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## Note

# Determination of albuterol in human serum by reversed-phase high-performance liquid chromatography with electrochemical detection

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Albuterol (salbutamol) is a $\beta$-adrenergic agonist that is widely used alone or in combination with methylxanthines, to treat bronchospasms in asthmatic patients. Inhaled albuterol produces substantial bronchodilation initially but its effects diminish rapidly and become unmeasurable after 2–5 h. Oral albuterol is more attractive because it is effective for up to 8 h after dosing [1]. However, the inhaled route is perceived to be superior to the oral route because very little is known about the disposition of oral albuterol and there is no pharmacokinetic basis for developing an oral dose strategy.

Pharmacokinetic data on oral albuterol are lacking because there has not been a convenient, yet sensitive, assay method for measuring serum albuterol concentrations after single-dose administration to a large number of normal volunteers. Previous pharmacokinetic studies of oral albuterol have used relatively small numbers of subjects and a highly technical gas chromatographic–mass spectrometric (GC–MS) assay method [2,3]. There are other methods of assay for albuterol in the literature including radioimmunoassay (RIA) [4], high-performance thin-layer chromatography (HPTLC) [5] and high-performance liquid chromatography (HPLC) [6–10]. Although these methods appear suitable for pharmacokinetic studies, they have certain limitations with respect to the assay of large numbers of study samples. The RIA method [4] requires a labor-intensive extraction procedure and the HPTLC [5] method requires derivatiza-

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tion prior to analysis. Some HPLC assays use ion-pair liquid–liquid extraction [7,8,10] that results in poor recovery of albuterol (< 70%) and a large number of interferences in the chromatograms [11]. Another HPLC procedure utilizes an amperometric detector with a glassy carbon electrode [6]; however, this detector requires frequent maintenance when used in the analysis of large numbers of serum specimens.

The purpose of this investigation was to develop a rapid, sensitive and specific analytical method that would be suitable for single-dose bioavailability and pharmacokinetic studies in large numbers ($\geq 30$) of subjects. HPLC was selected as the method of choice for convenience and, recognizing that serum albuterol concentrations were relatively low after oral administration, solid-phase extraction was utilized to optimize recovery of albuterol. An electrochemical detector was selected for this study to take advantage of the sensitivity provided by the oxidizable phenolic hydroxyl group in the albuterol molecule.

## EXPERIMENTAL

### *Materials*

Albuterol was obtained as salbutamol sulfate reference standard (99.6%, lot No. AWS 20B, Glaxo, Research Triangle Park, NC, U.S.A.). The internal standard, bamethane sulfate (lot No. 65F-0535), was purchased from Sigma (cat. No. B0382, St. Louis, MO, U.S.A.). These materials were used as received. Stock solutions of albuterol and internal standard were prepared in purified, deonized, distilled water in concentrations of 1 mg/ml (as bases). The stock solutions were stored at 4°C where they were stable for at least five weeks. A working albuterol solution (500 ng/ml) was obtained by diluting the albuterol stock solution and was used to prepare serum standards containing 1, 2, 5, 10, 20, 30 and 40 ng/ml albuterol, respectively. The serum was pooled serum from normal volunteers which had been previously shown to contain no endogenous substances capable of interfering with the assay. Phosphate-buffered saline (PBS) was obtained from American Bioclinical (Portland, OR, U.S.A.) as a concentrated solution containing 0.1% sodium azide as a preservative and was diluted according to the package instructions immediately before use. Dilution resulted in a final solution containing 0.9% sodium chloride in 0.01 *M* phosphate buffer (pH 7.5). A working internal standard solution (100 ng/ml) was prepared by diluting the internal standard stock solution with PBS. Methanol, monobasic ammonium phosphate and 85% phosphoric acid were HPLC grade and were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). A stock ammonium phosphate buffer solution was prepared by adding 0.25 *M* phosphoric acid (17.0 ml/l of 85% phosphoric acid) to 0.25 *M* monobasic ammonium phosphate (28.75 g/l) until the pH was adjusted to 2.6. This buffer solution was filtered through a 0.2-$\mu$m filter prior to use. The HPLC mobile phase was 8% methanol in 25 m*M* ammonium phosphate buffer. The mobile phase was filtered through a 0.2-$\mu$m filter and degassed by sonication under vacuum before using it.

*HPLC instrumentation*

A high-performance liquid chromatograph consisting of a syringe pump (Model 5700 solvent-delivery module, ESA, Bedford, MA, U.S.A.), operated at a flow-rate of 1.5 ml/min, an automatic sample injector (Model ISS-100, Perkin-Elmer, Norwalk, CT, U.S.A.), a reversed-phase analytical column (10 $\mu$m, $\mu$Bondapak $C_{18}$, 30 cm $\times$ 3.9 mm I.D., Waters Assoc., Milford, MA, U.S.A.) and a coulometric electrochemical detector (Model 5100A, ESA) were used for all analyses. The detector was equipped with a guard cell (Model 5020, ESA) operated at a potential of 900 mV and a dual-electrode analytical cell (Model 5010, ESA). Detectors 1 and 2 were operated at potentials of 500 and 800 mV, respectively. These chromatographic conditions resulted in a back-pressure of approximately 15 mPa and a background signal of 1.5 $\mu$A. The signal from detector 2 was amplified at a gain of 10 and integrated at an attenuation of 4 using an electronic integrator (Model HP-3392, Hewlett-Packard, Rockville, MD, U.S.A.). A signal of 160 nA resulted in full-scale deflection with these settings.

*Sample preparation*

Bond-Elut $C_{18}$ extraction columns (1 ml) (cat. No. 607101, Analytichem, Harbor City, CA, U.S.A.) and a Vac-Elut processing station (cat. No. A16000, Analytichem) were used for solid-phase extraction of serum samples. The vacuum pressure was maintained between 30 and 50 kPa when eluting the columns. Each serum sample was prepared for extraction by combining 1 ml of sample with 250 $\mu$l of working internal standard solution.

The extraction column was activated with 1.0 ml of methanol followed by 1 ml of water. Care was taken to prevent air from reaching the column during this activation step. The serum sample was transferred to the top of the column and vacuum was applied. The column was washed with 1 ml of water followed by 2 ml of 10% methanol (v/v) in water and allowed to air-dry for approximately 2 min under vacuum. Methanol (100 $\mu$l) was added to the column and eluted to waste. The column was allowed to air-dry again for 2 min. Albuterol and internal standard were eluted from the column with 1.0 ml of 75% methanol (v/v) in 0.25 *M* ammonium phosphate buffer. The eluates were evaporated to dryness under a stream of nitrogen at 50 °C. The residues were reconstituted with 250 $\mu$l of aqueous 10% methanol solution and 25–50 $\mu$l were injected into the HPLC system.

*Calculations*

Standard curves were prepared by plotting peak-height ratios (albuterol/internal standard) as a function of albuterol concentration and determining the best-fit line by least-squares linear regression analysis.

*Validation of assay*

Inter-day precision was determined by preparing standard curves using serum standards containing 1–40 ng/ml albuterol on five different days. Intra-day precision was determined by replicate assays ($n = 5$) of serum standards containing low (1 and 2 ng/ml) and high (30 ng/ml) concentrations of albuterol on two different days. Recovery was determined by comparing peak-height ratios ob-

tained from extracted serum standards to the peak-height ratios obtained from a solution of 80 ng/ml albuterol and 100 ng/ml internal standard in mobile phase that was injected directly into the HPLC system. An evaluation of the performance of the assay was conducted by administering two albuterol tablets (4 mg per tablet) to two normal, healthy volunteers and obtaining serial blood samples at frequent time intervals up to 24 h post-dose. Serum was harvested from these samples and frozen at $-20°C$ until analyzed for albuterol.

## RESULTS

Fig. 1 shows typical chromatograms of blank serum and serum containing 6.8 ng/ml albuterol analyzed by the procedure described in this report. The peaks corresponding to albuterol and internal standard were easily resolved from co-eluting endogenous substances. The retention times of albuterol and internal standard were approximately 9 and 13 min, respectively. The chromatograms from blank serum had no interfering peaks at retention times corresponding to either albuterol or internal standard.

Standard curves were linear over the range of 1–40 ng/ml albuterol with correlation coefficients consistently greater than 0.99. The recovery of albuterol was consistently greater than 84%. The sensitivity of the assay was 1 ng/ml which was the smallest concentration of albuterol that could be measured with acceptable ($\pm 15\%$) accuracy and precision. The precision of the assay is described by the data in Table I. The coefficient of variation ranged from 15–16% at low concentrations (1–2 ng/ml) of albuterol to $\leqslant 10\%$ at concentrations of 5–40 ng/ml. Standard curves of albuterol had a slope (mean $\pm$ S.D.) of $0.0452 \pm 0.0015$ and an intercept not significantly different from zero. The intra-day precision data determined on two occasions are shown in Table II. The precision was $\pm 10\%$ in almost all cases.

Fig. 2 shows the serum albuterol concentrations observed in two healthy vol-

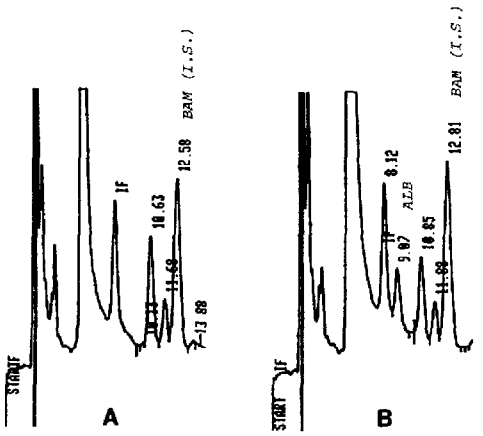


Fig. 1. Typical chromatograms of (A) blank human serum and (B) serum sample from human subject containing 6.8 ng/ml albuterol (ALB). Bamethane sulfate, internal standard (BAM), was added to both samples prior to analysis. (IF is integrator function mark: beginning of peak integration of chromatogram.)

TABLE I

INTER-DAY PRECISION DATA FOR ASSAY OF ALBUTEROL IN POOLED HUMAN SERUM STANDARDS

| Theoretical concentration (ng/ml) | Actual concentration (mean ± S.D., $n=5$) (ng/ml) |
|---|---|
| 40 | 39.8 ± 0.27 |
| 30 | 30.4 ± 0.37 |
| 20 | 19.8 ± 0.80 |
| 10 | 9.9 ± 0.57 |
| 5 | 4.9 ± 0.50 |
| 2 | 1.9 ± 0.32 |
| 1 | 1.3 ± 0.20 |

TABLE II

INTRA-DAY PRECISION DATA FOR ALBUTEROL ASSAY IN POOLED HUMAN SERUM STANDARDS

| Theoretical concentration (ng/ml) | Actual concentration (mean ± S.D., $n=7$) (ng/ml) | |
|---|---|---|
| | Day 1 | Day 2 |
| 30 | 31.1 ± 1.14 | Not determined |
| 2 | 1.8 ± 0.15 | 2.1 ± 0.16 |
| 1 | 1.1 ± 0.14 | 1.4 ± 0.09 |

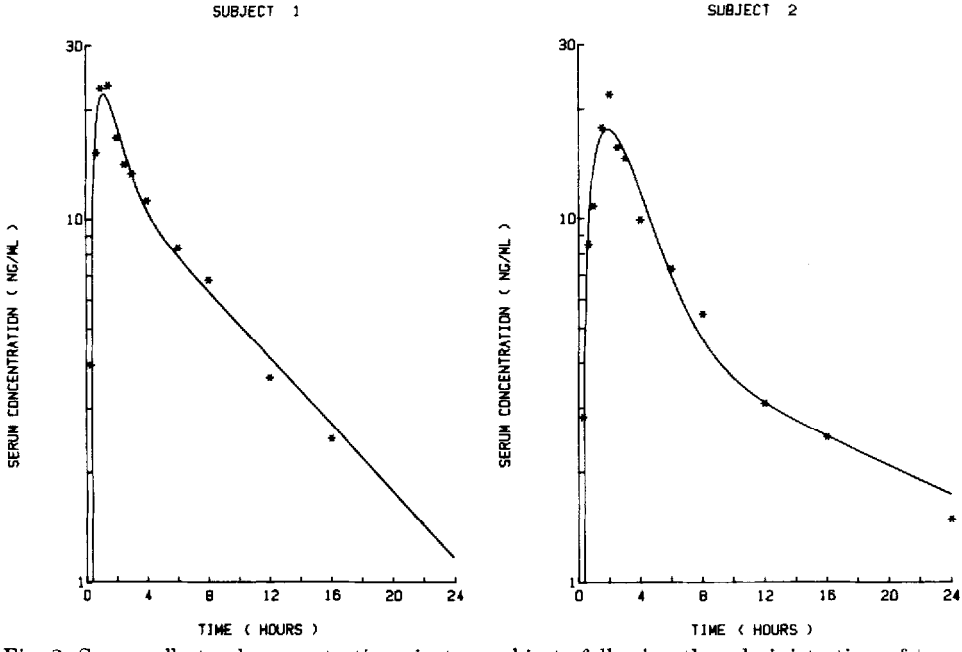


Fig. 2. Serum albuterol concentrations in two subjects following the administration of two 4-mg albuterol tablets.

unteers following the administration of 8 mg of oral albuterol. Serum albuterol concentrations were measured out to 16 h in subject 1 and to 24 h in subject 2. The time of maximum serum albuterol concentration ($T_{max}$) was 1.5–2.0 h and the serum albuterol concentration at $T_{max}$ ($C_{max}$) was 22–23 ng/ml in both subjects. The elimination half-life, as determined by linear regression analysis of the terminal, log-linear segment of each serum concentration–time curve, was 5.6 h in subject 1 and 11.4 h in subject 2.

## DISCUSSION

Oral albuterol has great potential for the therapeutic management of asthmatic patients. However, knowledge of its pharmacokinetics is essential to developing a strategy for effective oral dosing. Studies of the bioavailability and pharmacokinetics of oral albuterol in suitably large groups of normal volunteers has been hampered by the lack of a rapid, sensitive and specific assay for albuterol in serum. As a result there is a sparcity of data pertaining to the absorption and disposition of albuterol. The assay described in this report meets the analytical requirements necessary for single-dose bioavailability and pharmacokinetic studies in normal, healthy volunteers. It is relatively simple, rapid, sensitive ($\leqslant 1$ ng/ml) and specific for albuterol in normal human serum. It requires only 1 ml of serum, a compelling advantage in pharmacokinetic studies that include serial collection of multiple blood samples over short periods of time.

The strategy in developing this assay was to combine the efficiency of solid-phase extraction of serum specimens with the sensitivity of electrochemical detection. The extraction of albuterol from serum using traditional solvent extracting techniques was unsatisfactory because of low ($< 70\%$) and unreliable recovery.

The efficiency of extraction using solid-phase columns was at least 20% greater than with liquid–liquid extraction. Several SPE columns were tested for their selectivity and extraction efficiency including $C_{18}$, $C_8$, CN and DIOL columns. The $C_{18}$, $C_8$ and CN columns extracted albuterol equally well and better than the DIOL column; however, a $C_{18}$ extraction column was selected because of the aqueous nature of serum and the semi-polar nature of albuterol. Albuterol was eluted off the extraction column with a methanol phosphate buffer because the recovery was greater than that obtained using either methanol alone or combinations of methanol and acetonitrile. The electrochemical detector used in the HPLC system was stable throughout the course of the study and required minimal maintenance. The operating potential of the first detector cell was set at 500 mV because it selectively oxidized endogenous substances, without affecting the albuterol molecule, and markedly reduced background noise. The potential of the second detector cell was set to optimize the signal-to-noise ratio and obtain a limit of albuterol detection of 1 ng/ml.

The pilot study serum albuterol concentration–time data demonstrated that the assay is suitable for single-dose (4–8 mg) bioavailability and pharmacokinetic studies in healthy, normal volunteers. Drug interference studies were performed to assess the suitability of the assay for albuterol pharmacokinetic studies

in asthmatic patients. Theophylline, terbutaline, metaproterenol, isoproterenol and epinephrine did not interfere with the assay.

Caution should also be used in the selection of collection containers for blood samples which are to be analyzed for albuterol. Glass containers with no anticoagulants were found to be satisfactory in the human phase of this study, but additional studies need to examine potential interferences from heparin and/or other anticoagulants in the quantitation of albuterol.

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