Journal of Chromatography, 381 (1986) 205—208
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3194

Letter to the Editor

Determination of albuterol in human plasma by high-performance liquid chromatography with fluorescence detection

Sir,

Albuterol, or salbutamol is a beta$_2$-adrenergic agonist used in the treatment of asthma and other forms of reactive airways disease [1]. We describe a method for albuterol measurement that is sensitive to 1 ng/ml and selective for albuterol, and makes use of the simplicity and reliability of fluorescence detection and auto-injection of samples.

EXPERIMENTAL

Materials

Pure samples of albuterol sulfate and the internal standard bamethan sulfate were provided by Glaxo Pharmaceuticals (Ware, U.K.) and Sigma (St. Louis, MO, U.S.A.), respectively. All other reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification. Mobile phase components were filtered prior to and after mixing.

Apparatus and chromatographic conditions

The high-performance liquid chromatographic (HPLC) system consisted of a Waters solvent delivery system and a Perkin-Elmer 650-105 spectrofluorometer operated at excitation 220 nm and emission 309 nm (slit widths 15 and 20 nm, respectively). The separation system was a 15 cm × 4.6 mm stainless steel (5 μm particle size) reversed-phase C_{18} column (Supelco). Samples were injected automatically with a WISP-710 injector (Waters Assoc.). Mobile phase (water—acetonitrile, 92:8 adjusted to pH 2.5 with phosphoric acid) was run at a flow-rate of 1.6 ml/min. All analyses were performed at room temperature.

Preparation of samples

A 50-μl volume of solution of bamethan sulfate (2.5 μg/ml, 125 ng) was added to each of a series of 15-ml glass round-bottom tubes with PTFE-lined screw-top caps. A 1.0 ml sample of unknown plasma was added to each tube. Calibration standards for albuterol were prepared by adding 1, 2, 5, 10, 25, 50,

0378-4347/86/$03.50

75 and 100 ng of drug to consecutive tubes. Drug-free control plasma was added to each of the calibration tubes. One blank sample, taken from the subject prior to drug administration, was analyzed with calibration standards and each set of unknown samples.

Extraction procedure

To each tube were added 4 ml of a solution of 0.1 *M* di-(2-ethylhexyl) phosphate (DEHP) in chloroform. The tubes were agitated gently in the upright position on a vortex mixer for 1 min, then centrifuged at 20° C for 10 min at 600 *g*. The organic layer was transferred to a 15-ml glass tube containing 150 μl of 10 m*M* hydrochloric acid. The tubes were agitated gently, as before for 1 min, then centrifuged at 20° C for 10 min at 600 *g*. Aliquots of 50—90 μl of the acidic aqueous phase were then injected into the sample loop by auto-injection.

Single-dose pharmacokinetic study

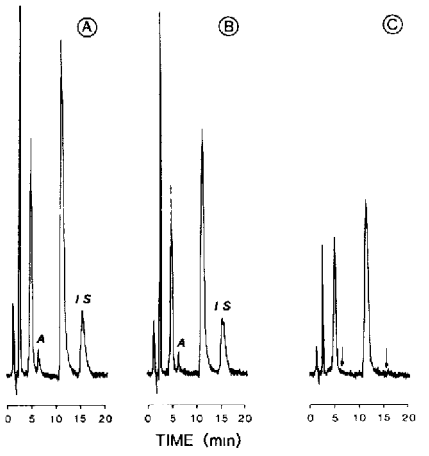
A healthy 32-year-old male volunteer participated. Albuterol (4 mg) was administered orally with water. Multiple venous blood samples were drawn into Venoject heparin-containing tubes over the following 12 h. Concentrations of albuterol were determined by the method described above.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, albuterol and the internal standard bamethan sulfate give symmetric well resolved chromatographic peaks (Fig. 1A—C) with retention times 6.6 min for albuterol and 15.9 min for bamethan. The blank plasma after extraction consistently contains no interfering peaks. An unidentified peak occurs at 5 min, and is clearly distinct from albuterol. A peak occurring at 11.5 min can be ascribed to DEHP, and also is distinct from albuterol and the internal standard.

We varied several aspects of the extraction conditions to examine their effect on peak size. Use of buffer such as that previously described [2, 3] did not improve recovery of albuterol or the internal standard, nor was there any effect after adding acid (100 μl 0.1 *M* hydrochloric acid) or base (100 μl 0.1 *M* sodium hydroxide) to the plasma. DEHP was found to be essential to achieve adequate extraction. Concentrations of DEHP ranging from 0.0001 to 0.1 *M* were examined and recovery was maximal at 0.1 *M*. For the re-extraction, concentrations of hydrochloric acid from 0.01 to 0.5 *M* were examined. There was no change in recovery with increasing hydrochloric acid concentrations.

The relation between albuterol concentrations and peak height ratio (vs. internal standard) is linear from at least 1 to 100 ng/ml ($y = 31x$). Correlation coefficients are always greater than 0.99. Relative standard deviations, calculated by doing six replicate analyses of known standards, were: 8.0% at 2 ng/ml, 4.3% at 5 ng/ml, 4.4% at 10 ng/ml, 5.5% at 25 ng/ml and 5.6% at 50 ng/ml. Minimum detectable concentration was 1 ng/ml (signal/noise 2:1). The between-day variability was assessed by inclusion of previously mixed quality-control samples containing 25 ng/ml of drug during extractions and



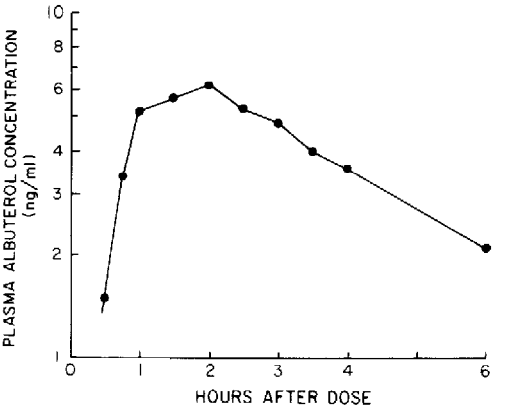


Fig. 1. (A) Chromatogram of an extract of a calibration standard containing albuterol 5 ng/ml and the internal standard, bamethan sulfate 12.5 ng/ml (B) Chromatogram of a single plasma from a subject taken 1 h after albuterol (4 mg) administration. (C) Chromatogram of a plasma sample taken from a subject prior to albuterol administration. Peaks: A = albuterol and I.S. = internal standard, bamethan sulfate.

Fig. 2. Plasma albuterol concentrations in a subject following a single oral dose of 4 mg albuterol.

analyses. The relative standard deviation for four analyses was 4.3% (25.8 ± 1.1 ng/ml). Extraction efficiency for both albuterol and bamethan was approximately 70%, similar to results from prior studies [2].

Fig. 2 shows plasma albuterol concentrations after a single 4-mg oral dose of atenolol. Peak plasma concentration was 6.2 ng/ml; time of peak was 2 h.

This report describes a reliable, sensitive, and selective method for the quantitation of albuterol in plasma using HPLC. The method combines excellent sensitivity with simplicity of extraction and the ease and reliability of fluorescence detection and sample auto-injection. In comparison to other HPLC methods for measurement of albuterol, the present method is substantially more sensitive than that of Kurosawa et al. [4]. Sensitivity is similar to that of Hutchings et al. [3] but these authors did not use an internal standard, and it is uncertain whether their method can be adapted to a standard. Tan and Soldin [2] report even greater sensitivity to 400 pg/ml, but their method involves a substantially more involved extraction procedure and uses electrochemical detection. In addition, their method is not usable with heparinized blood, since heparin co-elutes with albuterol. Oosterhuis and Van Boxtel [5] also report high sensitivity (500 pg/ml), but their method also requires electrochemical detection, using a specialized electrode not generally available. In summary, the present method combines adequate sensitivity with ease and reliability.

ACKNOWLEDGEMENTS

This work was supported in part by Grants MH-34223 and AG-00106 from the United States Public Health Service.

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(First received January 15th, 1986; revised manuscript received April 8th, 1986)