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Note

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

MARK J. HUTCHINGS

Department of Pharmaceutics, Victorian College of Pharmacy Ltd., Melbourne, Victoria (Australia)

JOHN D. PAULL

Royal Women's Hospital, Melbourne, Victoria (Australia)

and

DENIS J. MORGAN*

Department of Pharmaceutics, Victorian College of Pharmacy Ltd., Melbourne, Victoria (Australia)

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Salbutamol, 1-(4-hydroxy-3-hydroxymethylphenyl)-2-*tert.*-butylaminoethanol, also known as albuterol, is a β_2-adrenoceptor agonist widely used for the treatment of bronchial asthma and in obstetrics for the prevention of premature labour [1]. Previously reported assays for salbutamol have used liquid scintillation spectrometry [2], gas chromatography—mass spectrometry [3, 4] and more recently, high-performance liquid chromatography (HPLC) with amperometric detection [5]. However, these methods suffer from either a lack of adequate sensitivity [2], the use of time-consuming derivatization procedures [3, 4] or the need for elaborate equipment not routinely available in most laboratories [3—5]. We report a sensitive yet simple assay for salbutamol that uses ion-pair extraction and HPLC with detection by means of the drug's endogenous fluorescence. The method has been applied to a study of the plasma concentrations of salbutamol in pregnant women receiving the drug for the prevention of premature labour.

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EXPERIMENTAL

Reagents

Salbutamol sulphate was kindly supplied by Glaxo Australia (Melbourne, Australia). Di(2-ethylhexyl) phosphate (DEHP) was obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical reagent grade and obtained from Ajax Chemicals (Sydney, Australia). They were used as received. A phosphate buffer of pH 7.2 was used with a strength of 0.42 *M*.

Chromatographic system

A Laboratory Data Control Constametric IIG pump (Riviera Beach, FL, U.S.A.), a Rheodyne Loop injector No. 7120 (200 μl loop attached) (Berkeley, CA, U.S.A.) and a Zorbax® 5 μm ODS reversed-phase column (25 cm × 4.6 mm, DuPont, Wilmington, DE, U.S.A.) with pre-column (packed with Co:Pell ODS, Whatman, Clifton, NJ, U.S.A.) were used with a Perkin-Elmer 3000 fluorescence spectrometer (Norwalk, CT, U.S.A.) and Omniscribe (Houston Instrument, Austin, TX, U.S.A.) chart recorder. Maximum salbutamol fluorescence would be expected on excitation at 220 nm in accordance with UV spectrometry experience, however due to the limitations of the particular fluorimeter on hand, excitation was performed at 230 nm (15 nm slit) with emission being detected at 309 nm (20 nm slit).

The mobile phase was composed of 8% v/v acetonitrile in distilled water containing 0.15% v/v phosphoric acid BP. The flow-rate was 1.7 ml/min.

Plasma samples

Drug-free venous blood was obtained from healthy human subjects receiving no medication. Blood was also obtained from inpatients receiving salbutamol for the prevention of premature labour. Blood was collected into plastic tubes containing lithium heparin and centrifuged for 10 min at 1000 *g* in a refrigerated centrifuge. Plasma was separated and stored between −2 and −8°C in plastic tubes until assayed. A stability study revealed no degradation on storage after a 31-day period.

Ion-pair extraction of salbutamol

Plasma (1 ml) in a stoppered 10-ml glass tube was buffered at pH 7.2 using 0.2 ml of the buffer and extracted with a solution of 0.1 *M* DEHP in chloroform (6 ml) by vortexing for 2 min. The phases were separated by centrifugation (1000 *g* for 10 min) and the chloroform phase transferred to a clean tube containing 500 μl of 0.5 *M* hydrochloric acid into which salbutamol was extracted by vortexing for 2 min followed by centrifuging (1000 *g* for 5 min). A 200-μl aliquot of the acid phase was injected directly into the chromatograph.

RESULTS AND DISCUSSION

Salbutamol isolated from plasma chromatographed with a retention time of 6.0 min and was not interfered with by any peaks present in control plasma (Fig. 1). Although there is a peak at 23 min in the chromatogram from blank

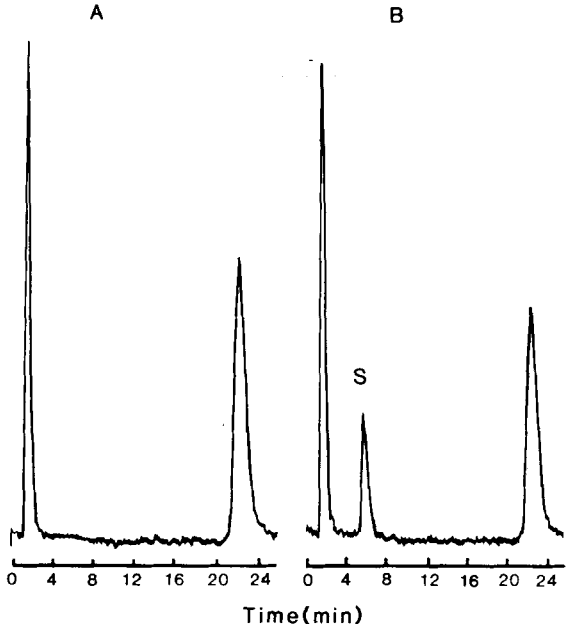

Fig. 1. Chromatograms obtained from plasma samples of patients. A: Blank; B: sample containing salbutamol (S), 12 ng/ml.

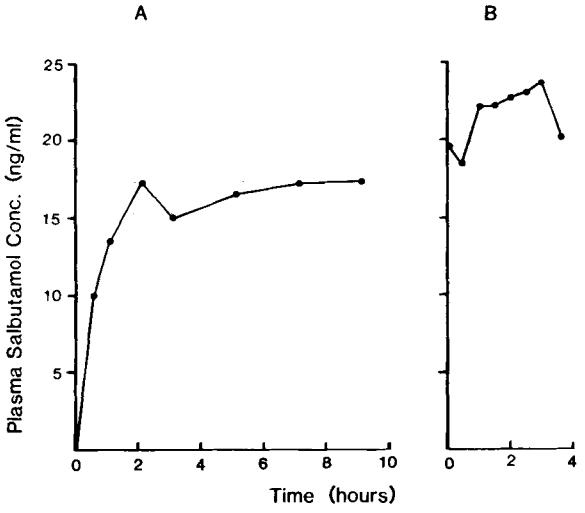

Fig. 2. Plasma salbutamol concentrations in a patient receiving the drug to arrest premature labour. (A) Intravenous infusion rate of salbutamol was 15.8 μg/min for the first 2 h, thereafter 7.8 μg/min; (B) subsequent oral maintenance therapy of 4 mg salbutamol 4-hourly.

plasma the analysis time can be reduced to about 15 min by injecting every second sample immediately after salbutamol elutes. Recovery of salbutamol, assessed by the injection of known amounts of salbutamol onto the chromatographic column, averaged 84 ± 3% (S.D.). The standard curve of peak height versus known concentration of salbutamol was linear over the range 0 to 100 ng/ml and the detection limit was 1.0 ng/ml (signal-to-noise ratio = 2:1).

Precision of the assay was assessed by replicate assays of known standards added to control plasma. At a plasma salbutamol concentration of 100 ng/ml the coefficient of variation (C.V.) was 2.5% (n=8) while at 5 ng/ml, the C.V. was 3.2% (n=7). The day-to-day C.V., assessed at 40 ng/ml, was 11% (n=5).

The assay was used to measure plasma salbutamol concentrations in pregnant women receiving the drug to arrest premature labour. Typical data, from a patient who received the drug intravenously and later orally, are shown in Fig. 2. No interference with our assay method was observed from the following drugs when they were given to patients whose salbutamol concentrations were monitored: betamethasone, chloral hydrate, paracetamol, pethidine, prochlorperazine mesylate, morphine, papaveretum, hyoscine HBr, aspirin, codeine and dexamethasone. Furthermore, theophylline, added to control plasma at a concentration of 20 μg/ml, also produced no interference. The data in Fig. 2 demonstrate that the method can accurately quantitate plasma salbutamol concentrations arising from both chronic intravenous and chronic oral dosage, with no interference from metabolites. The method possesses similar sensitivity but superior precision at low concentration than the other recently reported methods [3—5] and should therefore be equally useful in measuring plasma salbutamol concentrations after single intravenous or oral doses of the drug as those methods.

In summary, the assay described has the advantage over existing assays of being simple and not requiring elaborate and relatively expensive equipment. The use of the more widely available fluorimetric detection method should facilitate the acquisition of pharmacokinetic data on salbutamol.

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