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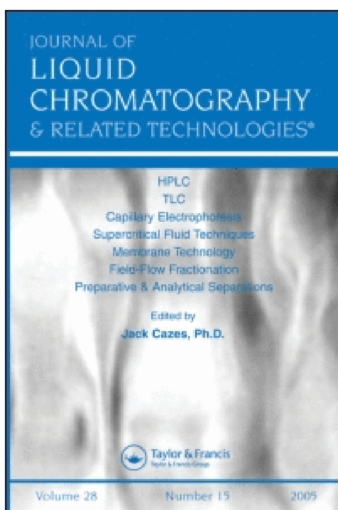

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR BASIC AMINE DRUGS IN PLASMA AND URINE USING A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE. II. CHLORPHENIRAMINE

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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 quantitation of chlorpheniramine in plasma and urine is presented. Alkalinized samples are cleaned by extraction with pentane (containing 1% CH_3CN), 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 an HPLC system with an Ultrasphere Si Column, an aqueous mobile phase at pH 7 containing 60% CH_3CN and 7.5 mM $(NH_4)_2HPO_4$, and UV detection at 200 nm. Although the average recovery of extraction is 58% ± SD 10%, the detection limit for the method is 0.7 ng/ml in plasma and 100 ng/ml in urine (s/n = 3) for 0.5 ml samples. The coefficients of variation (CV) on the results of samples run to measure interday and intraday precision and the bias on control samples were all 10% or less. We have used the method in a bioavailability study of a controlled release formulation involving over 1000 samples.

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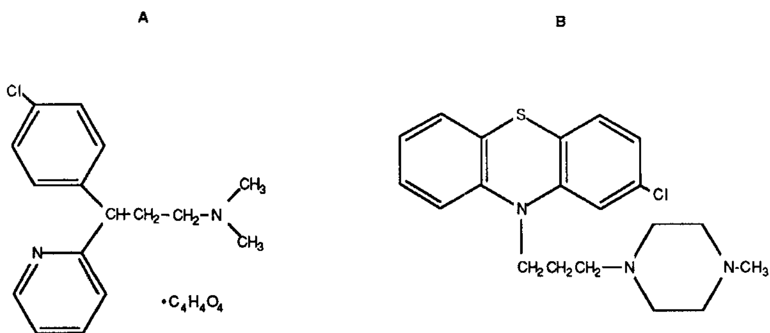

Figure 1: Chemical Structures of Chlorpheniramine Maleate and Prochlorperazine.

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INTRODUCTION

Chlorpheniramine maleate (Fig. 1), a potent antihistamine incorporated in a variety of pharmaceutical formulations, is widely used for relief of some symptoms of the common cold and allergic reactions. For pharmacokinetic and bioavailability studies, concentrations of this drug in plasma have been determined by gas chromatography (1), high performance liquid chromatography (HPLC) (2,3,4,5), GC-MS (6,7), and radioimmunoassay (3). The advantages of using a bare silica gel column run with an aqueous mobile phase have been demonstrated in our previous studies (8) in which a 0.5 ng/ml concentration of amiloride (8) and a 0.89 ng/ml concentration of metoclopramide (9) in plasma were easily detected in deproteined samples. In this system, the retention mechanism depends mainly on ion-exchange characteristics and only partly on lipophilic properties (10); endogenous neutral

lipid compounds and anionic compounds are not retained by the silica gel column. Since such interfering substances in biological fluids are eluted with the solvent front, the technique results in a cleaner base line, an improved signal to noise ratio (s/n) and a more sensitive detection of the drug. The advantages of this method are further demonstrated, where interfering peaks from plasma and urine samples would normally obscure a chlorpheniramine peak, by using UV detection at 200 nm.

MATERIALS AND METHODS

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Materials

Chlorpheniramine maleate (Sigma Chemical Co., St. Louis, MO) and prochlorperazine dimaleate (Smith, Kline & French Lab, Philadelphia, PA) were obtained commercially. Acetonitrile and pentane were HPLC grade (J.T. Baker Chemical Co., Phillipsburg, N.J.). Water was purified through a Nanopure apparatus (Barnstead Co., Boston, MA). All other reagents were analytical or reagent grade.

Apparatus

The HPLC system consisted of one pump (Model 110, Beckman Inc., San Jose, CA), an automatic sample processor (WISP 710B, Waters Associates, Milford, MA), a 5 μm Ultrasphere Si column, 4.6 mm X 25 cm (Beckman Inc., San Jose, CA), a variable wavelength UV detector (Kratos Analytical Instruments, Westwood, N.J.), and an integrator, Model 3390A, (Hewlett-Packard, Santa Clara, CA).

Chromatographic Conditions

The mobile phase was prepared by dissolving 3.96 g of dibasic ammonium phosphate in 1600 ml of water, adding 2400 ml of acetonitrile and adjusting the pH to 7.0 by adding phosphoric acid. The solvent was saturated with silica by pumping it through a silica gel column overnight, then was degassed and filtered before use. The flow rate was 1.0 ml/min. The UV detector wavelength was set at 200 nm and sensitivity was set at 0.002. The retention times for chlorpheniramine and prochlorperazine (internal standard) were 13.9 and 10.7 min, respectively (Fig. 2).

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Sample Preparation

The following were added to 0.5 ml plasma and urine samples: 100 μl of a saturated aqueous solution of sodium carbonate, 50 μl of the internal standard solution (prochlorperazine dimaleate) and 5 ml of pentane containing 1% CH_3CN. The mixture was rotated for 15 min at moderate speed and centrifuged at 3000xg for 10 min. The pentane layer was transferred and evaporated to dryness, and the residue was dissolved in 200 μl of mobile phase. Aliquots of 100 μl for plasma samples and 2 μl for urine samples were injected onto the column.

Recovery

Recovery of the drug from plasma samples was estimated by dividing the drug to internal standard peak height ratios of spiked samples that were extracted with pentane, blown down, and

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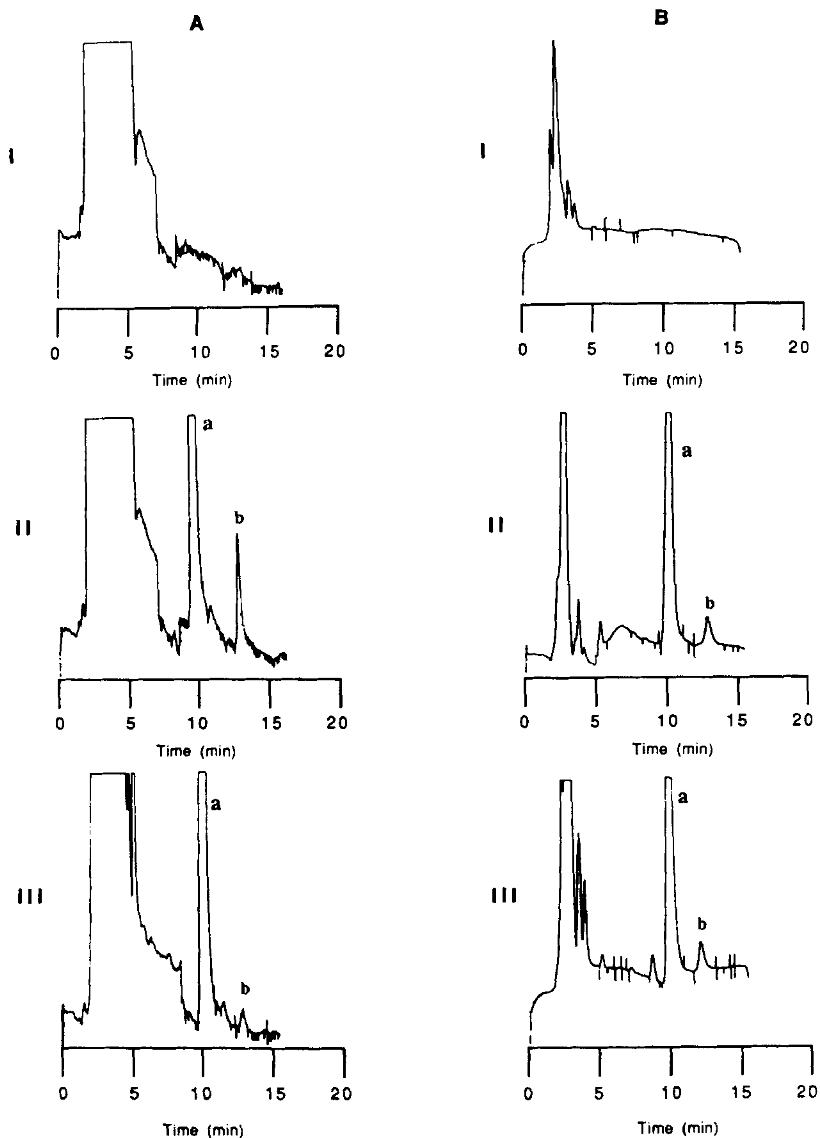

Figure 2: (Previous Page) HPLC Chromatograms of: A (I) Blank Plasma; A (II) Plasma Spiked to 16 ng/ml with Chlorpheniramine and with Internal Standard; A (III) Subject Plasma Sample Drawn at 24 Hours showing 2.26 ng/ml of Chlorpheniramine and Internal Standard; B (I) Blank Urine; B (II) Urine Spiked to 0.2 µg/ml with Chlorpheniramine and with Internal Standard; B (III) Subject Urine Sample for the 48-72 Hours Interval Showing 0.156 µg/ml of Chlorpheniramine and Internal Standard; (a) Internal Standard (Prochlorperazine) Peak; and (b) Chlorpheniramine peak.

reconstituted with mobile phase to the peak height ratios of aqueous solutions that had not been extracted. Samples for the recovery study were treated as described above, except that the internal standard was not added until the evaporation step.

RESULTS

Plasma and urine samples spiked with various amounts of chlorpheniramine maleate standard solution were analyzed. The linear relationship between the drug plasma concentration and the chlorpheniramine to internal standard peak height ratio for drug concentrations ranging from 0.70 to 28 ng/ml is given by the slope 0.0231, y-intercept -0.0003 and coefficiient of determination (r^2) 0.9985. For urine samples at concentrations from 0.10 to 6.0 µg/ml the linear relationship is given by the slope 0.3447, y-intercept -0.0117 and r^2 of 0.9998. Bias of control samples at four concentrations for urine (n=2) and plasma (n=6) remained at or below 10%. Precision of the method over the working range was determined by the analysis of replicate spiked samples. The interday and intraday precision was indicated by CV's of less than 10% for four different plasma (2.8 to 21 ng/ml) and urine (0.2 to 6.0 µg/ml) concentrations, as shown in Tables 1 and 2. The recoveries at four different concentrations averaged 58.3% with a standard deviation of 10%, as shown in Table 3.

DISCUSSION

Figure 2 shows, by the absence of interfering peaks near the drug peak, one of the advantages of using a silica gel column run

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TABLE I: PRECISION OF PLASMA ASSAY FOR CHLORPHENIRAMINE

SPIKED CONCENTRATION (ng/ml)	MEAN (n=6)	STANDARD DEVIATION	COEFFICIENT OF VARIATION
INTERDAY			
21.0	21.1	1.41	6.68
14.0	14.1	0.19	1.35
7.0	6.83	0.10	1.46
2.8	2.91	0.24	8.25
INTRADAY			
21.0	19.9	0.86	4.32
14.0	13.7	0.21	1.53
7.0	7.28	0.63	8.65
2.8	2.94	0.20	6.80

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with an aqueous mobile phase to separate basic amine drugs. According to the proposed retention mechanism (10), the silanol groups of the bare silica gel constitute the main engine for retention of compounds with cationic moieties such as drugs with basic primary, secondary, or tertiary amine groups. Consequently, neutral lipid compounds that would be retained by a C18 column and anionic compounds elute with the solvent front.

The major difficulty with this method, that the silica gel of a column dissolves when solvents with pH above 7.0 are used (11), is minimized when the mobile phase is saturated with silica.

TABLE II: PRECISION OF URINE ASSAY FOR CHLORPHENIRAMINE

SPIKED CONCENTRATION (μg/ml)	MEAN (n=6)	STANDARD DEVIATION	COEFFICIENT OF VARIATION
INTERDAY			
6.00	6.05	0.111	1.83
2.00	2.00	0.042	2.10
0.800	0.798	0.011	1.38
0.200	0.203	0.010	4.93
INTRADAY			
6.00	5.89	0.056	0.95
2.00	1.92	0.036	1.84
0.800	0.778	0.027	3.47
0.200	0.219	0.011	5.02

TABLE III: RECOVERY OF EXTRACTION (n=5)

Spiked Conc. (ng/ml)	Peak Height Ratio After Extraction	Peak Height Ratio Without Extraction	Percent Recovery
2.8	0.049	0.024	49.0
7.0	0.130	0.065	50.0
14.0	0.260	0.172	66.2
21.0	0.355	0.242	68.2
		MEAN =	58.3

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Also, if the mobile phase is recycled while the assay is running, silica saturation is easily maintained.

In this HPLC method the use of an unbonded silica gel column, an aqueous mobile phase, and UV detection at 200 nm yields satisfactory results for the determination of chlorpheniramine in plasma and urine samples. Although the average recovery of the method (by extraction with pentane containing 1% CH_3CN prior to injection) was only about 60% for plasma samples, the high absorptivity of the drug at 200 nm was sufficient to result in a detection limit of 0.7 ng/ml (s/n=3) for 0.5 ml plasma samples. The method is simple, precise, accurate, and sensitive and has been used in a bioavailability study of a controlled release chlorpheniramine formulation. Reliable measurements of chlorpheniramine concentration were obtained for up to 72 hours following four oral doses of 6 mg of chlorpheniramine maleate taken at 6 hour intervals and for a 24 mg dose of a sustained release formulation taken at 0 hour, as shown for one volunteer in Figure 3 for plasma. Data obtained from urine samples was used to determine rates of excretion as shown in Figure 4. Only 14.1 ± 8.0% (for 12 normal volunteers) of the chlorpheniramine was excreted unchanged by 72 hours following the four oral doses.

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The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby in-

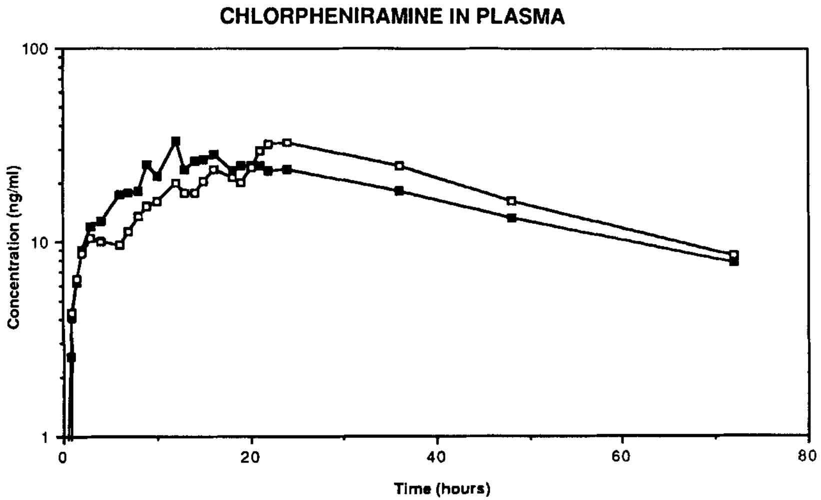

Figure 3: Chlorpheniramine Plasma Concentration - Time Profile for One Human Volunteer Given Chlorpheniramine Maleate: □ 6 mg at 0, 6, 12, and 18 hours and ■ 24 mg at 0 hour.

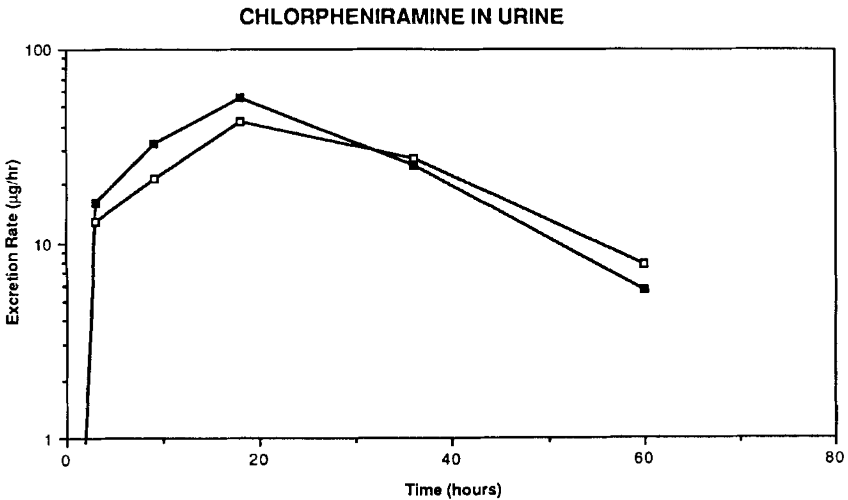

Figure 4: Chlorpheniramine Urine Excretion Rate - Time Profile for One Human Volunteer Given Chlorpheniramine Maleate: □ 6 mg at 0, 6, 12, and 18 hours and ■ 24 mg at 0 hour.

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terfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange (10) and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. Then, the interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug, even at a UV wavelength of 200 nm, as shown in Figure 2.

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