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Liquid chromatographic determination of amines in complex cough-cold formulations *

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Summary

A rugged, stability-indicating HPLC method has been developed and validated for pseudoephedrine hydrochloride, doxylamine succinate and dextromethorphan hydrobromide in a commercial cough-cold liquid formulation. Effects of mobile phase composition on the capacity factors of 6 amine drugs commonly used in many over-the-counter cough-cold formulations have been studied. Based on the mobile phase optimization study, we can easily select an optimized HPLC condition for this method.

Introduction

Most cough-cold products usually contain some combination of drugs that include an analgesic (acetaminophen), a decongestant (phenylpropanolamine hydrochloride (**I**) or pseudoephrine hydrochloride (**III**)), an antihistamine (diphenhydramine hydrochloride (**II**), doxylamine succinate (**IV**), or chlorpheniramine maleate (**VI**)), and an antitussive (dextromethorphan hydrobromide (**V**)). The structures of compounds **I** to **VI** are shown in Fig. 1. Combination products have been extensively used for the relief of symptoms of coughs and colds. In an extra strength liquid combination product, the analgesic, acetaminophen is usually formulated in a much greater amount (e.g. 1000 mg per dose) than the remaining active amine components. On the other hand, the presence of formulation excipients, such as sugars, preservatives, flavors and dyes, further increases the difficulty for the simultaneous determination of the lower-dosed amine components (e.g. 7.5–60 mg per dose) using a chromatographic method.

Several methods for the simultaneous determination of a wide variety of amine drugs in various cough-cold formulations have been reported in the literature. For example, an ion pair liquid chromatographic (LC) assay of decongestants and antihistamines was reported (Koziol et al., 1979). A liquid cough-cold product containing 3 active amines (**I**, **V** and **VI**) was assayed using a reversed-phase ion-pair LC method (Hal-

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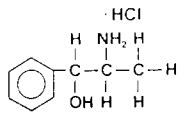
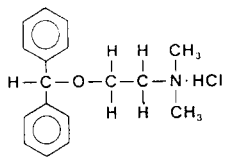
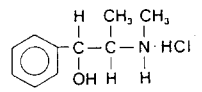
I.
Phenylpropanolamine Hydrochloride



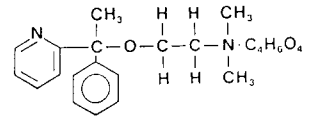
II.
Diphenhydramine Hydrochloride



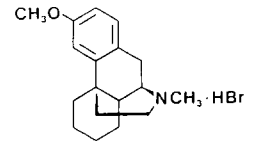
III.
Pseudoephedrine Hydrochloride



IV.
Doxylamine Succinate



V.
Dextromethorphan Hydrobromide



VI.
Chlorpheniramine Maleate

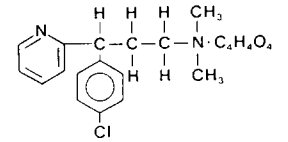


Fig. 1. Structures of 6 common amine drugs found in cough-cold products.

stead, 1982). Another combination product containing the same 3 amines and acetaminophen was assayed using a single column and 3 different mobile phases (Gupta and Heble, 1984). Most recently, a HPLC assay of an over-the-counter multisymptom cough-cold preparation was also reported (Achari and Stillman, 1986). These methods usually employed reversed-phase columns using octadecylsilane (ODS) and phenyl phases, and/or ion-pairing reagents to effect the separation of these drugs. Another recent paper (Hansen and Thompson, 1985) reported a HPLC method for the determination of various antihistamines, including doxylamine succinate, in laboratory animals and drug feed. This method employed a reversed-phase cyanopropyl column with acetonitrile, phosphate and trimethylamine buffer mobile phase. However, none of the reported procedures was found to be suitable for our new formulations, which contain the active components and various combinations of the following inactive components as excipients: sorbitol, alcohol, carbowax, citric acid, sodium citrate, sodium benzoate, mint, sodium saccharin, beet sugar, FD & C Red 40, FD & C Red 33, FD & C Blue 1, and water. This report describes a modified HPLC method which has been validated for **III, IV**, and **V** in a commercial cough-cold liquid formulation with respect to precision, accuracy, linearity, sensitivity, specificity and ruggedness. Also, effects of variation in the mobile phase composition on the capacity factors of **I–VI** have been determined as part of the mobile phase optimization exercise. Consequently, changes in the pH and other parameters of the mobile phase have been used to optimize the assay condition, and hopefully to gain a better control of the separation for these amine compounds under the method conditions developed.

Materials and Methods

Reagents and materials

HPLC grade acetonitrile, methanol, glacial acetic acid, and reagent grade potassium phosphate dibasic were obtained from J.T. Baker (Phillipsburg, NJ). HPLC grade triethylamine (TEA)

was obtained from Pierce Chemical (Rockford, IL). Pseudoephedrine hydrochloride standard was obtained from Ganes Chemicals (New York, NY). Other reference standards were obtained from the USP and NF (Rockville, MD).

Equipment

The HPLC system used in this study consisted of two Beckman Model 112 pumps and a Beckman Model 165 variable wavelength UV/VIS detector (San Ramon, CA). Automatic injection was performed with a Waters WISP 710B autosampler (Milford, MA). Integration was accomplished with a Beckman Model 450 Data System (San Ramon, CA).

Chromatographic conditions

The chromatographic column was 15 cm in length, 4.6 mm i.d., and packed with Sepralyte CN 5 μm size particles (Analytichem Int., Harbor City, CA). The mobile phase consisted of 75 parts by volume of acetonitrile, 10 parts by volume of methanol, and 15 parts by volume of aqueous buffer solution. The buffer solution was 5 mM potassium phosphate dibasic, 59 mM TEA, adjusted to pH 5.3 with glacial acetic acid. The flow rate was set at 2.0 ml/min with a typical back pressure of 1400 psi. 20 μl of the standard and sample preparations were injected onto the column and the analytes were detected by UV at a wavelength of 262 nm, and a sensitivity setting of 0.03 AUFS. Under these conditions, typical retention times are approximately 4.0, 4.9 and 5.7 min for pseudoephrine hydrochloride, doxylamine succinate and dextromethorphan hydrobromide, respectively.

Preparations of standards

Accurately weigh about 250, 31, and 125 mg of **III, IV**, and **V** analytical standards into the same 100 ml volumetric flask. Add water to dissolve the analytical standards, fill to volume with more water and mix well. This is the standard stock solution. Into 3 separate 50 ml volumetric flasks, pipette 3, 4, and 5 ml of the standard stock solution. Fill each flask with water to volume and mix well. These are the 80, 100 and 120% level working standard mixture solutions.

TABLE 1

Method precision for pseudoephedrine –HCl (III) doxylamine succinate (IV) and dextromethorphan –HBr (V) in a cough-cold liquid formulation

	Day	III	IV	V
	1	59.8	7.67	31.5
	1	59.9	7.64	31.3
	1	59.3	7.65	31.1
Mean ($n = 3$)		59.7	7.65	31.3
% CV (within-day)		0.5	0.2	0.6
	2	59.4	7.48	30.0
	2	59.3	7.52	30.0
	2	59.5	7.53	29.8
Mean ($n = 3$)		59.4	7.51	29.9
% CV (within-day)		0.2	0.2	0.4
	3	59.2	7.51	29.9
	3	59.0	7.47	29.8
	3	59.1	7.47	29.4
Mean ($n = 3$)		59.1	7.48	29.7
% CV (within-day)		0.2	0.3	0.9
Mean ($n = 9$)		59.4	7.55	30.3
% CV (total)		0.6	1.2	2.9

Values give mg found/30 ml.

Preparations of samples

Carefully pipette 10 ml of a representative sample with a 10 ml T.C. pipette and transfer it into a 100 ml volumetric flask. Rinse the inside wall of the T.C. pipette to ensure quantitative transfer of 10 ml sample. Add water to dilute the sample and make up to the volume with more water, mix well. Centrifuge a portion of this sample preparation for HPLC injections.

Results and Discussion

Method and validation

Nine sample solutions were prepared from a typical cough-cold liquid formulation. These solutions were assayed in sets of 3 on separate days, and each set assayed with an independent set of fresh standards. These constituted a 3×3 precision study. Table 1 shows that the within-day precision is less than 1% (CV) and the between-day precision as indicated by the total CV of 0.6%, 1.2% and 2.9% for amine drugs **III, IV** and **V**,

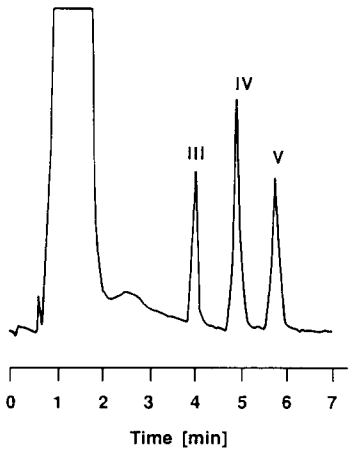


Fig. 2. Typical sample chromatogram of a commercial cough-cold liquid formulation. Acetaminophen and formulation excipients elute before **III, IV** and **V**.

respectively. Typical sample and placebo chromatograms are shown in Figs. 2 and 3. The accuracy was determined by wet spiking a set of cough-cold liquid placebos with amine drugs **III, IV**, and **V** at approximately 80%, 100%, and 120% of the normal dosage level. Three sets of spiked placebo liquids were prepared over the 3 concentration levels, with each set assayed on separate days with an independent set of standards. These constituted a 3×3 matrix study. Table 2 shows that good mean recoveries of 99.6%, 98.2% and 101.4% were obtained for amine drugs **III, IV**, and **V** respectively. The method is linear from 10% to 200% of the normal working concentration levels based on peak area responses. The detection limits are 4 μg/ml, 0.5 μg/ml and 2 μg/ml for amine drugs **III, IV**, and **V**, respectively, at the specified chromatographic conditions and based on a signal-to-noise ratio of three. Separation from chemically similar compounds illustrates the specificity of the method (see Fig. 4). This method has been used and found to be repeatable and reproducible by two different analysts in our laboratory for over a year. Our experience indicated that about 1500 injections of sample solutions can be expected from a typical analytical column.

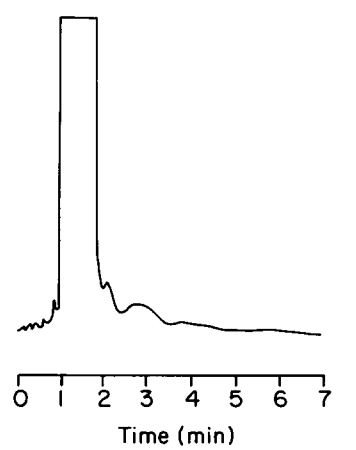


Fig. 3. Placebo chromatogram of a commercial cough-cold liquid formulation, showing no peaks detected in the region where **III, IV** and **V** elute.

TABLE 2

Recovery data based on 3-level spiking of placebos with pseudoephedrine·HCl (III), doxylamine succinate (IV) and dextromethorphan·HBr (V)

	Day	III	IV	V
80	1	100.8	99.5	102.9
100	1	101.0	99.3	103.0
120	1	100.3	98.5	101.7
Mean (n = 3)		100.7	99.1	102.5
% CV (within-day)		0.4	0.5	0.7
80	2	99.6	98.8	101.2
100	2	99.3	98.9	101.3
120	2	98.8	97.1	100.0
Mean (n = 3)		99.2	98.3	100.8
% CV (within-day)		0.4	1.0	0.7
80	3	99.9	97.2	101.2
100	3	98.8	97.5	101.3
120	3	98.3	97.2	99.7
Mean (n = 3)		99.9	97.3	100.7
% CV (within-day)		0.8	0.2	0.9
Mean (n = 9)		99.6	98.2	101.4
% CV (total)		1.06	1.11	1.26

Values give % recovery.

Mobile phase optimization

The effects of phosphate concentration, triethylamine concentration, pH and organic concentration on the capacity factors (k') of the 6 active components were systematically studied by changing one of these parameters in turn while keeping the others constant. Thus, the phosphate buffer

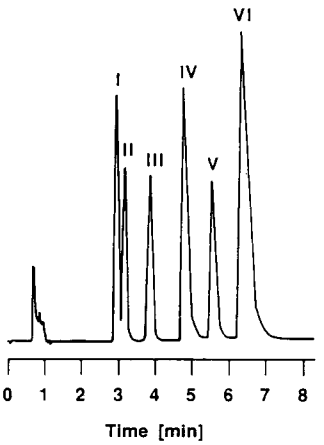


Fig. 4. Method specificity as shown by separation of compounds of similar type. (See Fig. 1 for compound identification.)

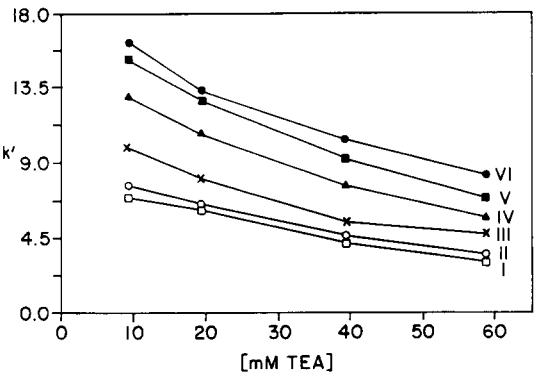


Fig. 6. Capacity factors as a function of triethylamine concentration. Mobile phase is acetonitrile : methanol : buffer (75 : 10 : 15). Buffer consists of 5 mM phosphate, various concentrations of TEA, and is finally adjusted to pH 5.3 with glacial acetic acid.

concentration in the aqueous solution of the mobile phase was studied at 1, 5 and 9 mM, while keeping the acetonitrile and methanol concentrations at the constant levels of 75 and 10% (v/v) respectively. Similarly, the TEA concentration was kept constant at 29.6 mM and the pH maintained at 5.3 in all cases. This is illustrated clearly in Fig. 5. No major changes in the k' values of the 6 amine drugs were observed. Triethylamine is added to the mobile phase to improve the peak shape of these amine drugs by reducing the peak tailing phenomenon. The effect of TEA concentration on k' values was studied at 10, 20, 40 and 59 mM while keeping the other parameters constant at 5 mM phosphate buffer, 75% acetonitrile, 10% methanol and pH 5.3. Significant decrease in the k' values of all 6 amine drugs were found as the concentration of TEA was increased. This is illustrated in Fig. 6. In the case of the pH study, the pH of the aqueous solution of the mobile phase was adjusted with glacial acetic acid while keeping all other parameters constant (see Fig. 7). It should be pointed out that dramatic changes were observed in the k' values and the elution orders of these 6 amine drugs. From the plot in Fig. 7, it is fair to conclude that the best resolution for these 6

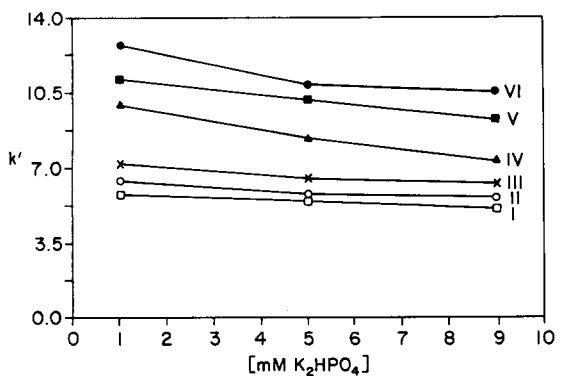


Fig. 5. Capacity factors as a function of phosphate buffer concentration. Mobile phase is acetonitrile : methanol : buffer (75 : 10 : 15). Buffer consists of 29.6 mM TEA, phosphate at various concentrations, and is finally adjusted to pH 5.3 with glacial acetic acid.

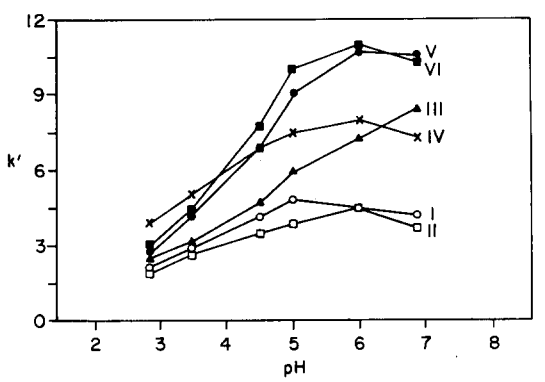


Fig. 7. Capacity factors as a function of pH. Mobile phase is the same as those for Fig. 5 with 5 mM phosphate and various pH adjustments using glacial acetic acid.

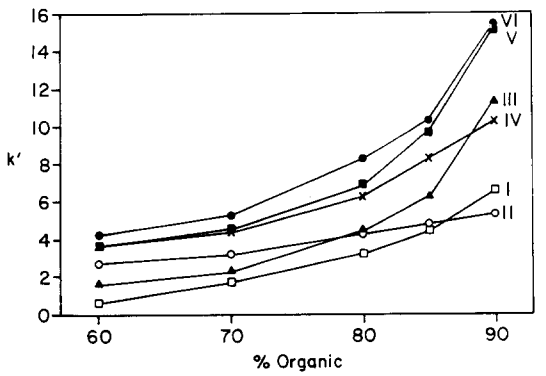


Fig. 8. Capacity factors as a function of organic solvents concentration. The ratio of acetonitrile to methanol is maintained at 75 : 10. The buffer consists of 5 mM phosphate, 29.6 mM TEA, adjusted to pH 5.3 using glacial acetic acid.

amines occurs around pH 5. As shown in Fig. 8, the effect of organic concentration on k' was studied from 60% to 90% (v/v) with the TEA concentration in the aqueous buffers being kept constant. It should be noted that the ratio of acetonitrile to methanol was kept constant at 75 : 10 in all mobile phases. This was accomplished by premixing these two organic solvents and pumping the resulting organic solution in one pump. The aqueous solution was prepared, kept constant and pumped by another pump. By programming the flow rates of these two pumps, the desired concentration of organic solution was varied from 60% to 90% systematically and efficiently. It should be pointed out that the ionic strength was not kept constant during these optimization experiments.

Figs. 5–8 illustrate the effect of changes in mobile phase composition on the capacity factors of 6 amine drugs, **I–VI**. Clearly, changes in the TEA concentration and the hydrogen ion concentration or the pH of the mobile phase will have a significant effect on the capacity factors and the resolution of these amine drugs. As a rule of thumb, most aliphatic amines (primary, secondary and tertiary) have pK_a values between 9.5 and 11.0 while those of aromatic amines are between 5 and 7. When the "observed pH" of the mobile phase is equal to the pKa of the amine, i.e. typically about 5 for an aromatic amine and about 9 for an aliphatic amine, a given sample solute becomes 50% in the ionic form (Yost, Ettre and Conlon, 1980). With regard to the effect of organic solvents in the mobile phase, our observation that the k′ values increase as a function of organic solvent content in the mobile phase is opposite to those usually found in a reversed-phase HPLC system. However, we are not totally surprised that this phenomenon exists. One possible and logical explanation is as follows. As discussed above, the retention of each amine, in general, increases as the pH increases. Further, we know that the "observed pH" of a mobile phase increases as the percent of organic solvent content increases. Also as the percent of organic solvent increases in the mobile phase, the 'effective concentration' of TEA in the mobile phase decreases. This is because the TEA concentration is constant only in the aqueous buffers. From Fig. 6, it is obvious that as the 'effective concentration' of TEA decreases, the k' values of these amine drugs increase. This contributes partly to the increase in retention of these amines as the percent of organic solvent increases. Since we don't have enough data to support an interpretation of the mechanism(s) of separation of these amines in the cyano propyl-bonded phase, we would prefer not to speculate it at this time.

Conclusion

In conclusion, a rugged, rapid, and stability-indicating HPLC method has been developed and validated for amine drugs **III, IV**, and **V** in a complex cough-cold formulation. The method can be optimized for maximum resolution and minimum elution time for a variety of amine drugs in various matrices by adjusting the key mobile phase parameters such as pH, TEA and the organic solvent concentrations.

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