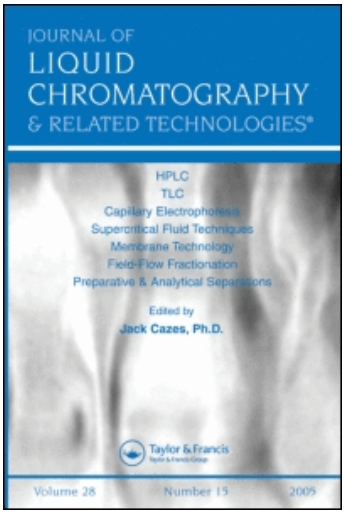


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# High-Performance Liquid Chromatographic Assay For Basic Amine Drugs in Human Plasma with A Silica Gel Column and an Aqueous Mobile Phase. IV. Albuterol

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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR BASIC AMINE DRUGS IN HUMAN PLASMA WITH A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE. IV. ALBUTEROL

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## ABSTRACT

A high performance liquid chromatographic (HPLC) assay is presented for the determination of albuterol in human plasma. Sample analysis is performed with a silica gel column and an aqueous mobile phase. Plasma samples are extracted with chloroform containing di(2-ethylhexyl) phosphate. Samples are back extracted into a hydrochloric acid solution. A fluorescence detector is used for this assay. The detection limit of the assay is 0.2 ng/ml for 0.5 ml plasma samples. This method has been used in a bioavailability study of albuterol sulfate tablets (4.8 mg) given to healthy volunteers.

## INTRODUCTION

Albuterol, 2(*tert*-butylamino)-1-(4-hydroxy-3-hydroxymethyl-phenyl)ethanol (Figure 1) is a relatively selective beta-adrenergic drug

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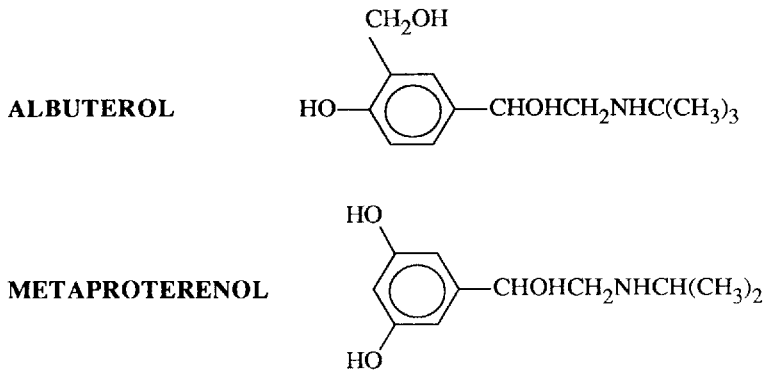


FIGURE 1: Structures of Albuterol and the Internal Standard, Metaproterenol.



which has been widely used for treatment of respiratory diseases (1). The pharmacokinetics of this drug following a therapeutic dose is incompletly known, largely due to the difficulty in measuring the drug in the hundred picogram per ml range in plasma.

In recent years, several GC-MS (1,2), HPLC (3-8, 10) with fluorimetric or electrochemical detection, and RIA (11) analytical methods have been developed to measure the concentration of albuterol or its analogue in plasma. These methods require complicated procedures, have high technical costs or are inadequately sensitive or selective. We demonstrate a simple and highly sensitive HPLC assay for determination of albuterol in human plasma that makes use of a silica gel column and an aqueous mobile phase. This chromatographic technique has been heavily used in our laboratory to successfully determine 0.2 ng/ml concentrations of amine drugs in plasma (9, 12, 13).

## MATERIALS AND METHODS

### Materials

Albuterol sulfate, lot no. 16029/M, was provided by Laborchim and the internal standard, metaproterenol, was furnished by LTI Co.,

(Menlo Park, CA). Di(2-ethylhexyl) phosphate (DEHP) was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical reagent grade, purchased from commercial sources and used without further purification.

## Chromatographic Conditions

An HPLC Model 110 solvent delivery system (Beckman Instruments, Berkeley, CA) equipped with an automatic sample processor WISP 710 B (Waters Associates, Milford, MA) and an Altex Ultrasphere Si column (4.6 x 250 mm, 5 μm particle size), (Beckman Instruments, Berkeley, CA) were employed. The detector system consisted of a Shimadzu RF 535 fluorescence detector (Shimadzu Scientific Instrument Inc. Columbia, MD), and an HP integrator Model 3392 (Hewlett-Packard, Santa Clara, CA) was used. Detector operating wavelengths were: excitation at 225 nm and emission at 310 nm. The extraction was carried out with a Glas-Col rotator (Glas-Col Apparatus Co. Terre Haute, IN).

The mobile phase consisted of 3 mM $(NH_4)_2HPO_4$ in methanol/water (65:35) with pH adjusted to 7.7 by 10% phosphoric acid. The solvent was filtered and degassed before use. The flow-rate was 1.0 ml/min, and all separations were performed at room temperature. Retention times for albuterol and metaproterenol, the internal standard, were 14.7 min and 11.5 min, respectively.

## Sample preparation

Plasma (0.5 ml) in a 15 ml glass test tube with a teflon cap was extracted with 5 ml of 0.1 M DEHP in $CHCl_3$ containing 15 ng of metaproterenol as the internal standard. The resulting mixture was rotated at moderate speed using a Glas-Col rotator for 10 min. Following centrifugation at 3000 *g* for 10 min, the organic phase was transferred to a capped glass tube. Albuterol and the internal standard were back extracted from $CHCl_3$ with 400 μl of 0.025 M HCl. Finally, 100-150 μl of the aqueous phase was injected onto the column.

Pooled drug free plasma spiked to various concentrations of albuterol were divided into 0.5 ml aliquots and stored in a -20°C freezer until assayed. These samples were used as controls during the assay of clinical samples and for the stability study.

## RESULTS

Under the described chromatographic conditions, albuterol and the internal standard produce symmetric chromatographic peaks. An unidentified endogenous peak occurring at 13.3 minutes is clearly separated from albuterol and its internal standard. (Figure 2).

The albuterol standard curve is linear for concentrations in the range 0.2 to 30 ng/ml. Coefficients of determination ($r^2$) of standard curves (n = 48) for the analyses described here were greater than 0.998. The sensitivity limit was 0.2 ng/ml (signal/noise ≥ 3).

The interday and intraday precision of the method determined by the analysis of spiked samples (n = 6) for four concentrations (0.4 to 10 ng/ml) representative of the standard curve range are presented in Tables 1 and 2. The coefficients of variation (CV) for the method ranged 1.76-11.9% for interday and 1.66-13.2% for intraday precision.

Recovery of albuterol from plasma was estimated by comparing the peak height ratio of albuterol to internal standard in plasma to that of albuterol to internal standard in 0.025 M HCl solution. The results appear in Table 3. The recovery at four concentrations representative of the standard curve range averaged 83.7% with a CV of 4.88%.

No appreciable degradation of albuterol in plasma frozen at -20°C was observed over a 40 day period.

## DISCUSSION

The separation of albuterol from the biological sample using organic solvent extraction is not entirely successful because of the amphoteric character of albuterol. An ion-pair extraction was found to be necessary for this assay, and DEHP was found to be essential to achieve an adequate extraction (8). Very good recovery was

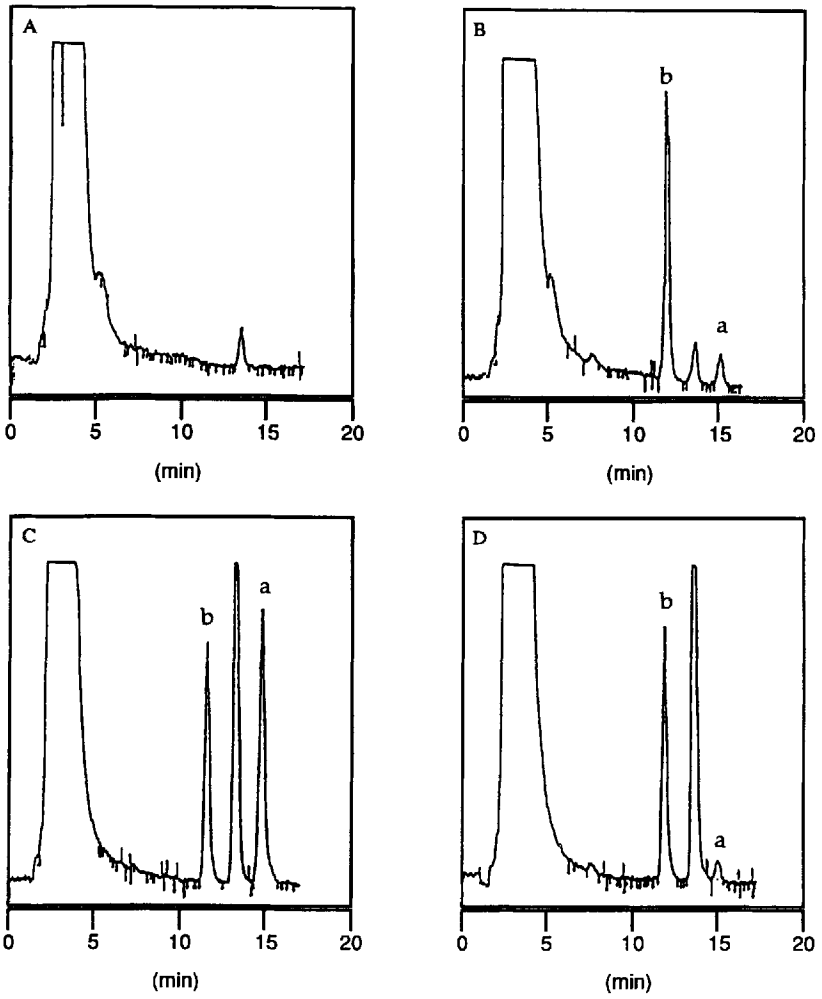


Figure 2: HPLC chromatograms of [A] blank plasma, [B] plasma spiked with 0.6 ng/ml albuterol, [C] plasma from a subject 1 h after an oral albuterol administration of an albuterol sulfate tablet (4.8 mg) (Conc. 11.2 ng /ml ) and [D] plasma sample taken 24 h after albuterol administration as in (C) (Conc. 0.202 ng/ml). a = albuterol; b = metaproterenol, internal standard.

TABLE 1: INTER-DAY PRECISION OF ALBUTEROL PLASMA ASSAY

| Spiked Concentration (ng/ml) | Mean (n=6) | SD | Percent CV |
|---|---|---|---|
| 10.0 | 10.1 | 0.488 | 4.83 |
| 6.00 | 5.96 | 0.105 | 1.76 |
| 2.00 | 2.04 | 0.063 | 3.09 |
| 0.400 | 0.420 | 0.050 | 11.9 |

TABLE 2: INTRA-DAY PRECISION OF ALBUTEROL PLASMA ASSAY

| Spiked Concentration (ng/ml) | Mean (n=6) | SD | Percent CV |
|---|---|---|---|
| 10.0 | 9.35 | 0.155 | 1.66 |
| 6.00 | 5.67 | 0.119 | 2.10 |
| 2.00 | 2.01 | 0.085 | 4.23 |
| 0.400 | 0.439 | 0.058 | 13.2 |



TABLE 3: RECOVERY OF ALBUTEROL PLASMA ASSAY

| Concentration (ng/ml) | Percent Recovery (n=3) |
|---|---|
| 10.0 | 80.5 |
| 6.00 | 79.8 |
| 2.00 | 87.4 |
| 0.600 | 87.0 |
| Overall Average Percent Recovery = 83.7 ± 4.08 | |

maintained, even with the simple back extraction by 0.025 M HCl, as shown in Table 3. Solid phase purification, such as Sep-Pak cartridge purification (4), usually increases assay cost, inconsistency in results and sample preparation time.

An increasing popular technique, use of a bare silca gel column with an aqueous mobile phase to separate the drug from other components of the biological fluid, was employed (9, 12, 13). In this study the effects of changes in the mobile phase methanol concentration, pH and ionic strength on the chromatographic behavior of albuterol, metaproterenol, and the major interfering substance were investigated. The methanol content in the mobile phase had a profound effect on the separation factors ($\alpha$) of the albuterol, metaproterenol and endogenous peaks, as shown in Figure 3. The retention times ($k'$) of albuterol and metaproterenol decreased, while the $k'$ of the unidentified interfering substance increased, with approximate linearity as the mobile phase methanol concentration was increased. At methanol concentrations between 65% and 75% or above 85% a good separation of the three substances could be achieved. Also, and in general, an organic modifier concentration of more than 50% is preferred in order to depress dissolution of bare silca in the column at higher pH values.



The effect of changes in the ionic strength of the mobile phase on retention and resolution was also determined. Figure 4 shows that at salt concentrations below 3.0 mM, separation between the two drugs and the interfering compound was inadequate and inconsistent. Increasing salt concentration from 3 mM to 12 mM reduced $k'$ for the two drugs and the interfering compound due to the competition of ionized basic molecules with buffer toward the active site(s) on the silica gel.

The effect of changes in mobile phase pH on the retention times of the drugs and the interfering compound is presented graphically in Figure 5. Changes in $k'$ followed a similar pattern for all three compounds; $k'$ increased with pH from pH 5.5 to 7.8, and decreased as pH increased above 7.8. An explanation of this behavior could be made in terms of the effect of pH upon the ionization of surface silanols and

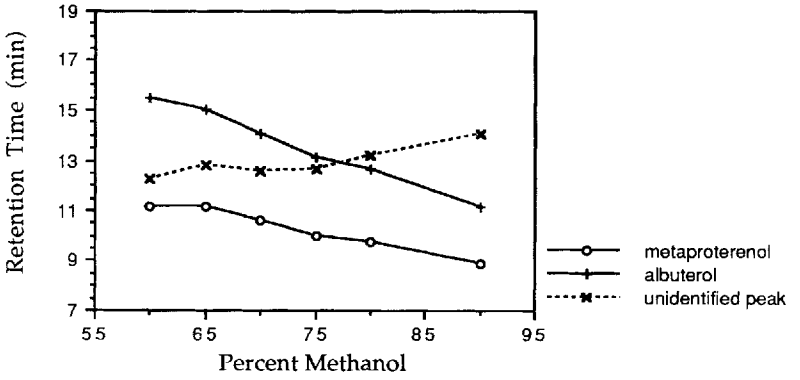


Figure 3: Effect of methanol upon retention. Mobile phase is methanol/water with 3.5 mM $(NH_4)_2HPO_4$ at pH of 7.7.

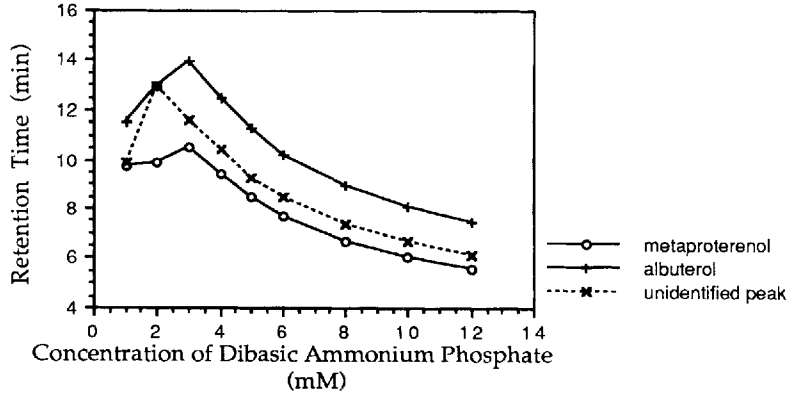


Figure 4: Effect of ionic strength upon retention time. Mobile phase is methanol/water (65:35) at pH of 7.7.

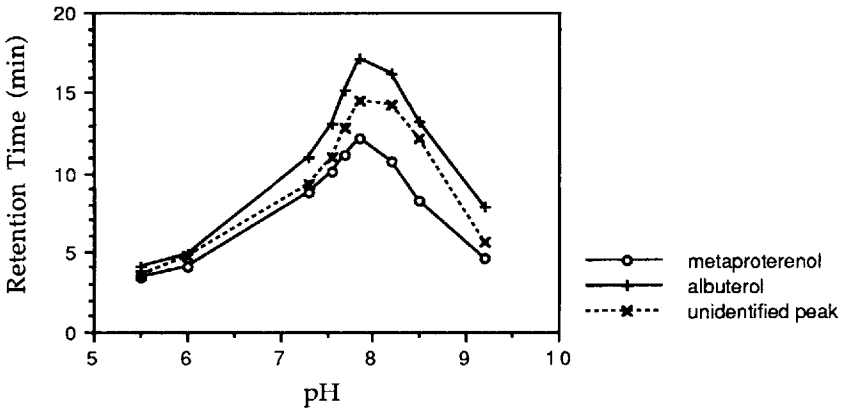


Figure 5: Effect of pH upon retention time. Mobile phase is methanol/water (65:35), 3.5 mM $(NH_4)_2HPO_4$.



of constituents of the samples (9). The $pK_a$ values of albuterol and metaproterenol are approximately 8 and 9.3, respectively. As pH is increased from 5.5 to 7.8 the gel's surface hydroxyls, which are increasingly ionized, attract the ions in the sample and the retention of the ionic amines is increased. Beyond the $pK_a$ of these amines, retention decreases due to the decreasing protonization of the amines.

The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most wildly used HPLC technique today in assays for drugs in biological fluids. In this type of system, the retention mechanism depends mainly on the liphophilic character of the substance to be analyzed. Such a system retains a considerable amount of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, as presented here, the retaining mechanism results mainly from ion exchange (9) and partially from a lipophilic interaction. 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. Interfering substances in biological fluids elute with the solvent front, leaving a very clean baseline for the drug of interest. In general, separation of

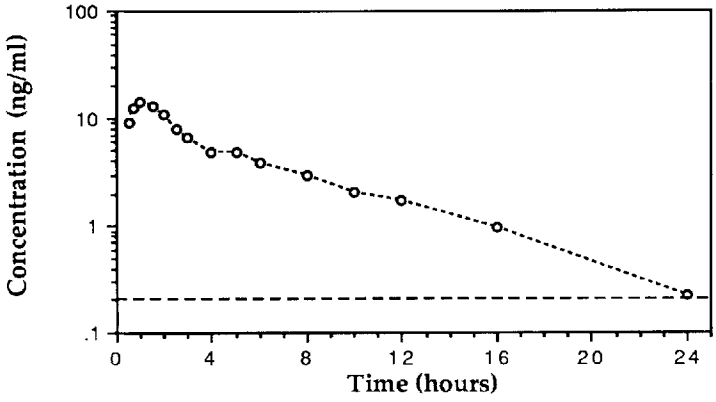


Figure 6: Human Plasma Concentration of Albuterol After Oral Administraion of Albuterol (4.8 mg)



TABLE 4: PHARMACOKINETIC DATA FOR ALBUTEROL FOLLOWING A SINGLE ORAL DOSE (4.8 mg) OF ALBUTEROL SULFATE*

| Parameter (n = 24) | Formulation A mean | Formulation A SD | Formulation B mean | Formulation B SD |
|---|---|---|---|---|
| $C_{max}$ (ng/ml) | 10.109 | 3.257 | 10.075 | 2.316 |
| $T_{max}$ (h) | 2.688 | 1.342 | 2.563 | 1.469 |
| $AUC_{0\ to\ t}$ (h·ng/ml) | 67.496 | 13.937 | 65.847 | 11.006 |
| $AUC_{0\ to\ \infty}$ (h·ng/ml) | 75.963 | 16.787 | 73.939 | 13.279 |
| $K_{el}$ | 0.088 | 0.019 | 0.090 | 0.023 |

*Formulation A = generic formulation; Formulation B = proventil (Schering)

basic drugs from biological fluids can be accomplished by adjusting the percentage of an organic modifier (acetonitrile, methanol), pH and ionic strength of the mobile phase, as presented in this paper.

By taking advantage of albuterol's native fluorescence and by using a silica gel column with an aqueous mobile phase, we were able to obtain a 0.2 ng/ml detection limit for 0.5 ml plasma samples. This result is comparable to a GC/MS assay.

In conclusion, we have successfully developed a simple and highly sensitive HPLC assay for determination of albuterol in plasma. The method has good selectivity, reproducibility and has better sensitivity than existing albuterol HPLC assays. Reliable measurement of albuterol concentrations were obtained for up to 24 hours following an oral dose of 4.8 mg of albuterol sulfate, as shown in Figure 6. Table 4 shows a pharmacokinetic summary between two formulations. There was no significant difference (p. 0.05). Currently, the method is used to evaluate controlled release formulations.



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