Journal of Chromatography, 339 (1985) 440—444
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2510

Note

High-performance liquid chromatographic assay for trilostane and its major metabolite, 17-ketotrilostane, in human plasma

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(First received September 13th, 1984; revised manuscript received November 21st, 1984)

Trilostane [(4α,5α,17β)-4,5-epoxy-3,17-dihydroxyandrost-2-ene-2-carbonitrile] is a novel steroid [1] which is effective in inhibiting adrenal hormone production in experimental animals [2]. In humans, the drug is an effective adjunct in the treatment of Cushing's syndrome [3].

This report describes a high-performance liquid chromatographic (HPLC) method for the determination of trilostane (Fig. 1, I) and 17-ketotrilostane [(4α,5α)-4,5-epoxy-3-hydroxy-17-oxoandrost-2-ene-2-carbonitrile, Fig. 1, II], its major metabolite [4], in human plasma. The assay was used to quantitate trilostane and 17-ketotrilostane in the plasma of a subject who had received trilostane by oral administration.

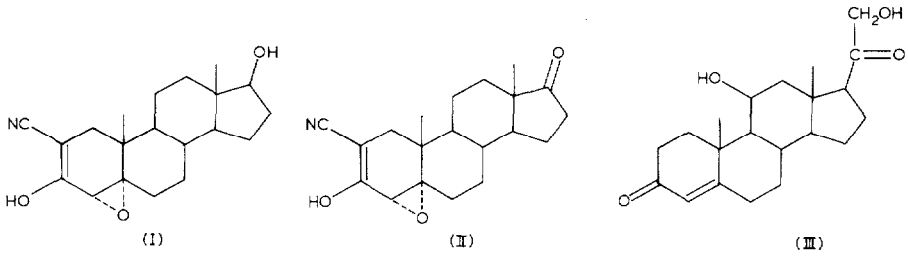

Fig. 1. The chemical structures of trilostane (I) 17-ketotrilostane (II) and the internal standard (III).

EXPERIMENTAL

Materials

Trilostane and 17-ketotrilostane were synthesized at the Sterling-Winthrop

0378-4347/85/$03.30

Research Institute. The internal standard corticosterone (4-pregnene-11β,21-diol-3,20-dione, Fig. 1, III) was obtained commercially from Sigma. Other chemicals were obtained commercially (reagent grade) and used without further purification.

Preparation of plasma standards and samples

Plasma standards were prepared by supplementing 1.0 ml of normal human plasma with a methanolic solution of trilostane and 17-ketotrilostane to achieve concentrations of 0 (methanol only), 0.050, 0.10, 0.25, 0.50, and 1.0 μg/ml. Duplicate standards at each concentration were prepared.

Two sets of randomized and coded samples were prepared for analysis under single-blind conditions, as above. Each plasma set contained triplicate samples at final concentrations of 0.12, 0.30 and 0.60 μg/ml. One set of plasma samples was analyzed immediately after preparation. The other set was stored in the laboratory freezer for five days before analysis.

Assay method

To a tube containing 1.0 ml of plasma (containing potassium oxalate as the anticoagulant) were added 50 μl of internal standard solution (0.065 mg/ml in methanol), 0.50 ml of 1 *M* phosphate buffer (pH 6.6) and 5.0 ml of diethyl ether. The samples were agitated on a rotary shaker for 10 min and centrifuged at 900 *g* for 10 min. The organic phase was transferred to a clean silanized 15-ml centrifuge tube. The aqueous phase was again extracted with 5.0 ml of diethyl ether as above. The organic phases were pooled, placed in a 60°C heating block, and were evaporated to dryness with the aid of a stream of nitrogen. The residue was dissolved in 300 μl of the HPLC mobile phase and 100 μl were injected into the HPLC system for analysis.

Chromatography

The HPLC system was operated isocratically at ambient temperature. The system consisted of an automatic injector (WISP, Waters Assoc., Milford, MA, U.S.A.), a pump (Model 45, Waters Assoc.), a 10 cm × 9.4 mm I.D. Partisil 5 CCS/C_8 RAC column (rapid analysis chromatography column, 5 μm particle size, Whatman, Clifton, NJ, U.S.A.), with a 37—50 μm particle size Phenyl/Corasil precolumn (Waters Assoc.), and a UV detector with a 280-nm filter (Model 440, Waters Assoc.). The mobile phase consisted of 0.1 *M* sodium acetate (adjusted to pH 5.0 with 0.1 *M* acetic acid)—methanol (40:60). The flow-rate was 2.0 ml/min.

The output of the detector was interfaced with a Model 3356 Laboratory Automation System (Hewlett-Packard, Palo Alto, CA, U.S.A.) computer for data acquisition and handling. Peak height ratios (trilostane:internal standard, 17-ketotrilostane:internal standard) for each standard and sample were calculated and least-squares regression analysis was performed on the peak height ratios versus nominal concentration. The concentrations of trilostane and 17-ketotrilostane in each sample were determined by inverse prediction from the linear regression [5]. The minimum quantifiable level (MQL) of the assay was estimated as that concentration at which the lower 80% confidence interval just encompassed zero concentration [6].

The observed concentrations for the prepared, spiked samples were expressed as percent differences from the nominal values. The range of these percent differences was used to define the accuracy of the assay. Precision was estimated from the standard deviation derived from the analysis of variance on the percent differences.

The percent recoveries of the extraction procedure for trilostane, 17-ketotrilostane and the internal standard were determined by comparing the peak heights of trilostane, 17-ketotrilostane and the internal standard with those obtained by direct injection.

Analysis of clinical samples

Plasma samples from one subject who had received two 60-mg capsules of trilostane were analyzed by the above procedure. Blood samples were taken at specified intervals by venipuncture. The plasma was separated by centrifugation and stored in the laboratory freezer until analyzed as described above.

RESULTS AND DISCUSSION

Analytical method

Representative chromatograms of an extracted plasma blank and an extracted plasma standard are shown in Fig. 2A and B, respectively. The results of the analysis of prepared plasma samples are summarized in Table I. A plot of peak height ratios (trilostane:internal standard, 17-ketotrilostane:internal standard) versus concentration for the plasma standards was linear over the range of 0—1.0 μg/ml of plasma, as determined by linear regression analysis ($y = 3.14x + 0.002$, $r = 0.999$ for trilostane and $y = 3.78x + 0.04$, $r = 0.999$ for 17-ketotrilostane).

An overall estimate of the assay precision, based on the derived standard

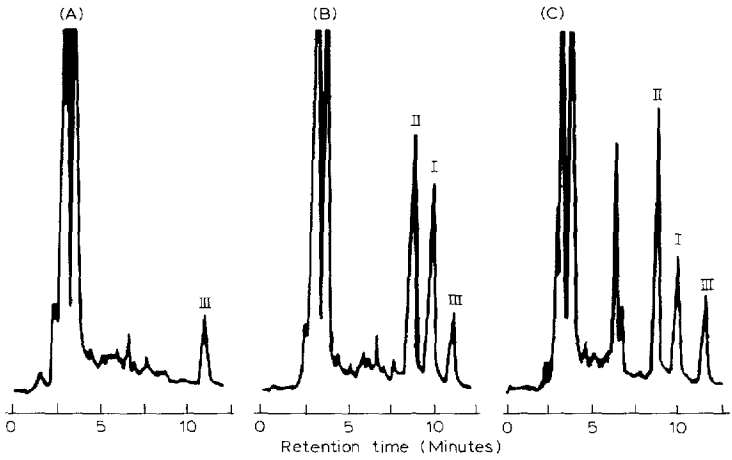

Fig. 2. Representative chromatograms of (A) an extracted plasma blank with internal standard (III) only; (B) an extracted plasma standard containing 1.0 μg/ml 17-ketotrilostane (II), 1.0 μg/ml trilostane (I), and internal standard (III); and (C) an extracted plasma sample from a human volunteer (taken 1 h after a 120-mg dose) containing 0.48 μg/ml trilostane (I), 1.3 μg/ml 17-ketotrilostane (II), and internal standard (III).

TABLE I

RESULTS OF THE ANALYSIS OF PREPARED PLASMA SAMPLES (n = 6)

Concentration added (μg/ml)	Mean concentration found (μg/ml)	Percent S.E.M.	Mean percent difference
Trilostane			
0.12	0.12	1.1	−1.2
0.30	0.30	0.6	1.4
0.60	0.60	0.5	−0.8
17-Ketotrilostane			
0.12	0.12	0.7	1.5
0.30	0.30	0.9	0.6
0.60	0.60	0.6	−0.1

deviation, was equal to 3.20% for trilostane and 3.89% for 17-ketotrilostane. The accuracy of the assay, defined by the range of the mean percent differences from the expected values, varied from −2.00% to 4.33% for trilostane, and from −0.44% to 2.22% for 17-ketotrilostane. The mean (± S.E.M.) MQLs of the two sets were 0.026 (± 0.006) μg/ml for trilostane and 0.028 (± 0.004) μg/ml for 17-ketotrilostane.

The fresh and frozen sample sets were compared to determine whether freezing affected the sample concentrations. The observed concentrations for the triplicate determinations were expressed as percent differences from the nominal values and analyzed in a two-way analysis of variance with replication to test for a concentration effect, a time (fresh versus frozen) effect, and a concentration-by-time interaction. No significant differences ($p > 0.05$) were observed for trilostane or 17-ketotrilostane.

The extraction efficiency studies in plasma indicated mean recoveries of 88% for trilostane, 84% for 17-ketotrilostane and 102% for the internal standard.

TABLE II

PLASMA CONCENTRATIONS OF TRILOSTANE AND 17-KETOTRILOSTANE IN CLINICAL SAMPLES FROM ONE SUBJECT RECEIVING 120 mg OF TRILOSTANE

Time after administration (h)	Trilostane concentration (μg/ml)	17-Ketotrilostane concentration (μg/ml)
0	<MQL*	<MQL**
0.5	0.08	0.09
1.0	0.48	0.83
2.0	0.35	1.33
3.0	0.45	1.33
4.0	0.24	0.98
6.0	0.04	0.31

*Less than the minimum quantifiable level of 0.02 μg/ml.
**Less than the minimum quantifiable level of 0.02 μg/ml.

The results of the analysis of the clinical plasma samples from one subject receiving 120 mg of trilostane are given in Table II. The maximum concentration found for trilostane was 0.48 μg/ml in the 1.0-h sample and the maximum level for 17-ketotrilostane was 1.3 μg/ml in the 2.0- and 3.0-h samples. Fig. 2C is a chromatogram of the 1.0-h sample.

CONCLUSION

In summary, an accurate, selective, reproducible and precise HPLC assay has been developed for the determination of trilostane and 17-ketotrilostane concentrations in human plasma. This method has proven useful for analysis of specimens obtained during clinical trials; details of these results will be reported elsewhere.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Jerome Edelson, Senior Director for Drug Safety Evaluation for his continuing interest and support.

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