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HPLC ASSAY FOR BASIC AMINE DRUG IN PLASMA USING A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE -- APPLICATION IN A PILOT BIOAVAILABILITY STUDY OF CHLORPHENIRAMINE CONTROLLED-RELEASE DOSAGE FORM

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ABSTRACT

A high performance liquid chromatographic (HPLC) method that involves the use of a silica gel column and an aqueous mobile phase for simultaneous separation of chlorpheniramine, pseudoephedrine and terfenadine in plasma is presented. Alkalized samples are cleaned by extraction with n-hexane, and the extraction is followed by evaporating the solvent and reconstituting the residue in a small amount of mobile phase. An aliquot of this solution is analyzed by a HPLC system with a silica gel column, an aqueous mobile phase containing 55% CH_3CN and 45% $(NH_4)H_2PO_4$(pH 4.0), and UV detection at 210 nm. The low detection limits of the method in plasma are 1 ng, 4 ng and 0.5 ng for chlorpheniramine, pseudoephedrine and terfenadine, respectively. In this study, terfenadine acts as an internal standard. The coefficient of variance

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on the results of intraday and interday precision and the accuracy on control samples of chlorpheniramine and pseudoephedrine were all within 10%. We have used this method successfully in a pilot bioavailability study of a newly developed controlled-release formulation.

INTRODUCTION

The group of amines used as antihistamines, antititussives, and decongestants are frequently formulated together for the symptomatic relief of the common cold, nasal allergies, acute rhinitis and rhinosinusitis. However, basic amine drugs frequently cause problems when analyzed by using reversed-phase column because of their interaction with acidic silanol groups on the surface of the stationary phase (1). On the other hand, the unbound silica gel, with the maximum concentration of surface silanol groups, is a preferable stationary phase for these compounds. The use of an aqueous mobile phase on a silica gel column has been utilized to determine several amine drugs in plasma (2-10) and in the pharmaceutical dosage form (11).

Chlorpheniramine maleate, an antihistamine, and pseudoephedrine hydrochloride, a nasal decongestant are often used together in some cough-cold-allergy dosage form. High performance liquid chromatographic (HPLC) method for determination of chlorpheniramine and pseudoephedrine in pharmaceutical dosage forms (11-13) and in urine (14) have been reported. None of the reported HPLC methods is applicable for the simultaneous determination of these two basic amine drugs in plasma. Terfenadine is a relatively new H_1-histamine receptor antagonist which has less CNS effects than other antihistamines (15-17). The radioimmunoassay, with a detection limit of 0.2 ng/ml, has been used to quantity terfenadine in plasma (16,18). The HPLC method for determination of terfenadine in plasma was not available in literature, except in unpublished data mentioned in an review article (16).

In the present study, an increasingly notable technique using a bare silica gel column run with an aqueous mobile phase to separate drugs in

biological fluids was employed. A simple, selective and sensitive HPLC method for quantization of chlorpheniramine, pseudoephedrine and terfenadine in plasma is presented in here. We have successfully applied this method to a pilot bioavailability study of newly developed chlorpheniramine controlled-release pellets in three normal healthy volunteers.

MATERIALS AND METHODS

Materials: Chlorpheniramine maleate was purchased from In-Fa company (Milan, Italy), pseudoephedrine hydrochloride from Gane-S company (U.S.A), and terfenadine from the Farmos-Group company (Finland). The controlled-release chlorpheniramine pellets was formulated and prepared using Furend (Japan) CF-granulator (Kingdom, Pharmaceutical Co., Taiwan, R.O.C.). The reference chlorpheniramine tablet was purchased from Veterans Pharmaceutical Plant (Lot T8102, Taiwan R.O.C.). The analytical grade ammonium dihydrogen phosphate was purchased from Merk (Darmastadt, F.R.G.). Acetonitrile and n-hexane were HPLC grade (MallincKrodt, Kentucky, U.S.A.). Water was prepared through a Mill-RO 60 water purification system (Millipore, Bedford, MA, U.S.A.). All other reagents were analytical or reagent grade.

Apparatus: The HPLC system consisted of a pump (Model 510, Water assoc., CA, U.S.A.), an automatic sample processor (Autosampler 23, SIC, Japan), a 10-μm uporasil Si column, 3.9 mm x 30 cm (Waters Assoc., CA, U.S.A.), a column oven (Model LC-22A, BAS, U.S.A.), a variable-wavelength UV detector (Model 783A, Kratos, Ramsey, NJ, U.S.A.) and an integrator (model data module 740, Waters Assoc., CA, U.S.A.).

Chromatographic Conditions: The mobile phase was prepared by dissolving 207 mg of ammonium dihydrogen phosphate in 1800 ml of water and adjusting the pH to 4.0 by adding phosphoric acid. This solution was then mixed with 2200 ml of acetonitrile. The solution was saturated with silica gel packing materials, then degassed and filtered through a 0.45 m membrane filter before use. The flow rate was 1.0 ml/min,

and a column oven was thermostated at 45 °C. The UV detector wavelength was set at 210 nm.

Sample Preparation: To one ml plasma or serum spiked with chlorpheniramine maleate and pseudoephedrine hydrochloride in a 20 ml screw-capped tube were add 50 µl of 1 µg/ml terfenadine as internal standard and 250 µl of 5% aqueous potassium hydroxide. Five ml of n-hexane was then added followed by a 30 min rotation and centrifugation at 2000 g for 10 min. The organic layer was transferred to a clean test tube and evaporated to dryness with a stream of filtered compressed dry air. The sample was reconstituted with 300 µl mobile phase. A portion of 200 µl of the reconstituted solution was injected into HPLC apparatus.

Assay Validation and Recovery: Precision and accuracy were determined by constructing the within-day (n=3) and between-day standard curve (n=6). To assess the precision and accuracy of the within-day assay, five extractions of plasma sample in low and high concentrations, 2 and 30 ng/ml for chlorpheniramine and 20 and 200 ng/ml for pseudoephedrine, were performed on a single day. The between-day standard curve was carried out for two weeks. The mean peak height ratio used throughout this study was calculated by dividing the chlorpheniramine and pseudoephedrine peak height by the terfenadine peak height in plasma. The overall extraction recovery (ER) of chlorpheniramine, pseudoephedrine and terfenadine from plasma was calculated from the following equation :

$$ER = \frac{PHp}{PHs} \times 100\%$$

where PHp is the peak height of chlorpheniramine, pseudoephedrine or terfenadine in plasma, and PHs is the peak height of equivalent amount of chlorpheniramine, pseudoephedrine or terfenadine in stock solution without extraction.

Application in a Pilot Bioavailability Study of Chlorpheniramine Controlled-release Pellets: The comparison of chlorpheniramine maleate controlled-release pellets and the reference tablet in three normal healthy male Chinese volunteers was performed with a one week washout period. All subjects were shown by medical examination to be in good physical condition with normal blood and urine chemistry. Informed consent was obtained from all subjects. Each volunteer was instructed to refrain from taking any medication for one week prior to and during the studies. The subject fasted 10 hr prior to the drug administration. Each subject received either one controlled-release capsule containing 8 mg chlorpheniramine maleate pellets or a 4 mg conventional tablet q6h, twice with 200 ml of water. Blood samples were collected from a catheter in the forearm vein immediately before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 25, 28, 33, 48 hr. Serum was obtained after centrifugation, and all serum samples were stored at -20 ^{o}C until HPLC assay.

RESULTS AND DISCUSSION

Typical chromatograms for blank plasma and for plasma spiked with drugs are presented in Figure 1. The retention times for chlorpheniramine, pseudoephedrine and terfenadine were approximated 6.5, 8.5, and 10.6 min, respectively. In the system consisting of bare silica gel with an aqueous mobile phase, the retention mechanism results mainly from ion-exchange characteristics and only partially from lipophilic properties.[1] Endogenous non-ionic neutral lipid compounds and anionic compounds in biological fluids will not be retained on the silica gel column. Thus, the interfering substances in biological fluids are eluted at the solvent front, leaving a clean baseline around the drug retention time, even at a UV wavelength of 210 nm, as shown in Figure 1. Additionally, this HPLC system successfully and simultaneously separates chlorpheniramine, pseudoephedrine and terfenadine in plasma, each of which can be the internal standard of the other. In this study, terfenadine was chosen as the internal standard to the assay system.

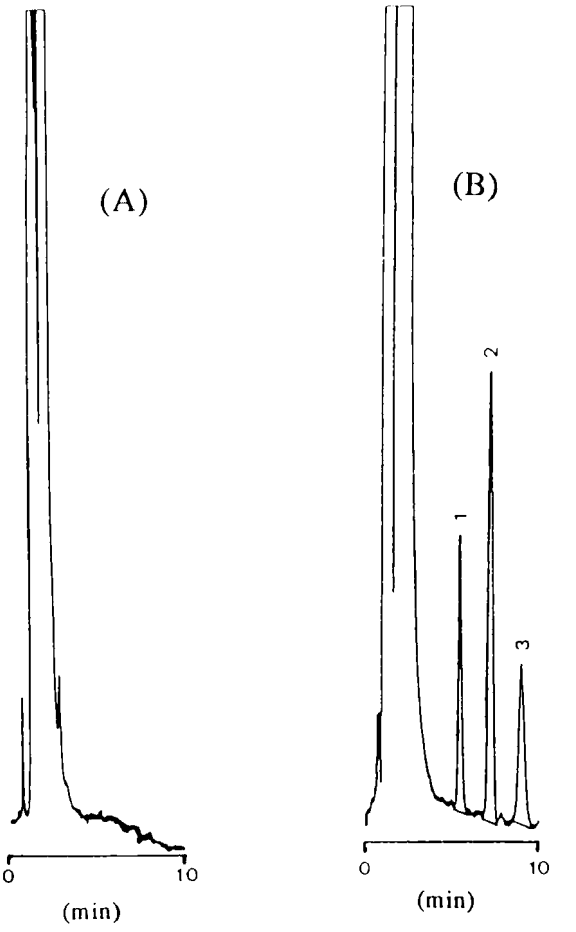

FIGURE 1

Chromatograms of (A) blank plasma and (B) plasma spiked with (1) terfenadine, internal standard, (2) pseudoephedrine and (3) chlorpheniramine using silica gel column at UV-210 nm.

The standard curves of both within-day (Figure 2) and between-day (Figure 3) using nine different concentrations, 1-50 ng/ml for chlorpheniramine and 10-400 ng/ml for pseudoephedrine in plasma, were obtained by plotting the ratio of the peak height verse concentration. Over these concentration ranges, the standard curves were linear for both amine drugs. The correlation coefficients were both higher than 0.999, and

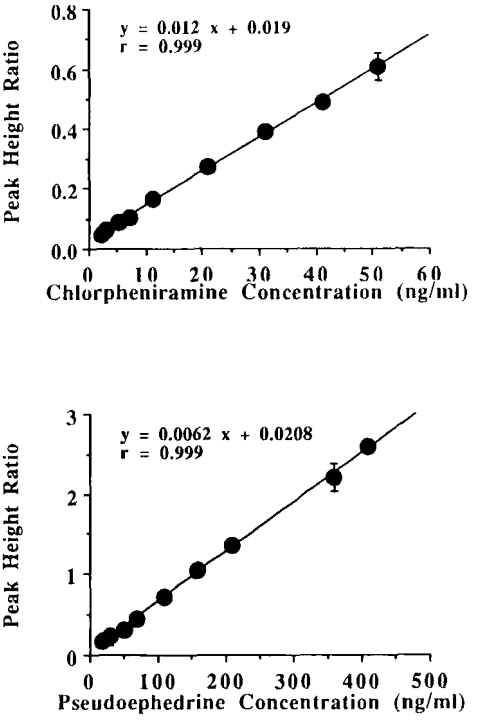

FIGURE 2

Within-day standard curve of chlorpheniramine and pseudoephedrine (n=3).

the linearity of response was determined by least-square analysis of data points. The coefficients of variation (CV) of within-day and between-day assay for above concentration ranges were all within 10 %.

The accuracy was confirmed by determining spiked samples using the calibration curve. The results are presented in Table 1. The maximum error for chlorpheniramine and pseudoephedrine both at low and high concentrations were below 10%. The recoveries at four different concentrations in plasma averaged 91.41% for chlorpheniramine maleate (Table 2), and the low detection limit of chlorpheniramine in plasma was 1 ng. The high absorptivity of pseudoephedrine and terfenadine at 210 nm was sufficient to result in respective detection limits of 4 ng and 0.5 ng for

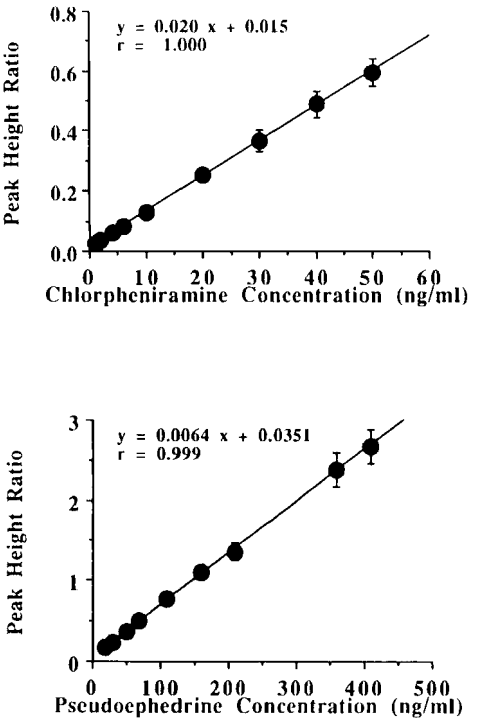

FIGURE 3

Between-day standard curve of chlorpheniramine and pseudoephedrine (n=6)

TABLE 1

Precision and Accuracy of Plasma Assay for Chlorpheniramine and Pseudoephedrine

Spiked Concentration (ng/ml)	Mean Observed Concentration (ng/ml), (n=5)	Standard Deviation	Percent Coefficient of Variation	Percent Error
Chlorpheniramine				
2	1.932	0.189	9.795	3.384
30	31.34	1.045	3.335	4.457
Pseudoephedrine				
20	20.42	0.086	4.209	2.121
200	216.0	1.439	6.666	7.972

TABLE 2

Absolute Recovery of Chlorpheniramine Maleate, Pseudoephedrine Hydrochloride and Terfenadine from Plasma

Chlorpheniramine		Pseudoephedrine		Terfenadine	
Concentration (ng/ml)	Percent Recovery	Concentration (ng/ml)	Percent Recovery	Concentration (ng/ml)	Percent Recovery
4	95.87	40	38.09	50	28.55
10	86.27	100	35.52	50	33.39
20	92.07	200	39.45	50	31.98
40	91.45	400	38.83	50	28.58
Mean	91.41		37.97		30.63
S.D.	3.950		1.730		2.450
CV%	4.320		4.550		8.010

S.D.: Standard Deviation

CV%: Percent Coefficient of Variation

pseudoephedrine and terfenadine, respectively, although the average recoveries of the method by extraction with n-hexane prior to injection were low for pseudoephedrine and terfenadine (Table 2).

In this HPLC system, the use of an unbound silica gel column, an aqueous mobile phase, and UV detection at 210 nm with only one step extraction yields satisfactory results for simultaneous determination of chlorpheniramine and pseudoephedrine in plasma samples. The method is simple, precise, accurate, sensitive and has been successfully used in a pilot bioavailability study of newly developed chlorpheniramine controlled-release pellets. Reliable measurements of chlorpheniramine concentrations in serum were obtained for up to 48 hours following oral doses of 4 mg of chlorpheniramine maleate conventional tablet taken at 6 hr intervals twice and 8 mg dose of controlled-release pellets, as shown for three volunteers in Figure 4. The tested controlled-release dosage form did show a lower peak concentration and prolong release characteristics.

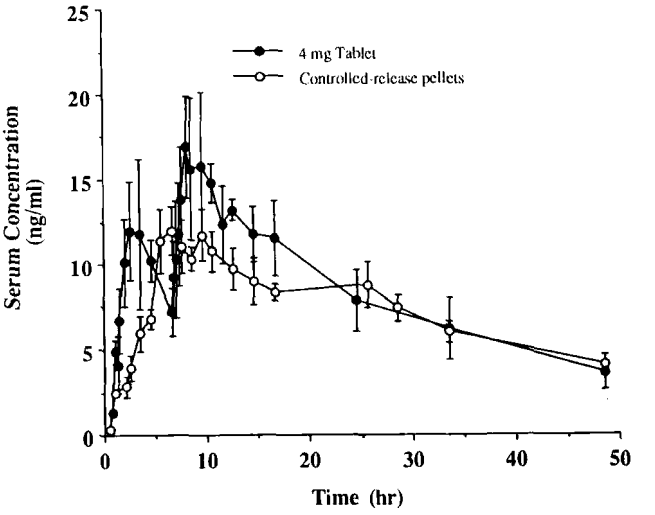

FIGURE 4

Mean serum concentration-time curve of 4 mg immediate release tablet at 0 and 6 hr and 8 mg controlled-release pellets of chlorpheniramine for three normal healthy volunteers.

The major difficulty of using an unbound silica gel column run with an aqueous mobile phase is that the silanol groups of the stationary phase may dissolve when a solvent system with a pH value above 7.0 is used. This risk can be minimized by saturating the mobile phase with silica. A lower pH value of the mobile phase can also stabilize the stationary phase, but it shortens the drug retention and may distort the separation. Under the conditions described in this paper, the column was stable, and no change in column efficacy was noted throughout the study.

CONCLUSIONS

We have developed a simple, selective and sensitive HPLC method by using a bare silica gel column run with an aqueous mobile phase for quantization of chlorpheniramine, pseudoephedrine and terfenadine in plasma is presented in here. We have also successfully applied this method

to a pilot bioavailability study of newly developed chlorpheniramine controlled-release pellets in three normal healthy volunteers.

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