Determination of Serum Tulobuterol Concentrations by Mass Fragmentography: Comparison with an Electron-Capture Gas Chromatographic Method

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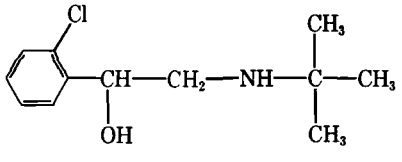
Abstract □ A simple and sensitive method is reported for the quantitative determination of the bronchodilator tulobuterol in human serum. Tulobuterol and an internal standard were extracted from alkalinized serum with ether and then back-extracted into dilute hydrochloric acid. After alkalinization and extraction of the aqueous solution, the extract was evaporated to dryness. The residue was silylated and subjected to mass fragmentography.

Keyphrases □ Tulobuterol—serum, extraction, derivatization, mass fragmentography □ Bronchodilator—tulobuterol, serum analysis, mass fragmentography □ Mass fragmentography—analysis, tulobuterol, serum

Tulobuterol (I) is a newly synthesized bronchodilator having a potent and long-lasting effect (1, 2). An electron-capture GLC determination of this drug in human serum was reported previously (3). This method was specific and sensitive, but the procedure was tedious and time consuming. Application of mass fragmentography to the detection of plasma albuterol and terbutaline, which possess similar chemical structures to tulobuterol, has been reported (4–6). This paper describes a sensitive and rapid analytical method for determining tulobuterol in human serum by mass fragmentography. The analytical results are compared with those obtained by electron-capture GLC.



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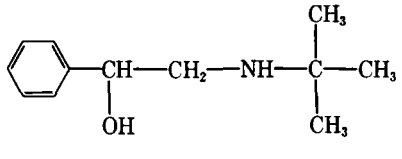

II

EXPERIMENTAL

Materials—Tulobuterol hydrochloride was synthesized previously (7). The internal standard, α-[(*tert*-butylamino)methyl]benzyl alcohol (II), was synthesized using a previously described method (8). *N,O*-Bis(trimethylsilyl)acetamide[1] (III) was employed as the derivatization reagent. Other reagents and solvents were of analytical grade and were used without further purification.

Drug Administration and Sample Collection—Four healthy, male volunteers each received a 2-mg oral dose of tolubuterol hydrochloride in the form of a syrup solution[2] or tablets. Blood samples of ~8 ml were withdrawn before the administration and at 0.5, 1, 1.5, 2, 3, 4, and 6 hr.

[1] Nakarai Chemical, Ltd., Kyoto, Japan.
[2] Prepared by dissolving the granulated drug in 20 ml of water.

Table I—Typical Tulobuterol Calibration Curve

Drug Concentration, ng/ml	Peak Area Ratio[a]	Ratio[b]
1	0.096	0.096
2	0.141	0.071
4	0.295	0.074
7	0.573	0.082
10	0.766	0.077

[a] Ratio of drug–internal standard. [b] Peak area ratio divided by drug concentration.

After standing for ~30–60 min, the serum was separated by centrifugation (2000×*g*, 10 min) and stored frozen until analysis.

Apparatus—GLC[3]-MS[4,5] determinations were performed using a glass column of 2-m length and 2-mm i.d., packed with 2% OV-1 on 100–120 mesh chromosorb G (HP)[6], operating at 165°. The injector, separator, and ion source were held at 210, 290, and 150°, respectively. The ionization energy and trap current were 70 eV and 30Q μA, respectively. Measurements were performed by single-ion monitoring at *m/z* 86.

Extraction Procedure and Sample Preparation—A solution of internal standard (1 ml of an 8-ng/ml solution in methanol) was evaporated to dryness under reduced pressure in a 15-ml glass centrifuge tube, and 1 ml of serum and 0.5 ml of 1 *N* NaOH were added. The tube was stoppered and extracted with 6 ml of ether by a reciprocating shaker at 160 strokes/min for 5 min. After centrifugation (2000×*g*, 5 min), the ether layer was transferred to a second tube containing 5 ml of 0.1 *N* HCl. The tube was shaken, centrifuged, and the ether layer removed.

Four milliliters of the aqueous layer was transferred to a third tube and alkalinized with 1 ml of 1 *N* NaOH. After extraction with 6 ml of ether, the tube was shaken and centrifuged. Five milliliters of the ether phase was transferred to a fourth tube and evaporated to dryness in a water bath at 50°. The residue was dissolved in 100 μl of III (1:4 solution in ethyl acetate), and 1 μl was chromatographed.

RESULTS AND DISCUSSION

Figure 1 shows a mass fragmentogram obtained from a serum sample to which a known amount of tulobuterol hydrochloride had been added, together with those obtained from a serum blank and from a volunteer receiving tulobuterol hydrochloride by the oral route. The peaks of tulobuterol and internal standard were well resolved and no interfering peaks from endogenous materials were present.

The mean percent recovery and standard deviation of tulobuterol hydrochloride were 65.02% and ±3.45, respectively. The corresponding values for the internal standard were 63.82% and ±2.52. Results were obtained from six determinations using serum with 5 ng/ml of tulobuterol hydrochloride and 8 ng/ml of the internal standard added. Although the recoveries of tulobuterol hydrochloride and internal standard in the present assay are low, they are reproducible. Attempts to improve the drug recovery led to either added complexities or irreproducibility.

The ratio of the peak area of tulobuterol to that of the internal standard was calculated. Statistical analysis indicated excellent linearity in the range of 1–10 ng/ml serum with a correlation coefficient of 0.998, a slope of 0.0775, and an intercept of 0.002 (Table I).

[3] JGC-20K, JEOL, Tokyo, Japan.
[4] JMS D-300, JEOL, Tokyo, Japan.
[5] JMA-2000, JEOL, Tokyo, Japan.
[6] Wako Pure Chemical Industries, Ltd., Osaka, Japan.

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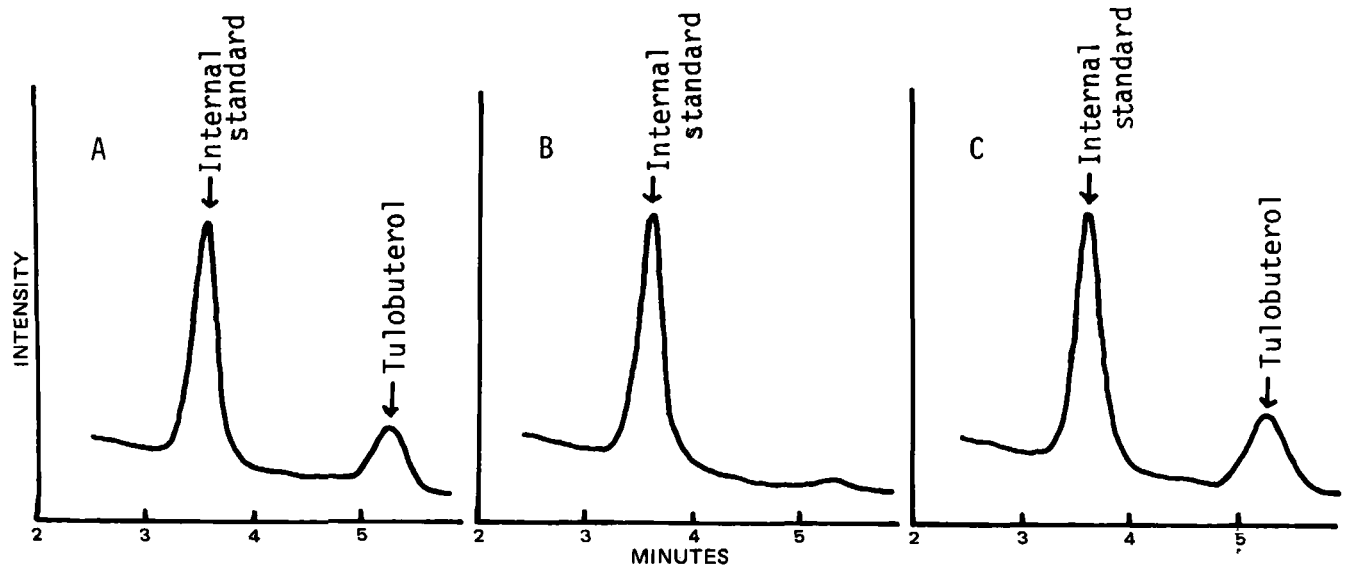

Figure 1—*Mass fragmentograms of serum extracts. Key: (A) serum to which known amounts of tulobuterol hydrochloride (4 ng) and internal standard (8 ng) were added; (B) serum free from drug; and (C) serum of volunteer who took 2 mg of tulobuterol hydrochloride orally (Subject 1, 3 hr).*

Although a previous assay (3) has been used extensively to determine serum levels of tulobuterol <10 ng/ml in humans, the present assay was found to be linear up to 50 ng/ml. When six replicate 1-ml samples of serum each containing 7 ng of tulobuterol hydrochloride/ml were analyzed, the mean value was 7.03 ng of tulobuterol hydrochloride/ml with a standard deviation of ±0.17 and a coefficient of variation of 2.42%. When serum samples containing 2 ng of tulobuterol hydrochloride/ml were analyzed, the mean value was 2.03 ng of tulobuterol hydrochloride with a standard deviation of ±0.10 ng/ml and a coefficient of variation of 4.93%.

To show the applicability of the method described, a serum concentration–time profile of tulobuterol was established from four volunteers following an oral dose of tulobuterol hydrochloride (Fig. 2). The among-subject variability was estimated by examination of the differences in the peak concentration and time to reach the peak.

Tulobuterol and some urinary metabolites can be determined simultaneously by mass fragmentography (9). Two major modifications were made in the present assay, compared with the single-extraction procedure used previously for tulobuterol in urine, *viz.*, ethyl acetate–acetone (3:1, v/v) extraction from a solution made alkaline with ammonium hydroxide. The extraction solvent was changed to ether, and back-extraction into the aqueous layer (pH ~1.0) and reextraction into the ether layer were added. Interference from endogenous materials in the serum was avoided with the improved cleanup procedure, although this was time consuming and lowered the overall recovery of tulobuterol. This method was inapplicable for determinations in human serum due to the low serum levels (<10 ng/ml) at a therapeutic dose (1–2 mg).

Albuterol and terbutaline were quantitated at low plasma levels using the relatively high mass ion $[M - 86]^+$ after forming the *tert*-butyldimethylsilyl derivatives (4, 5). In the case of tulobuterol, however, the relative intensity of $[M - 86]^+$ was <0.1%, which does not ensure adequate sensitivity.

To validate the present mass fragmentographic method, serum specimens were assayed by both mass fragmentography and electron-capture GLC. The analytical data obtained by both methods were correlated by linear regression analysis, yielding a correlation coefficient of 0.905 and a slope of 0.987 (Fig. 3).

In the latter method, an additional step was needed for the removal of excess derivatizing reagent (trifluoroacetic anhydride). Moreover,

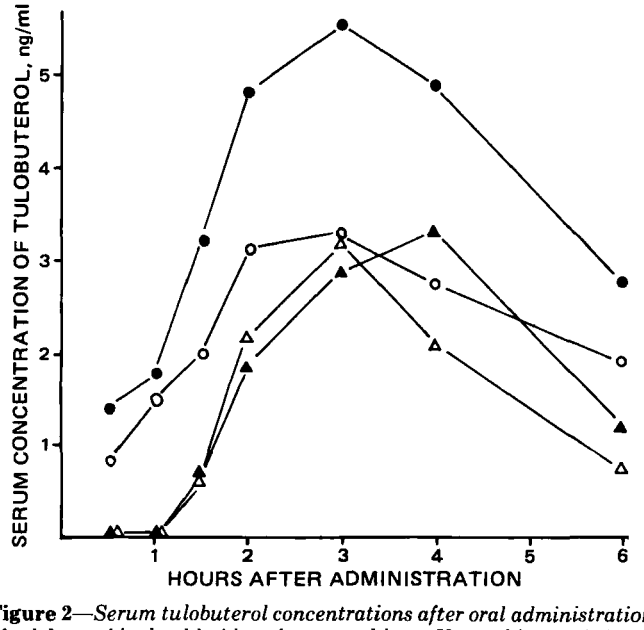

Figure 2—*Serum tulobuterol concentrations after oral administration of tulobuterol hydrochloride to human subjects. Key: subjects 1 (●) and 2 (○), administered in the form of a syrup solution; subjects 3 (▲) and 4 (△), administered in the form of tablets.*

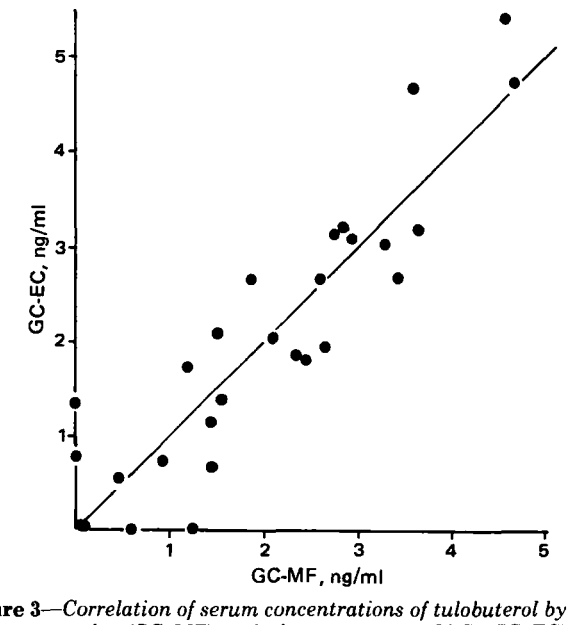

Figure 3—*Correlation of serum concentrations of tulobuterol by mass fragmentography (GC–MF) and electron-capture GLC (GC–EC). The line was calculated by least-squares linear regression analysis; y = 0.987x ± 0.060 and r = 0.905.*

measurement time of one sample in the electron-capture GLC (~15 min) is longer than that in the mass fragmentography. The mass fragmentography appears to be more readily adaptable to large numbers of samples.

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COMMUNICATIONS

Pharmacokinetics of Drugs in Blood III: Metabolism of Procainamide and Storage Effect of Blood Samples

Keyphrases □ Pharmacokinetics—procainamide metabolism, storage effect of blood samples, *N*-acetylprocainamide □ Procainamide metabolism—pharmacokinetics, storage effect of blood samples, *N*-acetylprocainamide

To the Editor:

The distribution kinetics and metabolism of drugs in blood are of importance in pharmacokinetic studies. For instance, if a drug equilibrates slowly between plasma and blood cells, the time elapsed between collection and centrifugation of a blood sample could have a significant effect on the plasma concentration measured, which might differ considerably from the true *in vivo* concentration. This has been recently shown with gentamicin and furosemide (1, 2). A similar problem might also be anticipated if a drug undergoes *in vitro* metabolism in the blood. The above phenomenon has been referred to as the storage effect of blood (1, 2). This communication reports our preliminary studies on the metabolism of procainamide in blood and the storage effect of blood samples for procainamide and its metabolites.

Freshly withdrawn[1], heparinized control blood from five healthy subjects, aged 25–41 years, was used. None of the subjects received any medication for at least 1 month prior to the study. The whole blood (10 ml) in test tubes[2] from each subject was spiked with procainamide[3] stock solution (2 mg/ml of free base) to yield a concentration of 20 μg/ml. After manually mixing for 30 sec, each blood sample was quickly divided (1.0 ml) into 10 vials. The sealed vials were then stored (time zero) in a refrigerator (5°). To prevent any interaction between the drug and the stopper, the screw caps of the test tubes and vials used were all lined with aluminum foil. Similar preparations were carried out with blood samples kept at ambient temperature (25 ± 1.0°). These temperatures were chosen in order to simulate common procedures for handling blood samples. The vials were removed and centrifuged immediately at various times up to 48 hr. All the plasma samples were frozen until analyzed for procainamide and its metabolite, *N*-acetylprocainamide, using a modified high-performance liquid chromatographic (HPLC) method which was developed earlier in this laboratory (3).

Briefly, the assay involved the addition of 0.25 ml acetonitrile to 0.1 ml of plasma sample in a test tube[2]. After vortex-mixing and centrifugation, 20 μl of the supernatant solution was injected directly onto the column. The instrumentation consisted of a solvent delivery pump[4], a syringe-loading sample injector[5], a cation exchange column[6], and a fixed-wavelength detector[7] with 254-nm filter. The mobile phase was made of 75% (v/v) 0.12 *M* ammonium phosphate acidified with phosphoric acid (0.2%) and 25% acetonitrile; the flow rate was 2.5 ml/min. By this method, the detection limit was 0.2 μg/ml for both procainamide and *N*-acetylprocainamide in plasma.

Figure 1 shows the typical plasma concentration profiles of procainamide from the above studies in three subjects. Assuming that procainamide initially was only confined to plasma, based on the individually determined hematocrits, one could estimate its concentrations from the three subjects to be 40, 40, and 35 μg/ml, respectively. However, the results (Fig. 1) show much lower concentrations (15.6–18.7 μg/ml) obtained immediately after spiking and brief mixing, indicating an initial rapid and extensive distribution of the drug to blood cells. Adsorption of the drug onto the glass tube could be ruled out, since there was no initial loss of procainamide from the whole blood in subsequent studies.

The plasma concentrations, in general, decreased with time (Fig. 1). However, fluctuations in the measured concentration were usually found during the initial 12 hr of

[1] Vacutainer, Division of Becton, Dickinson and Co., Rutherford, N.J.
[2] Fisher Scientific Co., Pittsburgh, Pa.
[3] Hydrochloride salt, E. R. Squibb & Sons, Inc., Princeton, N.J.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model 7125, Rheodyne, Berkeley, Calif.
[6] Partisil PXS 10/25 SCX, Whatman, Clifton, N.J.
[7] Model 440, Waters Associates, Milford, Mass.

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