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Determination of 17β-Hydroxy-1α-methyl-17α-propyl-5-α-androstan-3-one in Plasma by Gas Chromatography–Mass Spectrometry with Single-Ion Detection

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Abstract □ The topical anti-androgen 17β-hydroxy-1α-methyl-17α-propyl-5α-androstan-3-one is determined in plasma samples by extracting with ether and subsequent mass fragmentography with single-ion detection at *m/z* 303. 17β-Hydroxy-1α-methyl-17α-pentyl-5α-androstan-3-one, added to the samples before extraction, is used as the internal standard. Reproducibility was calculated to be ±5.9% at the 5-ng/mL level and 0.4% at the 20-ng/mL level. The limit of detection is ~1 ng/mL. Total gas chromatography–mass spectrometry analysis time is ~10 min/sample.

Keyphrases □ 17β- Hydroxy -1α-methyl-17α- propyl -5α- androstan -3-one—determination in plasma, gas chromatography—mass spectrometry, single-ion detection □ Mesterolone—17α-propyl analogue, determination in plasma, gas chromatography–mass spectrometry, single-ion detection

17β-Hydroxy-1α-methyl-17α- propyl -5α-androstan-3-one, the 17α-propyl analogue of mesterolone, is currently under investigation as a topical anti-androgen for the treatment of acne and seborrhea. Pharmacological data of this compound, which may act similarly to the 17α-propyl analogue of testosterone (1), have been reported earlier (2).

Topical anti-androgens act locally in the skin, and the systemic availability of this class of compounds should be as low as possible in order to avoid endocrinological disorders. Therefore, any analytical method applied to the determination of these drugs in plasma must be very sensitive to be able to detect very small portions of absorbed substances. Because the drug molecule may be metabolized during passage through the skin, there are also certain requirements regarding specificity of the assay. In the present report an analytical method for the determination of plasma concentrations of 17β-hydroxy-1α-methyl-17α-propyl-5α-androstan-3-one (I) is described, which is devoid of tedious extraction or derivatization procedures and may, therefore, be useful in clinical tolerance studies of this compound when relatively high doses of drug are administered. Furthermore, it may serve for controlling the specificity of an RIA procedure.

EXPERIMENTAL

Subjects and Medication—Two healthy male volunteers (40 and 31 years of age, and 88 and 70 kg, respectively) were treated topically two times daily with 10 mL of a 3% solution of I in 70% aqueous ethanol. The drug was spread over the total body of the volunteers. During the first week the skin area to which the drug has been applied was kept under occlusion for 22 h/d. Blood was drawn into heparinized syringes before the treatment and in weekly intervals up to 8 weeks after the beginning of the 6-week treatment. The samples were immediately centrifuged, and the plasma stored frozen until analysis.

Chemicals—All solvents (ethanol, *n*-hexane, and ether) were of analytical reagent grade[1] and were used without further purification.

Extraction Procedure—Fifty nanograms of the internal standard (17β-hydroxy-1α-methyl-17α-pentyl-5α-androstan-3-one) dissolved in 50 μL of *n*-hexane was transferred into a 15-mL stoppered test tube and evaporated to dryness under a stream of nitrogen. One milliliter of plasma was added, followed after a short mixing time by 3 mL of ether. After thoroughly mixing on a vortex mixer for 1 min and centrifugation at 1200×*g* for 5 min, the organic phase was transferred to another test tube and evaporated to give a final volume of ~10 μL. One microliter of this solution was analyzed by gas chromatography–mass spectrometry (GC–MS). The recovery of the extraction procedure was determined by spiking 1-mL plasma samples with either 20 or 50 ng of radiolabeled I, with subsequent determination of the radioactivity in the organic phase.

Gas Chromatography–Mass Spectrometry—A GC–MS system[2] with the following components was used: a gas chromatograph[3], a quadrupole mass spectrometer[4], and a data system[5]. The gas chromatograph was equipped with a CP-SiL 5-coated open-tubular glass column (25 m × 0.25 mm) used in the direct-coupling mode or, alternatively, a CP-SiL 5 CB wall-coated open-tubular fused silica column (25 m × 0.23 mm), which was directly introduced into the ion source.

The temperature program applied to the column was 240°C for 1 min, then rising to a final temperature of 260°C at a rate of 10°C/min. Using the fused silica column the program was 200°C for 2 min, then 10°C/min up to 300°C. The injector was kept at 290°C, the direct coupling device at 280°C. Helium was used as carrier gas with a pressure of 14 psi in front of the column. The split ratio was 1:5.

Calibration Curve—The standard curve was constructed with 1-mL blank plasma samples containing 0, 5, 10, 15, 20, and 50 ng of I and 50 ng of the internal standard. These samples were extracted by the method described above. The peak areas of the internal standard and drug were measured, and the calibration curve (peak area ratio of drug/standard *versus* the concentration of I) was constructed. The precision of the assay was calculated from consecutive determinations of the drug within 1 d and by comparison with the results obtained on different days.

[1] E. Merck, Darmstadt, West Germany.
[2] Finnigan 4021.
[3] Finnigan 9610.
[4] Finnigan 4000.
[5] Incos-type 2100.

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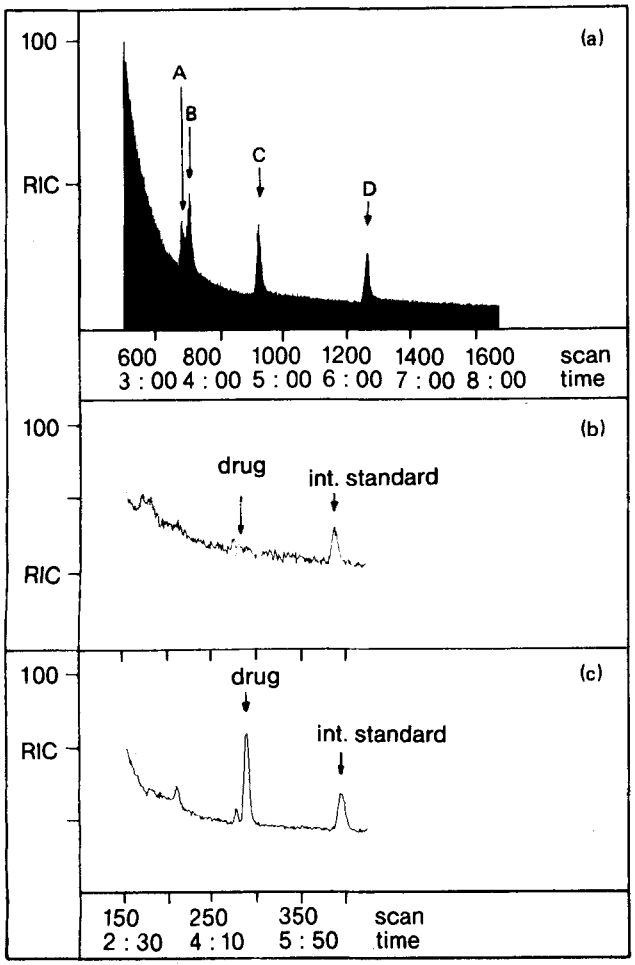

Figure 1—*Mass fragmentograms of (a) a mixture of mesterolone (A) and its 17α-methyl- (B), 17α-propyl- (C), and 17α-pentyl (D) analogues; (b) a plasma extract containing 5 ng of internal standard; and (c) a plasma extract with 7.5 ng of I and 5 ng of internal standard. The chromatograms were obtained with a CP-SiL 5 glass capillary column.*

RESULTS AND DISCUSSION

Assay—This report describes a sensitive and highly selective method for the determination of I in plasma utilizing GC–MS with single-ion detection. Plasma samples are extracted with ether after the addition of the internal standard, and the extract is evaporated to dryness, taken up in *n*-hexane, and injected directly into the GC–MS system.

Detection of the substances eluted from the chromatographic column is performed by means of single-ion measurements at *m/z* 303. This mass constitutes the base peak of the spectrum of both the drug and the internal standard, and it probably results from cleavage at the propyl and pentyl side chains.

The retention times of I and the internal standard using the glass column were 4.8 and 6.7 min, respectively. Figure 1 shows chromatograms of a blank plasma sample, of a sample containing drug and internal standard, and of some reference compounds.

Plasma concentrations of I were determined by comparing the peak areas of the drug and the internal standard added to the plasma before extraction.

Table I—Calibration Curves of the Assay Obtained on Five Different Days

Day	Calibration Curve[a]	Correlation Coefficient (r)
1	$y = -0.020 + 0.022x$	0.9990
2	$y = 0.001 + 0.019x$	0.9995
3	$y = 0.005 + 0.020x$	0.9997
4	$y = -0.007 + 0.021x$	0.9990
5	$y = -0.006 + 0.021x$	0.9997
Mean	$y = -0.005 + 0.021x$	0.9994

[a] Data obtained with the fused silica column; y = peak area of the drug/peak area of the internal standard and x = concentration of the drug (ng/mL).

Table II—Interassay Variation of Different Concentrations of I Using the Fused Silica Column

Amount Added ng/mL	Peak Area Ratio (Drug/Standard)	n	*RSD*, %
5	0.100 ± 0.016	5	16.0
10	0.201 ± 0.012	10	5.7
15	0.300 ± 0.011	5	3.7
20	0.394 ± 0.009	14	2.2

Table III—Plasma Concentrations of I Determined After Repeated Daily Topical Applications of 600 mg of Drug to Two Male Volunteers

Week	Subject 1	Subject 2	Mean ± *SD*
0	0	0	0 ± 0
1	16	14	15 ± 1
2	—	8	—
3	18	12	15 ± 4
4	20	15	18 ± 4
5	12	11	12 ± 1
6	11	11	11 ± 0
7	0	0	0 ± 0
8	0	0	0 ± 0

Typical calibration curves of the assay are described in Table I. The recovery of the extraction process was determined by measuring the radioactivity in the organic phase of samples spiked with ^{3}H-labeled drug. With 20 and 50 ng/mL, 83 ± 4 and 80 ± 1% were recovered in the ether phase (n = 5 each), respectively.

The recovery of the internal standard was identical with that of I, as could be demonstrated by establishing a calibration curve of unextracted drug. The data obtained were identical with that described in Table I.

The overall accuracy of the assay, expressed as the standard deviation of seven consecutive plasma determinations of 5 ng/mL, was 5.9%. With 20 ng/mL, an accuracy of 0.4% was calculated. The interassay variation, determined by comparing peak area ratios on different days, is given in Table II. The limit of detection was found to be ~1 ng/mL.

Plasma Concentrations—After daily repeated topical applications of 600 mg of I to two male volunteers, plasma levels of the drug were <20 ng/mL (Table III). During the first week of treatment the body was kept under occlusion for 22 h/d, while in the following weeks there was no occlusion. However, there were no differences in plasma levels between the two parts of the study. One week after the last treatment, the concentration of I had dropped below the limit of detection of ~1 ng/mL.

The analytical assay described differs from other methods for the detection of steroids by its simple extraction and, more importantly, lack of derivatization for a GC analysis. For similar molecules, such as testosterone and dihydrotestosterone, less simple procedures have been described in the literature (3–6). These compounds are extracted with ether, purified on Sephadex LH 20 and Lipidex 5000, derivatized with silylating agents, and finally detected in a high-resolution mass spectrometer either by single-ion monitoring or by metastable peak determination.

In the present report, on the other hand, ether extracts are taken to dryness, the residue is dissolved in *n*-hexane, centrifuged, and the supernatant injected onto the column; the drug concentration is assayed by single-ion detection in a quadrupole mass spectrometer. Deterioration of the capillary column, either the glass or the fused silica columns used, was not observed as might be expected from the very simple extraction procedure. The column was heated every evening at 300°C for 1 h. Therefore, we were able to analyze >300 plasma samples without any loss of resolution. The method described, therefore, seems to be suitable for routine analysis of I in clinical studies.

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