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Note

Determination of tulobuterol in human plasma by capillary gas chromatography

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Tulobuterol hydrochloride (*o*-chloro-α-[*tert.*-butylaminomethyl]benzyl alcohol hydrochloride, Fig. 1) is a sympathomimetic amine. It has potent and long-acting bronchodilator activity [1–3]. Tulobuterol has been measured in urine [4] and serum [5] after extraction and derivatisation by gas chromatography–mass spectrometry (GC–MS) and in serum by packed-column GC with electron-capture detection [6].

This paper describes a more simple, rapid and sensitive procedure for the measurement of tulobuterol in plasma and urine, using hexane extraction and trifluoroacetyl derivatisation. The method is capable of measuring circulating levels of tulobuterol in plasma and in urine after administration of clinically used doses of the drug. The assay is highly specific for tulobuterol and there is no interference from tulobuterol metabolites.

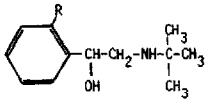

Fig. 1. Chemical structures of tulobuterol (R = Cl) and deschlorotulobuterol (R = H, internal standard).

EXPERIMENTAL

Materials and reagents

Hexane and ethyl acetate were glass-distilled grade (Rathburn Chemicals, Walkerburn, U.K.), trifluoroacetic anhydride was purchased from Fluorochem

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(Dinting Vale, U.K.). Tulobuterol hydrochloride was supplied by UCB (Brussels, Belgium). 2-(N-*tert.*-Butylamino)-1-phenylethanol hydrochloride (deschlorotulobuterol hydrochloride), for use as an internal standard, was synthesised using standard procedures. Standard solutions of tulobuterol hydrochloride and internal standard were prepared in freshly glass-distilled water at concentrations equivalent to 0.1 and 0.5 μg free base per ml, respectively. All solutions were stored in the dark at 4°C.

Sample preparation procedure

Plasma or serum samples (1 ml) were transferred into disposable glass extraction tubes (10 ml capacity), and internal standard solution (20 μl containing 10 ng deschlorotulobuterol) was added, followed by sodium hydroxide solution (2 *M*, 0.2 ml). Samples were then extracted with hexane (6 ml) in a rotating extraction apparatus for 10 min. After centrifugation (2000 *g* for 10 min), the organic layer was carefully transferred into another extraction tube and was evaporated just to dryness at 40°C under a stream of nitrogen. The residue was mixed on a vortex mixer with ethyl acetate (1 ml, containing trifluoroacetic anhydride, 1% v/v) and heated at 70°C for 30 min. The reaction mixture was then evaporated at 40°C under nitrogen, and water (2 ml) added to the residue, which was finally extracted with hexane (1 ml). After centrifugation (2000 *g* for 10 min), the hexane layer was transferred to an autosampler vial and an aliquot (1–3 μl) injected into the chromatograph. MS showed that bis(trifluoroacetyl) derivatives were formed from tulobuterol and the internal standard.

Samples of urine, appropriately diluted with distilled water, were extracted using the same procedure.

Capillary gas chromatography

A Hewlett-Packard 5880A gas chromatograph (Hewlett-Packard, Wokingham, U.K.) equipped with a 15-mCi ^{63}Ni electron-capture detector operated in the splitless mode and containing a fused-silica capillary column (25 m×0.31 mm I.D.) coated with cross-linked 5% phenylmethyl silicone (0.17-μm film) was used to measure derivatised tulobuterol in the biological extracts. The carrier gas was dry oxygen-free nitrogen at a flow-rate of 1 ml/min, and the detector supplementary gas flow-rate (also nitrogen) was 30 ml/min. The injector and detector temperatures were 110 and 300°C, respectively. The initial oven temperature was 50°C which immediately after injection was programmed to increase at the rate of 20°C/min to 195°C. This temperature was maintained for 1 min, after which time it was programmed to increase at the rate of 30°C/min to 280°C. This final temperature was maintained for 3 min, after which time the oven was automatically cooled to 50°C prior to the next injection. Under these conditions, the trifluoroacetyl derivatives of tulobuterol and the internal standard were eluted from the column with retention times of 6.6 and 5.8 min, respectively (Fig. 2). Output from the chromatograph was recorded on a Hewlett-Packard 5880A recorder. The amounts of tulobuterol were quantified by the peak-height ratio technique, with the peak heights being measured manually. Chromatograms of the same stan-

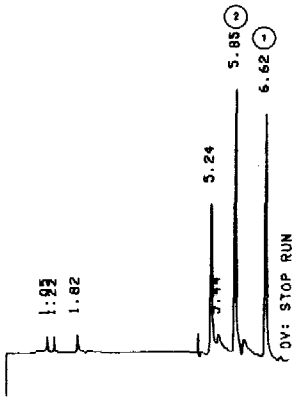

Fig. 2. Chromatogram of trifluoroacetyl derivatives of tulobuterol (peak 1) and deschlorotulobuterol (peak 2).

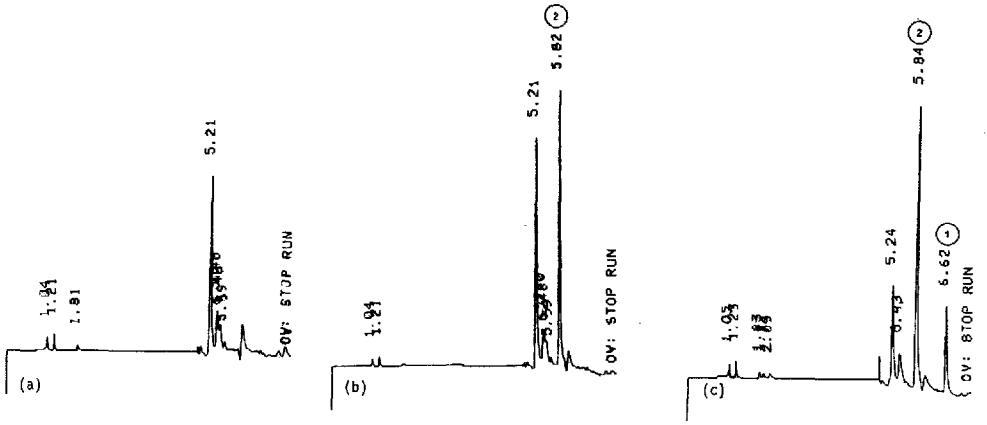

Fig. 3. Chromatograms of (a) pre-dose control plasma, (b) control plasma containing internal standard and (c) plasma containing tulobuterol at a concentration of 1.4 ng/ml. Peaks: 1 = tulobuterol derivative; 2 = internal standard derivative.

TABLE I

BETWEEN-DAY PRECISION MEASUREMENTS OF TULOBUTEROL IN PLASMA

Concentration of tulobuterol (ng/ml)	Peak-height ratio tulobuterol/internal standard (mean ± S.D.)	Coefficient of variation (%)
0.2	0.05 ± 0.01	20
0.4	0.09 ± 0.01	11
1	0.21 ± 0.02	10
2	0.40 ± 0.02	5
3	0.64 ± 0.04	6
5	1.02 ± 0.05	5

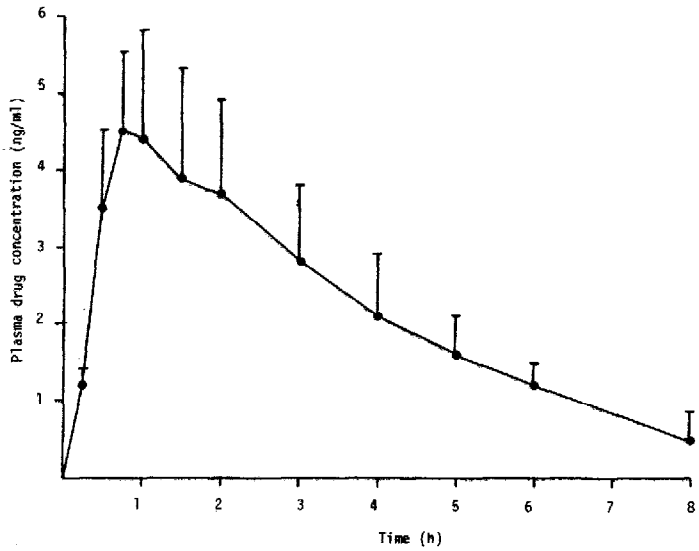

Fig. 4. Mean plasma drug concentrations after oral administration of tulobuterol hydrochloride equivalent to 4 mg free base to human subjects ($n=4$); vertical bars indicate standard deviations.

dards from either plasma, serum or urine were essentially identical and plasma was used for the calibration standards.

Calibration procedure

The calibration line for the measurement of tulobuterol was constructed from samples of blank plasma (1 ml) containing tulobuterol at concentrations of 0.2, 0.4, 1, 2, 3 and 5 ng/ml and internal standard at a fixed concentration of 10 ng/ml. These samples were taken through the extraction and derivatisation procedure described above.

Studies in human

Plasma and urine samples were obtained from four human subjects dosed with an aqueous solution (100 ml) containing tulobuterol hydrochloride (equivalent to 4 mg tulobuterol free base) after fasting overnight. The study was conducted under conditions similar to those described elsewhere [7].

RESULTS AND DISCUSSION

Precision and accuracy of measurement

Derivatisation and measurement was repeated on five occasions at each concentration over the calibration ranges during a three-day period. The between-day precision of the method, as indicated by the coefficient of variation (C.V.) of peak-height measurements of drug to internal standard, ranged from $\pm 5\%$ at 5 ng/ml to $\pm 11\%$ at 0.4 ng/ml (Table I).

The calibration line for the determination of tulobuterol in plasma (and urine), constructed from five replicate measurements at six concentrations in the range

0.2–5 ng/ml, was linear ($y=0.2054x$, where y is the peak-height ratio of tulobuterol/internal standard derivatives and x is the concentration of tulobuterol added to serum). The standard error of the calibration line at its mid-point was ± 0.3 ng/ml. In quality-control samples, concentrations of tulobuterol in the range 0.5–5 ng/ml were measured with a mean accuracy of $102 \pm 7\%$ (C.V.) of the true value.

Recovery and limit of detection

The recovery of tulobuterol and internal standard from plasma was estimated after extracting samples of control plasma and adding tulobuterol (4 ng) and internal standard (10 ng) to the organic phase prior to derivatisation. The peak-height ratios of tulobuterol to internal standard on the chromatograms of these extracts were compared to those from plasma to which tulobuterol (4 ng) or internal standard (10 ng) were added.The recovery (mean $\pm$ S.D.) of tulobuterol from plasma was $88 \pm 4\%$ ($n=4$) and that of internal standard was $87 \pm 4\%$ ($n=4$).

No interfering peaks with retention times similar to either tulobuterol or internal standard were present in predose (blank) plasma or urine (Fig. 3). The limit of detection of tulobuterol was set by instrumental noise at 50 pg/ml. The reliable limit of accurate measurement was regarded as 200 pg/ml, the lowest datum point on the calibration line.

Selectivity of the analytical method

No peaks interfering with the analysis were present in any control plasma, serum or urine samples investigated. There was no interference from the major metabolites of tulobuterol, which, being phenolic in nature, were not extracted under the conditions used. Similarly there was found to be no interference from two other clinically used bronchodilators, salbutamol and terbutaline.

Concentrations of tulobuterol in human plasma and urine

The mean concentrations of tulobuterol in the plasma of four volunteers after single oral doses of tulobuterol hydrochloride (equivalent to 4 mg tulobuterol free base) reached a peak of 4.5 ng/ml (Fig. 4) at 0.75 h after dosing and thereafter declined to below the limit of accurate measurement at 10 h with a half-life of 2.7 ± 0.3 h (mean $\pm$ S.D.). A similar terminal half-life for tulobuterol in plasma of 3.2 h has been reported previously [5].

Urinary excretion of unchanged tulobuterol accounted for only $2.5 \pm 1.4\%$ (mean $\pm$ S.D.) of the dose excreted by these subjects during five days. Enzymatic hydrolysis studies indicated that tulobuterol was not excreted as a glucuronic acid or sulphate conjugate, in contrast to the results obtained by Matsumura et al. [4] who reported that tulobuterol was excreted extensively (up to 50% dose) both unchanged and conjugated. These data reflect the extensive biotransformation of tulobuterol in man, since the drug is well absorbed from the gastrointestinal tract with over 80% of a radioactive dose being recovered in the urine during five days [8].

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