six ingredients, I, II, V, VI, IX, and X, was prepared by mixing 10.0 ml of each stock solution and then bringing to volume (100.0 ml) with the appropriate chromatographic solvent. This mixture was diluted further with the chromatographic solvent as needed.

**Dilutions of Commercial Dosage Forms for Analysis**—A 10.0-ml aliquot of the dosage form was diluted to 100.0 ml with water. An appropriate quantity of this solution was diluted further with an appropriate chromatographic solvent to obtain a concentration of 250.0 μg of I/ml. For the analysis of II, which was present in only one dosage form, the first dilution in water, *i.e.*, 10.0–100 ml, was used. This dilution contained 100 μg of II/ml.

**Assay**—A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions (chromatographic Solvent B). For comparison, an identical volume of the appropriate standard

[1] Parke-Davis & Co., Detroit, Mich.
[2] Schering Corp., Bloomfield, N.J.
[3] Merck & Co., Rahway, N.J.
[4] Hoffmann-La Roche, Nutley, N.J.
[5] Winthrop Laboratories, New York, N.Y.
[6] Wyeth Laboratories, Philadelphia, Pa.
[7] Waters ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.
[8] Spectroflow monitor SF770, Schoeffel Instrument Corp., Westwood, N.J.
[9] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[10] Autolab minigrator, Spectra-Physics, Santa Clara, Calif.
[11] Waters μBondapak $C_{18}$, Catalog No. 27324.
[12] Model 4500 digital pH meter, Beckman Instruments, Irvine, Calif.



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**Table I—Results on Potassium Guaiacolsulfonate and Phenylephrine Hydrochloride in Various Commercial Dosage Forms**

| Dosage Form (Expectorant) | Color | pH[a] | Concentration of I, mg/ml | Percent[b] of Label Claim Found: On I Using Developed Method | On I Using Literature Method | On II Using Developed Method |
|---|---|---|---|---|---|---|
| 1[c] | Green | 5.2 | 8.8 | 99.5 | 100.0 | —[d] |
| 2[e] | Yellowish brown | 5.2 | 8.8 | 99.4 | 124.4[f] | 97.0 |
| 3[g] | Yellow | 5.2 | 8.8 | 99.9 | 100.5 | —[d] |
| 4[h] | Dark red | 5.2 | 8.8 | 88.7 | 100.5 | —[d] |
| 5[i] | Red | 5.7 | 16 | 90.9 | 95.3 | —[d] |
| *RSD*, %[j] | | | | 1.14 | 1.29 | |

[a] After diluting 10 ml to 100 ml with water. [b] Average of two. [c] Other listed ingredients per 5 ml of the dosage form were: IX, 5 mg; ipecac fluid extract, 0.01 ml; chloroform, 0.015 ml; citric acid anhydrous, 60 mg; and sodium citrate, 197 mg. [d] Did not contain phenylephrine hydrochloride. [e] Also contained all ingredients listed under footnote *c* plus 1 mg of II/ml. [f] Results are high due to interference from II (see Ref. 1). [g] Also contained all ingredients listed under footnote *c* plus 1.5 mg of dextromethorphan hydrobromide/ml. [h] Also contained all ingredients listed under footnote *c* plus 2 mg of VI/ml. [i] Other listed ingredients per 5 ml of the dosage were: IV, 3.75 mg; VIII, 8.75 mg; ammonium chloride, 80 mg; methanol, 0.5 mg; and codeine sulfate, 10 mg. [j] Based on five injections of the standard mixture. Similar deviations were recorded on different days of the experiment.

solution (containing 250 μg of I/ml or 100 μg of II/ml) or mixture was injected after the assay solution eluted.

**Calculations**—Since preliminary investigations indicated that the peak areas (peaks heights also) were related directly to the concentrations (range of 1.25–5.0 μg) of each ingredient, the results were calculated using:

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

where $A_a$ is the peak area of the assay solution and $A_s$ is the peak area of the standard solution.

With I, the area of the larger peak (first peak from one of the two isomers) was used. The second peak was too small for accurate results. The results are presented in Table I, and the sample chromatograms are presented in Figs. 1A–1C and 2. A chromatogram that was developed using Solvent A is presented in Fig. 1D. Potassium guaiacolsulfonate also was assayed using the colorimetric method (1). The results are presented in Table I.

## RESULTS AND DISCUSSION

The results clearly indicate that paired ion HPLC is useful for the separation of a number of active ingredients (Fig. 1C): chlorpheniramine maleate, potassium guaiacolsulfonate, phenylephrine hydrochloride, and sodium benzoate. Under identical conditions without the addition of the counterion tetrabutylammonium, this separation was not possible (Fig. 1D). The addition of the counterion not only separated these ingredients but also the two isomers of potassium guaiacolsulfonate from each other. A number of other ingredients, bromdiphenhydramine hydrochloride, codeine phosphate, diphenhydramine hydrochloride, dextromethorphan hydrobromide, methylparaben, propylparaben, promethazine hydrochloride, colorants, and flavors, did not interfere with analysis. The concentrations tested were much higher (5 mg/ml, based on the original dosage form) than are usually found in commercial dosage forms.

The addition of a counterion such as III increases the retention times of weak acids (8, 9). However, it appears that it considerably decreased the retention time of phenylephrine hydrochloride, a zwitterion (Figs. 1C and 1D).

The developed method can be used for the quantitative determination of I in pharmaceutical dosage forms (Table I). The relative percent standard deviation based on five injections of the standard mixture was 1.29. Phenylephrine hydrochloride in combination with I also may be determined (Table I). The separation of I and II from each other appears to be excellent (Figs. 1C and 2A). It was not possible to determine the concentration of I and II separately using the method reported earlier (1).

Furthermore, it was estimated that the first four expectorants (Table I) contained ~0.02% sodium benzoate as the preservative while the fifth expectorant did not contain sodium benzoate. It was possible to determine the concentration of sodium benzoate without a separate analysis or additional injections into the chromatograph. With HPLC, additional ingredients can be determined without additional labor or cost.

The results on potassium guaiacolsulfonate in Expectorants 4 and 5 (Table I) were lower with the developed method than with the literature method (1) because the developed method separated the two isomers of I. Neither the literature method (1) nor the NF method (3) distinguishes between the two isomers. In the developed method, the larger peak (first peak from I) was used to determine the concentrations of I because the second peak was too small for accurate determinations. The ratio of peak heights of the two isomers was about 7:1 in the standard mixture (Fig. 1C, peaks 3 and 4) and in other expectorants (Figs. 2A and 2B, peaks 2 and 3) except 4 and 5 (Table I), both of which contained codeine.

In Expectorants 4 and 5, the ratios were significantly different, 5:1 (Fig. 2C, peaks 2 and 3) and 6.3:1, respectively. Therefore, there may be a stability problem in the presence of codeine. The difference in the ratios of the two isomers in the presence of codeine in the two samples may have been due to: (*a*) pH differences (Table I); (*b*) the difference in codeine

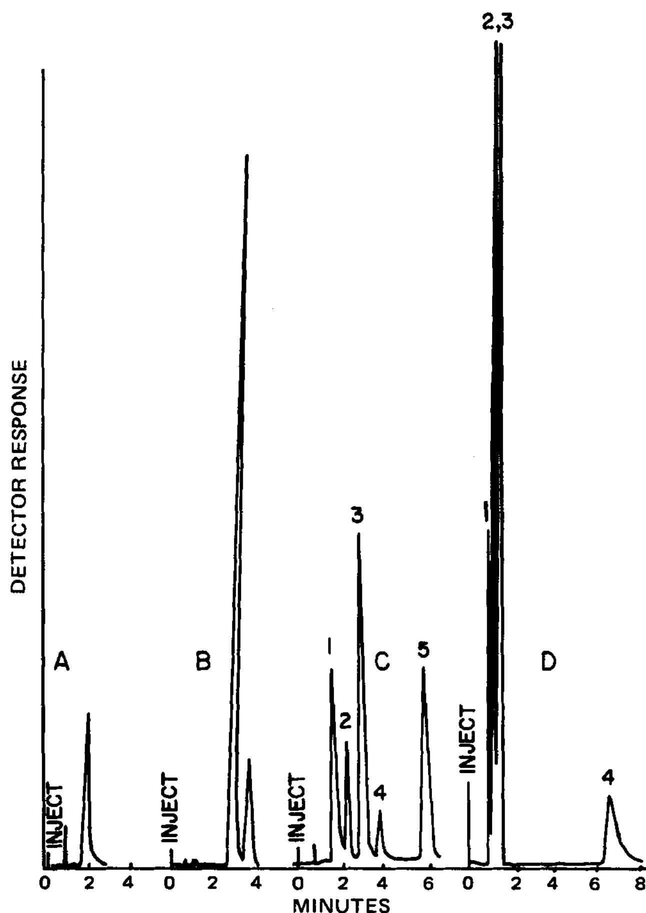


**Figure 1**—*Sample chromatograms. Solvent B was used for A–C, and Solvent A was used for D. Chromatogram A is from a standard solution of II in water (100 μg/ml). Chromatogram B is from a standard solution of I (250 μg/ml). Both peaks are from I. Chromatogram C is from a mixture containing 125 μg/ml each of I, II, V, VI, IX, and X. Peak 1 is from V, 2 is from II, 3 and 4 are from I, and 5 is from X. No peaks were recorded from the other two ingredients. Chromatogram D is the same as C, except that the solvent was A and the concentration of each ingredient was 250 μg/ml. Peak 1 is from V, 2 is from I, 3 is from X, and 4 is from II.*

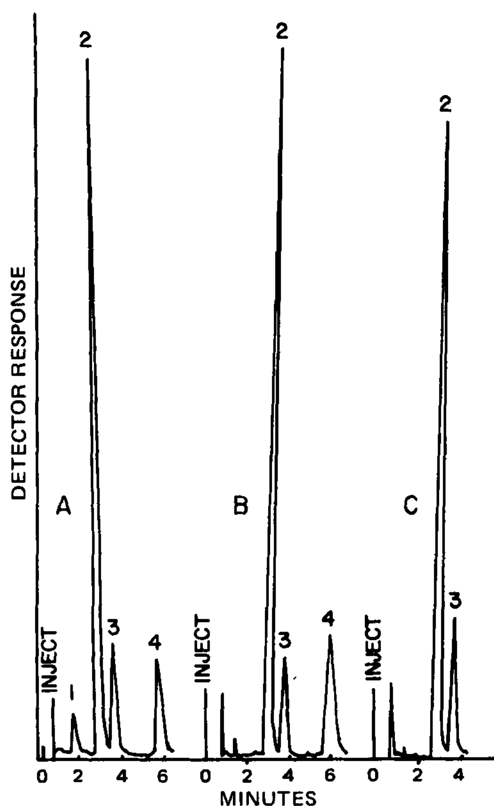


**Figure 2**—*Sample chromatograms developed using Solvent B. Chromatogram A is from a commercial expectorant (2 in Table I). Peak 1 is from II, 2 and 3 are from I, and 4 is from X. Chromatogram B is from Expectorant 1 in Table I. Peaks are the same as in A except that it did not contain II. Chromatogram C is from Expectorant 4 in Table I. Peaks are the same.*

salts: codeine phosphate in Expectorant 4 *versus* codeine sulfate in Expectorant 5; (*c*) a higher concentration of codeine in Expectorant 4 relative to the concentration of potassium guaiacolsulfonate, *i.e.*, codeine was 2 mg/ml in each sample *versus* only 8.8 mg of I/ml in Expectorant 4 and 16 mg of I/ml in Expectorant 5; (*d*) different buffering systems: citrates in Expectorant 4 *versus* ammonium chloride in 5; and (*e*) the ages of the samples, which were not determined. The standard mixture did contain codeine, and the ratio (about 7:1) of the peak heights of the two isomers had not changed even after standing for about 7 days. Without further intensive investigations, it is not possible to determine whether this change in ratio affects the therapeutic value.

Expectorants 1–4 (Table I) gave an additional unidentified peak after about 13.5 min. Expectorant 5 gave at least two unidentified peaks after about 7 and 13.3 min. These peaks, which were recorded using Solvent B, could be from the colorants, flavors, or preservative. This matter was not pursued further because detailed formulas of the dosage forms were not available.

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# Molecular Connectivity Analyses of Structure Influencing Chromatographic Retention Indexes

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Received March 6, 1978, from the **Department of Pharmaceutical Chemistry, Medical College of Virginia, Richmond, VA 23298,* and the *‡Department of Chemistry, Eastern Nazarene College, Quincy, MA 02170.* Accepted for publication June 15, 1978.

**Abstract** □ The molecular connectivity indexes of various aliphatic alcohols, ketones, ethers, and esters were used to describe structural features influencing chromatographic retention indexes. Good correlations were obtained within chemical classes for a particular stationary phase.

**Keyphrases** □ Molecular connectivity indexes—correlated with structural features influencing chromatographic retention indexes, various organic compounds □ Chromatographic retention indexes—correlated with molecular connectivity indexes, various organic compounds □ Topological indexes—molecular connectivity indexes correlated with structural features influencing chromatographic retention indexes

The chromatographic retention index is a quantification of a dynamic physicochemical process involving the equilibration of a solute between two liquids passing each other at an interface (liquid–liquid chromatography) or the interchange state between gas and solution phases (gas–liquid chromatography). The retention index for a particular molecule in a particular system depends on the structure of that molecule and the nature of that system. A rigorous definition of the structure of a molecule should make it possible to arrive at an accurate value for a retention index for a given system.

## BACKGROUND

Previously (1), it was shown that definition of molecular structure at the level of topology could provide sufficient information for close correlations with numerous physicochemical properties. This definition of molecular structure is called molecular connectivity (2). To the extent that chromatographic retention indexes are influenced by topological



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