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Note

Simultaneous determination of chlorpheniramine and diphenhydramine in cough syrups by reversed-phase ion-pair high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) has become a powerful tool for the analysis of pharmaceutical products. Cough and cold syrups are usually complex mixtures containing several active ingredients including antihistamines, decongestants, analgesics, preservatives, dyes and flavours. A number of conventional methods such as base extraction or non-aqueous titration[1,2], gas–liquid chromatography (GLC)[3–5], UV spectroscopy[6–8] and combined methods involving thin-layer chromatography (TLC) and spectrophotometry[9,10] have been applied to the determination of cough–cold amines. Spectrophotometric, GLC or methods involving TLC separation when applied to cough mixtures can be lengthy and/or subject to interferences from the matrix of the sample, and they are generally not suitable for simultaneous assay. Ion-pair reversed-phase high-performance liquid chromatography (HPLC) has become a widely used technique for the determination of both acidic and basic pharmaceutical products. The theory and application of the technique have been reviewed[11–13]. HPLC methods have also been reported for several cough–cold preparations[14–18]. A number of HPLC methods have been applied to the determination of chlorpheniramine in plasma[19–21]. A method was developed for the rapid quantitative analysis of chlorpheniramine in plasma, saliva and urine using HPLC on a reversed-phase column[22]. Urinary excretion of chlorpheniramine in humans[23] and in children[24] has been determined by HPLC. The separation of diphenhydramine and some of its metabolites was achieved with appropriate variation of the pH, alkali metalhalide concentration and cation and/or anion species in the mobile phase[25]. The determination of diphenhydramine using ion-pair HPLC with addition of salt to the mobile phase has been reported[26]. 2-Naphthyl chloroformate was found to be a suitable fluorescent reagent for the determination of diphenhydramine[27]. Reversed-phase, ion-pair and competing-base HPLC was used for the determination of cough–cold ingredients, including chlorpheniramine[28].

Although chlorpheniramine has been determined[29] in a cough mixture containing diphenhydramine, the simultaneous assay of these two cough–cold amines has not yet been reported. This paper describes a rapid and accurate ion-pair re-

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versed-phase HPLC method for the simultaneous determination of chlorpheniramine and diphenhydramine, which frequently occur in combination in cough–cold syrups.

EXPERIMENTAL

Apparatus

A Spectra-Physics Model SP 8000 B chromatograph controlled by a microprocessor that allows the selection of constant pressure or constant flow for quantitation, with an automatic injector and also having provision for manuel injection, a dual-channel plotter/printer and Model SP 8440 UV–VIS variable-wavelength detector was used. A Spectra-Physics 25 cm × 4 mm I.D. stainless-steel column packed with octylsilane chemically bonded to silica gel (10 μm) was used. The UV detector was set at 254 nm (0.04 a.u.f.s.).

Mobile phase

Methanol (960 ml) was added to dioctyl sodium sulphosuccinate (12 g) with constant stirring, followed by addition of distilled water (435 ml), tetrahydrofuran (210 ml) and 4.8 ml of 85% phosphoric acid. The pH was adjusted to 2.5 ± 0.05 with ammonia solution. The solution was filtered through a 0.45-μm FH filter prewetted with methanol. The solution was thoroughly degassed in an ultrasonic bath. The chromatography was conducted at ambient temperature at a flow-rate of 2.0 ml/min.

Chemicals and reagents

Methanol was purchased from Merck (India) and was glass distilled. Tetrahydrofuran (THF) and phosphoric acid were obtained from IDPL (India) and the former was glass distilled before use. Ammonia solution was a product of Merck (India). Dioctyl sodium sulphosuccinate was purchased from Fluka (Switzerland). Standard samples of diphenhydramine hydrochloride and chlorpheniramine maleate used for the preparation of the calibration graphs and in synthetic formulations were obtained from Park Davis (India) and the Central Drugs Laboratory (India), respectively.

Preparation of standards

A set of ten standard solutions each for diphenhydramine hydrochloride and chlorpheniramine maleate was prepared containing 1.2-0.12 mg/ml of active ingredient, and were stored at 20°C in the dark in air-tight flasks.

Assay procedure

The instrument was set as indicated previously and the column was equilibrated for 20 min with the mobile phase flowing. Before being subjected to HPLC analysis, each commercial cough–cold syrup was appropriately diluted and filtered through a sample clarification kit with 0.5-μm filters. The filtered solution (75 μl) was injected into the chromatograph with a 250-μl syringe. The concentrations of chlorpheniramine and diphenhydramine in different syrup samples were determined by comparison with a calibration graph for each antihistamine (Fig. 1) of peak height *versus* amount of compound injected.

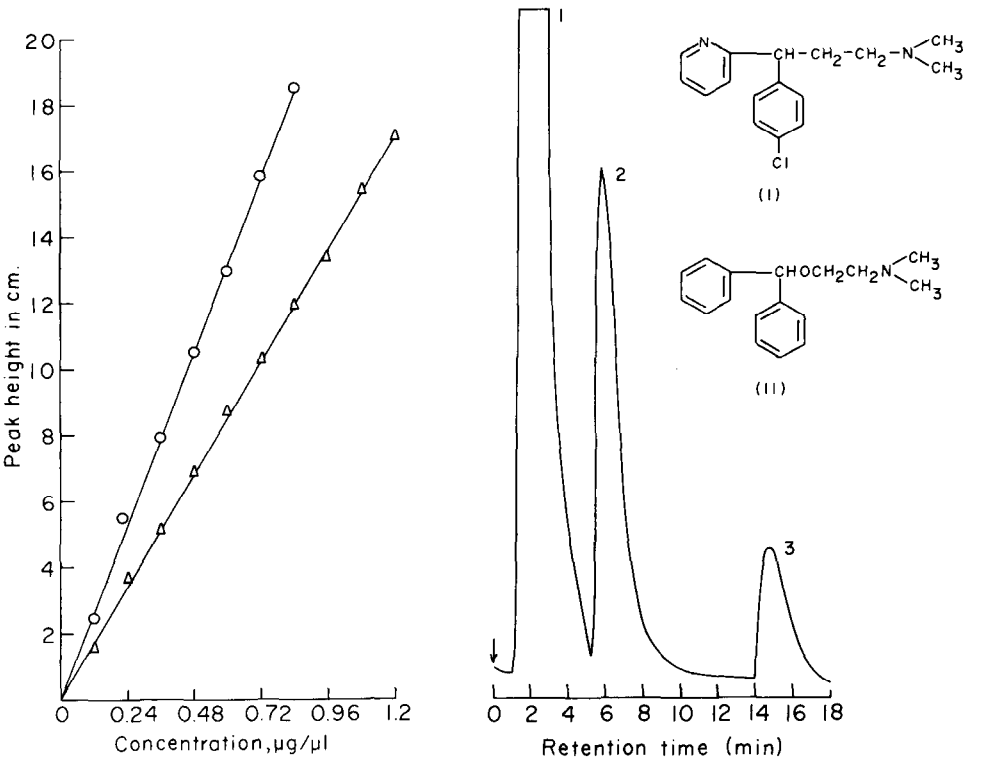

Fig. 1. Calibration graphs for chlorpheniramine (○) and diphenydramine (△).

Fig. 2. Representative chromatogram from a cough–cold syrup. Peaks: 2 = diphenhydramine; 3 = chlorpheniramine. Conditions: Column, RP-8 (25 cm × 4 mm I.D.); mobile phase, as described under Experimental; flow-rate, 2 ml/min; pressure, 1350 p.s.i.; detection, UV (254 nm) (0.04 a.u.f.s.); chart speed, 0.5 cm/min; injection volume, 75 µl. Structures: I, chlorpheniramine; II, diphenhydramine.

RESULTS AND DISCUSSION

A representative chromatogram from a cough syrup containing diphenhydramine hydrochloride and chlorpheniramine maleate is shown in Fig. 2. The detector response was linear for both compounds between 50 and 140% of the theoretical sample level and the deviations of the points from linearity for the compounds were $\leqslant 3.5\%$. The theoretical sample levels of diphenhydramine hydrochloride and chlorpheniramine maleate for manufacturers A, B and C were 2.5, 14.5; 2.5, 9.5; and 2.0, 10.0 mg per 5 ml, respectively.

For the determination of recoveries, synthetic formulations were prepared containing 75, 100 and 125% of the theoretical amount of diphenhydramine hydrochloride and chlorpheniramine maleate. The assay results are summarized in Table I. The reproducibility of the method was determined for both the compounds on the basis of five replicate measurements of a synthetic syrup formulation. The coefficient of variation (C.V.) was 1.19% for diphenhydramine hydrochloride and 0.84% for chlorpheniramine maleate.

The HPLC method was used to assay ten diphenhydramine hydrochloride–

TABLE I

RECOVERY OF DIPHENHYDRAMINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE FROM SYNTHETIC FORMULATIONS

Amount added as % of theoretical	*Recovery (%)**	
	Diphenhydramine hydrochloride	*Chlorphenhydramine maleate*
75	100.9	98.2
100	102.5	99.4
125	99.4	99.8
Mean	100.93	99.13
Standard deviation	1.2	0.83
C.V. (%)	1.19	0.84

* Calculated from five replicate determinations.

chlorpheniramine maleate syrups representing three different manufacturers. The results are summarized in Table II. The retention times under the conditions for diphenhydramine hydrochloride and chlorpheniramine maleate were 6.8 and 14.9 min, respectively. Dioctyl sodium sulphosuccinate was added to the mobile phase as a better separation of the peaks was achieved. Although the peaks corresponding to the two compounds could be satisfactorily separated, tailing of the chlorpheniramine maleate peak presented a serious problem. However, addition of tetrahydrofuran to

TABLE II

RESULTS OF ASSAYS (% OF DECLARED VALUE) OF DIPHENHYDRAMINE HYDROCHLORIDE (DH) AND CHLORPHENIRAMINE MALEATE (CM) IN COMMERCIAL SYRUP PREPARATIONS

Absolute amounts of diphenhydramine hydrochloride and chlorpheniramine maleate present in the formulations from manufacturers A, B and C were 2.5, 14.5; 2.5, 9.5; and 2.0, 10.0 mg per 5 ml, respectively.

Sample	*Manufacturer A*		*Manufacturer B*		*Manufacturer C*	
	DH	*CM*	*DH*	*CM*	*DH*	*CM*
1	100.1	99.0	102.3	98.4	101.5	100.2
2	100.8	98.7	100.6	98.6	101.7	98.5
3	101.6	100.2	101.5	99.2	100.8	98.7
4	99.5	98.5	101.3	100.5	101.2	99.4
5	102.1	100.4	100.2	98.4	100.7	98.2
6	101.5	99.6	99.7	98.5	99.8	99.1
7	98.7	100.5	100.4	99.1	100.4	100.3
8	100.3	98.2	100.1	99.0	101.1	100.7
9	102.0	99.2	99.5	98.2	99.7	98.8
10	99.8	98.8	102.0	99.3	100.5	100.4
Mean	100.64	99.31	100.80	98.92	100.74	99.43
Standard deviation	1.05	0.82	0.96	0.67	0.66	0.90
C.V. (%)	1.09	0.82	0.95	0.67	0.65	0.90

the mobile phase considerably improved the peak shape and reduced tailing. The coefficient of variation of the method for the syrup samples was $\leqslant 1.09\%$ for diphenhydramine hydrochloride and $\leqslant 0.90\%$ for chlorpheniramine maleate (Table II).

In conclusion, the described method is a rapid and accurate procedure for the simultaneous determination of diphenhydramine hydrochloride and chlorpheniramine maleate present in cough–cold syrups with potential for application to other cough–cold preprations containing similar ingredients.

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