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Chromatographic Behavior of Doxylamine Succinate, Phenylpropanolamine Hydrochloride, Chlorpheniramine Maleate, Dextromethorphan Hydrobromide, Paracetamol, and Guaifenesin in Ion Pair Reverse-Phase High Performance Liquid Chromatography

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A study of the chromatographic behavior of some cough syrup ingredients has led to the optimum chromatographic separation of four ingredients (doxylamine succinate, phenylpropanolamine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide). The paracetamol and guaifenesin were overlapping under all chromatographic conditions. The application of 1% chlorotrimethylsilane in methanol to the column (Partisil 5 CCS/C_8) was found to improve the column efficiency significantly. This separation can be applied for the analysis of cough syrup for these ingredients after a study of the interferences due to normal excipients.[2]

INTRODUCTION

The separation of some cough syrup ingredients by HPLC has been conducted (*1–13*). However, some compounds, e.g., doxylamine succinate (I), are very hard to deal with chromatographically due to severe tailing. This paper presents a study of the chromatographic behavior of doxylamine succinate, phenylpropanolamine hydrochloride (II), chlorpheniramine maleate (III), dextromethorphan hydrobromide (IV), paracetamol (V), and guaifenesin (VI). The study involved optimization of various parameters affecting the separation leading to optimum separation. The elution time is approximately 6 min. The separation can be utilized for the determination of these ingredients in commercially available cough syrups.

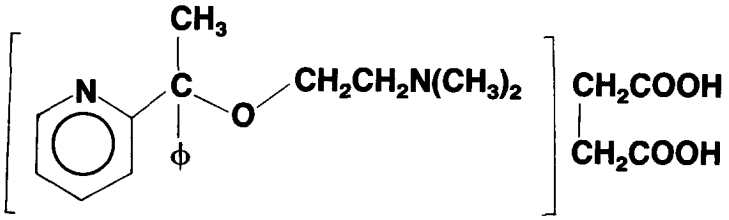
EXPERIMENTAL

Materials

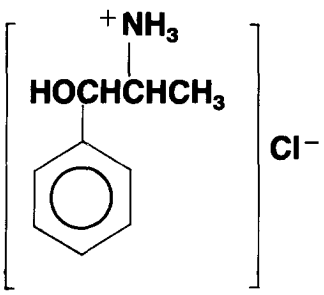
Generally, all chemicals were the purest grade available and were used as obtained without further purification. Methanol and acetonitrile HPLC grade (Koch–Light)[3] were 99.8%. Ammonium hydroxide, tetrahydrofuran (THF), so-

[1] To whom correspondence should be addressed.
[2] Excipient: any inert substance used as a diluent or vehicle for a drug.
[3] Koch–Light Limited, Haverhill, England.

0026-265X/87 $1.50



I



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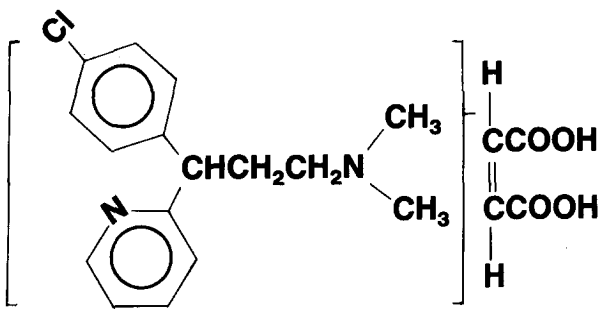

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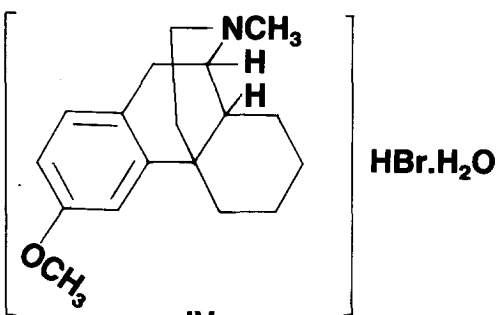

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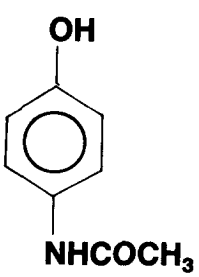

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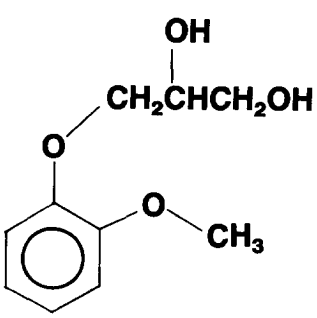

VI

dium dioctyl sulfosuccinate, and phosphoric acid (Fluka)[4] were 30, 99, 98, and 85%, respectively. The water used was always distilled and deionized.

The various cough–cold ingredients. **I, II, III, IV, V, VI,** and the internal standard (dextropropoxyphene hydrochloride, **VII**) (USP standards)[5] were 99.6, 99.6, 98.7, 100.0, 99.0, 99.4, and 98.6%, respectively.

Apparatus. A Varian Associates HPLC[6] Model 5030 equipped with a variable wavelength detector,[7] a 10-μl manual loop injector,[8] and a digital integrator[9] was used. A reverse-phase column (250 × 4.6 mm i.d.) Partisil 5 CCS/C_8 (Whatman)[10] was used at ambient temperature.

Chromatographic Conditions

The mobile phase consists of 7.0 m*M* sodium dioctyl sulfosuccinate in an acetonitrile/methanol/THF/water/phosphoric acid (370/300/300/30/0.7, v/v) solution. The pH was adjusted to 4.0 using dropwise addition of ammonium hydroxide.

The mobile phase and the sample solutions were filtered using 0.45-μm membrane filters (Gelman).[11] The mobile phase was degassed by vacuum prior to use.

The detector sensitivity was 0.16 AUFS. The flow rate was 2 ml/min. The wavelength was 258 nm. The chart speed was 0.5 cm/min.

Preparation of the Standard Solutions

Internal standard solution. Dextropropoxyphene hydrochloride (150 mg) was dissolved in 100 ml of methanol.

Standard solutions of I–IV for linearity. **(I)** (12 mg), **II** (105 mg), **III** (12 mg), and *IV* (75 mg) were dissolved in 100 ml of the internal standard solution. From this solution, six different standard solutions of different concentrations have been prepared covering the range 50–150% of the expected working range. That is 0.2, 0.4, 0.6, 10.8, 1.0, and 1.2 μg of **I** and **III**; 1.75, 3.50, 5.25, 7.00, 8.75, and 10.5 μg of **II**; and 1.25, 2.50, 3.75, 5.00, 6.25, and 7.50 of **IV** were injected per 10 μl.

THEORETICAL

A brief outline of the ion-pairing principle as developed by Higuchi and Michaelis (*14*), Schill *et al.* (*15*), Horvath *et al.* (*16, 17*), Kraak and Huber (*18*), and Karger *et al.* (*19, 20*) is given in the following.

If a cation R^+, in aqueous phase, comes in contact with a counter ion I^-, and is extracted in an organic phase, the equilibrium can be expressed as

$$R^+_{aq} + I^-_{aq} \rightleftharpoons R^+ I^-_{org}. \quad (1)$$

[4] Fluka AG, Chemische Fabrik, Switzerland.
[5] The U.S. Pharmacopeia Convention, Inc., Rochville, MD 20852.
[6] Varian, Palo Alto, CA.
[7] DuPont Co., Wilmington, DE.
[8] Valco Instruments Co., Houston, TX.
[9] Sp-4100, Spectra Physics, San Jose, CA.
[10] Whatman Chemical Separation, Inc., Clifton, NJ 07014.
[11] Gelman Instruments, Ann Arbor, MI.

The extraction coefficient, E, is measured as

$$E = \frac{[RH]_{org}}{[R^+]_{aq}\,[I^-]_{aq}}. \tag{2}$$

$[R^+I^-]_{org}$ is the concentration of the ion pair, $[I^-]_{aq}$ is the concentration of the pairing ion, and $[R^+]_{aq}$ is the concentration of the protonated amine.

The capacity factor (k') is expressed as

$$k' = \frac{V_s}{V_m}\frac{[R^+I^-]_{org}}{[R^+]_{aq}}$$

$$k' = \frac{V_s}{V_m} E\,[I^-]_{aq}. \tag{3}$$

V_s and V_m are the volumes of the stationary phase and the mobile phase, respectively.

RESULTS AND DISCUSSION

After some preliminary investigation involving compounds (I–VII), a reverse-phase 5 CCS/C_8 (a Whatman fully silanized reversed phase; C_8 groups bonded to Partisil via CCS bonding with a carbon load of approximately 9%; surface coverage 90%) was selected. A primary requirement of the chromatographic system was its ability to separate the amine drugs in a reasonable time without tailing. The pairing ion sodium dioctyl sulfosuccinate was selected due to its ability to produce adequate retention of doxylamine succinate, phenylpropanolamine-HCl,

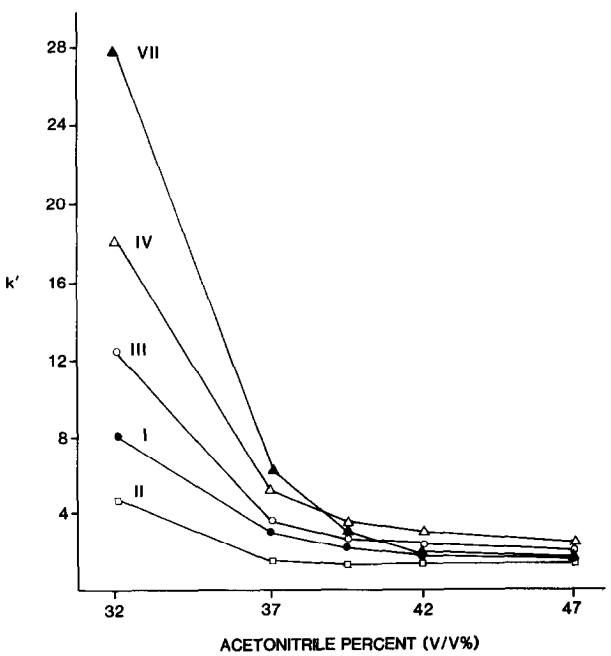

FIG. 1. Plots of the capacity factor versus the acetonitrile percentage in the mobile phase.

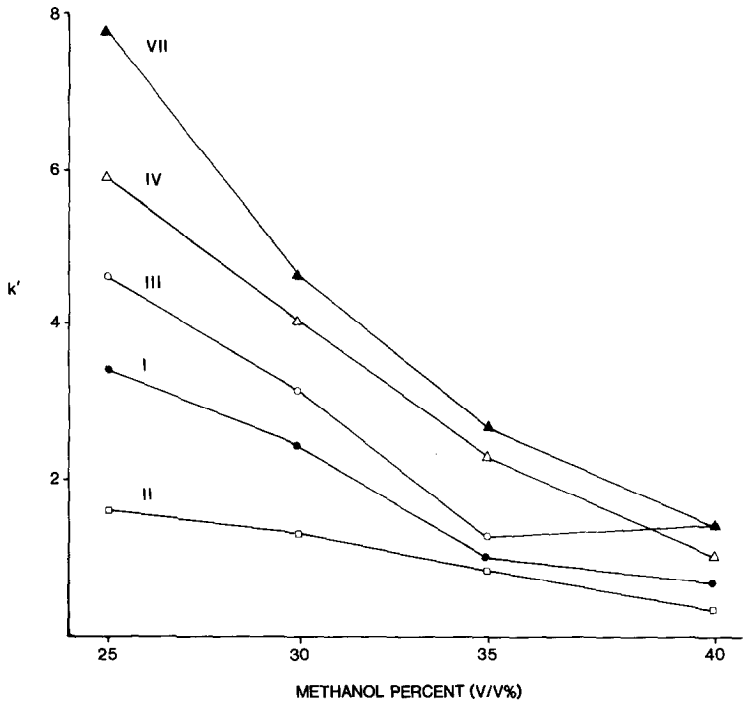

FIG. 2. Plots of the capacity factor versus the methanol percentage in the mobile phase.

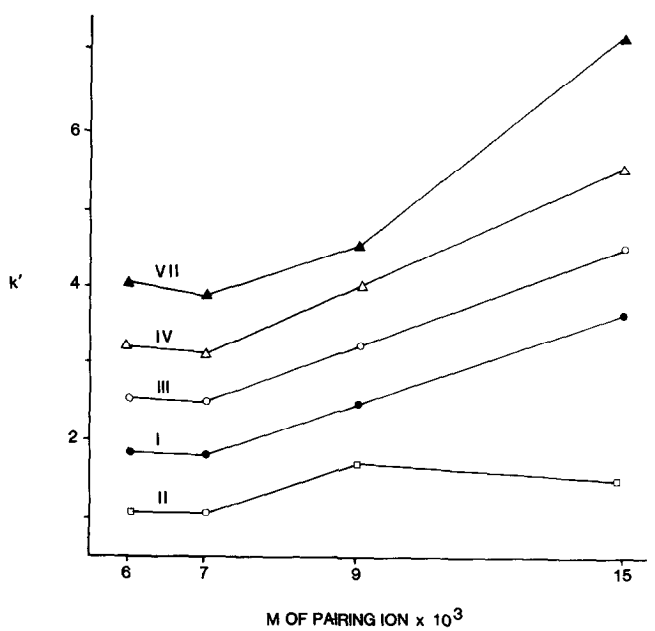

FIG. 3. Plots of the capacity factor versus the molarity of pairing ion.

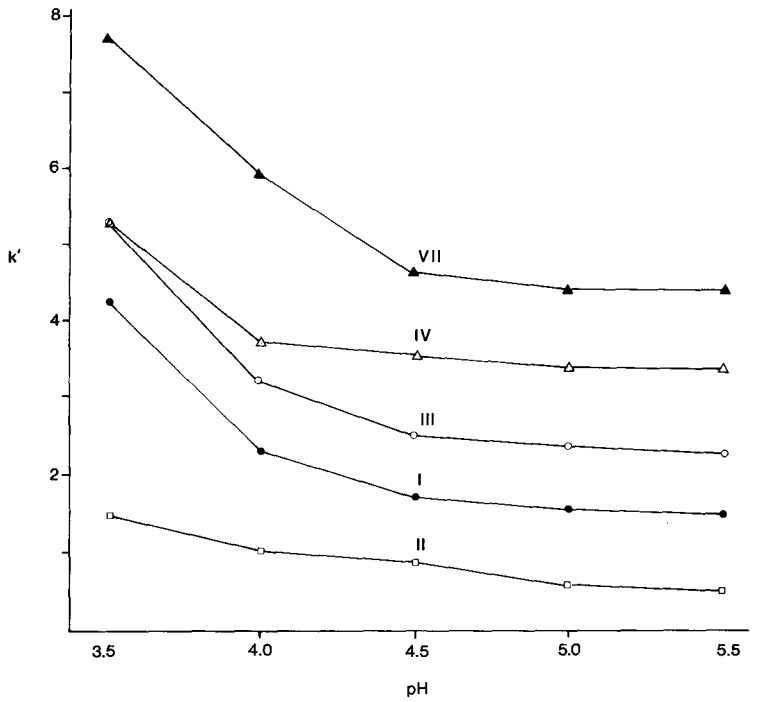

FIG. 4. Plots of the capacity factor versus the pH.

dextromethorphan-HBr, and chlorpheniramine maleate and its solubility, availability, and low cost.

In order to optimize the chromatographic conditions the effects of percentage of acetonitrile and methanol on the capacity factor (k') were studied. Also, a study of pH effects and pairing ion concentrations on the k' was carried out (Figs. 1–4). The k' values of I–IV were affected by the variation of acetonitrile concentration; all the compounds gave sharp peaks very close to each other, with low values of capacity factors; i.e., the resolution was poor at high acetonitrile percentage. At low acetonitrile concentration, k' values became very high, leading to longer t_R and broader peaks, i.e., lower efficiency. Therefore, a mobile phase of 37% acetontrile was selected, since it provides sharp peaks and reasonable t_R (Fig. 1). By lowering the methanol content of the mobile phase, the resolution of all compounds can be increased, but only at the expense of long retention times. Therefore, the optimum methanol concentration selected was 30% (Fig. 2).

The relationship between the capacity factor (k') and the pairing ion concentration is shown in Fig. 3. Equation (3) predicts k' to be proportional to the pairing ion concentration, and this behavior is observed with all compounds except II. In such a complicated mobile phase it is extremely difficult to explain clearly the role of each individual component. Chromatograms obtained in the absence of the pairing ion reagent were characterized by broad peaks and tailing, particularly with compounds II, III, and IV.

The k' values for I to IV and VII were affected by the variation of the pH of the mobile phase (Fig. 4). In the pH range 4.5–5.5, no change was observed, while in the pH range 3.5–4.5 for phenylpropanolamine and dextromethorphan slight changes in k' were observed. Sharper changes, however, were observed for chlorpheniramine, doxylamine, and dextropropoxyphene. The amine groups of phenylpropanolamine and dextromethorphan (pK_a 9) are fully protonated in this pH range and are behaving as monocations with respect to the ion-pairing system. Chlorpheniramine and doxylamine are capable of being protonated twice with reported pK_a values of 4.0 (pyridinium group) and 9.2 (tertiary amine group) (*21*). In the pH range 4.5–5.5, chlorpheniramine and doxylamine exhibit retention behavior more compatible with that of monocations, while at pH range 3.5–4.5 the rapid increases in their retentions are due to large concentrations of the dication forms. For bivalent sample ions, k' values are predicted to be proportional to the square of the pairing ion concentration (*22*), and the rapid increase in retention of chlorpheniramine and doxylamine at lower pH is consistent with this explanation. A mobile phase of pH 4.0 was chosen since it provides baseline separation with sharp peaks in a reasonable retention time.

The linearity of the detector response was determined by preparing calibration standard solutions as described under Experimental. The ranges of linearity for I–IV were 0.2–1.2 μg for I and III, 1.75–10.5 μg for II, and 1.25–7.50 μg for IV with correlation coefficients of 0.9997, 0.9993, 0.9997, and 0.9989 for I, II, III, and IV, respectively.

The detection limits for I–IV were 3.0 ng for I, II, and III, and 20 ng for IV as determined by diluting the standard solution with the mobile phase and injecting 10 μl into the column.

Figure 5 shows a typical chromatogram of a 10-μl injection of a synthetic mixture of I–VII utilizing the optimal chromatographic conditions. However, it was found that the column loses some of its efficiency after about 100 injections. The

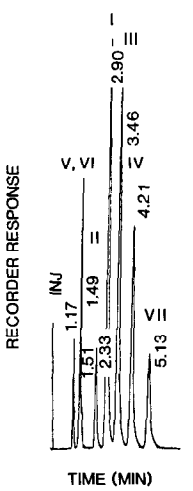

FIG. 5. A typical chromatogram of a 10-μl injection of a synthetic mixture of I–VII.

silanization of the column by pumping 1% solution of chlorotrimethylsilane[12] in methanol for 30 min through the column was found to restore efficiency completely, clearly because the chlorotrimethylsilane combines with some of the silanol groups (Si-OH) responsible for tailing.

In conclusion, the optimal chromatographic conditions described in this paper provide a baseline separation of some of the components of cough syrup. Under all conditions tried guaifenesin and paracetamol could not be separated. The separation can be used for the determination of these ingredients (I–IV and V or VI) in cough syrup but only after study of the interferences due to normal excipients utilized.

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