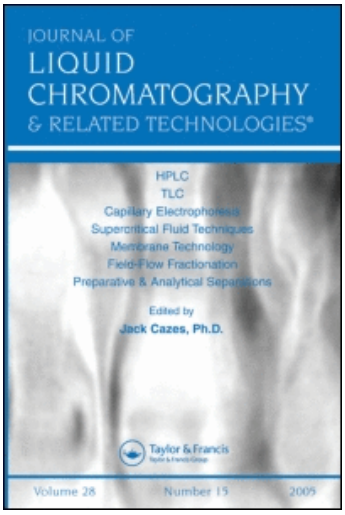

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DETERMINATION OF STEROIDAL HORMONES IN URINE SAMPLES BY MICELLAR LIQUID CHROMATOGRAPHY FOLLOWING SOLID-PHASE EXTRACTION

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ABSTRACT

Steroidal hormones were determined in spiked urine samples using micellar mobile phases of sodium dodecyl sulphate (SDS)-pentanol, solid-phase extraction (SPE), and detection in the UV region. In the optimized procedure, a 10 mL aliquot of urine sample is loaded into a C_{18} cartridge and washed with 2 mL of 50:50 (*v/v*) methanol-water, followed by 200 μL of 70:30 (*v/v*) methanol-water. The retained steroids are eluted with 2 mL of methanol and the eluate evaporated to dryness under nitrogen at 50°C.

The residue is redissolved with 200 μL of the micellar mobile phase used in the chromatographic separation and injected into the chromatograph. The performance of the procedure was checked for 13 steroidal hormones: dehydrotestosterone, methandienone, methenolone enanthate, methyltestosterone, dydrogesterone,

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medroxyprogesterone, medroxyprogesterone acetate, nandrolone, nandrolone decanoate, progesterone, testosterone, testosterone enanthate, and testosterone propionate.

Limits of detection were below 5 ng/mL for all steroids except nandrolone, nandrolone decanoate, and testosterone enanthate, whose elution from the SPE C_{18} column was not quantitative.

INTRODUCTION

Steroidal hormones are frequently administered in clinical practice, but they are also misused as anabolic drugs in humans and animals.[1] The use and abuse of these and other drugs have affected the bases of professional and amateur sport in the last decades.[2,3] In addition to ethical considerations, the negative side effects related to steroids intake have given rise to a strict regulation of their use. The availability of analytical methods to determine, unequivocally, steroidal hormones and their metabolites in physiological fluids is, thus, of primary importance.

Urine is the physiological fluid of choice for doping control because of convenience in taking the samples, relative simplicity, and accumulated experience. Steroidal hormones are widely metabolized in the organism. Consequently, detection of the parent drugs in urine samples requires procedures that reach limits of detection (LODs) down to 10 ng/mL. Gas chromatography, coupled to mass spectrometry (CG-MS), is a usual technique for the analysis of steroids in physiological fluids.[3–5]

Other reported procedures utilize high performance liquid chromatography (HPLC), usually coupled to very selective and sensitive detection systems.[6] The procedures are, however, too laborious and time-consuming, as they require extremely clean extracts and preconcentration of the steroids before assay. Several steps of liquid-liquid and solid-phase extraction (SPE), and even preparative HPLC, are often combined. SPE is usually carried out using C_{18} columns, and methanol or methanol-water mixtures as eluents.[3,6–9]

The wide range and high hydrophobicities of steroids (octanol-water coefficients, log $P_{o/w} = 3–8$)[10] present a challenge to the liquid chromatographic analysis of their mixtures. Amin *et al*.[11] reported a procedure for the analysis of four steroids: bolasterone, methyltestosterone, progesterone, testosterone, and testosterone acetate, with micellar mobile phases of sodium dodecyl sulphate (SDS) and acetonitrile, using a C_{18} column. Advantages, in terms of sensitivity, selectivity, simplicity, and cost, were found compared to normal phase and conventional reversed-phase chromatography. Mobile phases of SDS and acetonitrile are, however, too weak to elute at adequate retention times, highly hydrophobic steroids, such as clostebol acetate, nandrolone, nandrolone decanoate, progesterone,

testosterone enanthate, and testosterone propionate. We have shown in previous work, that micellar mobile phases containing a low amount of pentanol instead of acetonitrile can, in contrast, elute and resolve mixtures of steroids of diverse hydrophobicity.[12]

Sensitized fluorescence was proposed for the chromatographic detection of steroids in urine samples using SDS-acetonitrile mobile phases.[11] Reported LODs were 10–50 ng/mL when 200 μL of spiked urine sample was directly injected into the chromatograph without any previous treatment. A more recent report, which uses UV detection, gives LODs of 100 ng/mL for testosterone, medroxyprogesterone, and progesterone.[13] Using sensitized fluorescence or UV detection, we obtained in our laboratory, similar or higher LODs, owing to the high signal of the matrix, insufficient sensitivity, and background noise of the detection system.[14] These values are not sufficiently low. Previous cleaning of the sample and preconcentration of the steroids is needed before chromatographic analysis. Moreover, injection of a large volume of urine (200 μL) can produce rapid damage to the chromatographic column. The volume of physiological fluid injected into a micellar chromatographic system should not exceed 10 – 20 μL.

In this work, an SPE procedure with C_{18} columns, previous to chromatography with micellar mobile phases of SDS and pentanol, and detection in the UV region, is optimized for the separation of steroids from urine samples. The performance of the procedure is examined for 13 steroidal hormones: dehydrotestosterone, methandienone, methenolone enanthate, methyltestosterone, dydrogesterone, medroxyprogesterone, medroxyprogesterone acetate, nandrolone, nandrolone decanoate, progesterone, testosterone, testosterone enanthate, and testosterone propionate.

EXPERIMENTAL

Reagents and Columns

Stock solutions of 100 μg/mL of the following steroids were prepared in 0.10 M sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany): 1-dehydrotestosterone (DH), methandienone (MTD), methenolone enanthate (ME), methyltestosterone (MT) (Sigma, Madrid, Spain), dydrogesterone (DY) (Kalifarma, Barcelona, Spain), medroxyprogesterone (MD) (Frumtos-Zyma, Barcelona), medroxyprogesterone acetate (MX) (Cusí, Barcelona), nandrolone (ND) (Fher, Barcelona), nandrolone decanoate (DND) (Organón, Barcelona), progesterone (PG) (Seid, Barcelona), testosterone (T) (Schering, Madrid), testosterone enanthate (TE) (Schering, Madrid), and testosterone propionate (TP) (Schering, Madrid).

All compounds, except those of Sigma, were kindly donated by the pharmaceutical laboratories. Some steroids required some drops of methanol prior to the addition of the surfactant solution to be dissolved.

The mobile phases contained SDS and 1-pentanol (analytical grade, Merck). Methanol and chloroform (HPLC grade, Scharlau) were used to wash the chromatographic system and eliminate strongly retained solutes. The micellar mobile phases and the injected steroids solutions were filtered through Nylon membranes (0.45 μm, Micron Separations, Westboro, MA, USA). Nanopure water (Barnstead, Sybron, Taunton, MA, USA) was used throughout.

Solid-phase extraction of the steroids was carried out through cartridges containing 100 mg of C_{18} sorbent (Bond Elut, Varian, Harbor City, CA, USA), using methanol or SDS micellar solutions as eluents. Chromatographic separation was made with a Spherisorb ODS-2 column (5 μm, 120 mm × 4.6 mm i.d., Scharlau). A shorter Spherisorb ODS-2 column (35 mm x 4.6 mm i.d.) was used before the injector to saturate the mobile phase with silica.

Apparatus

A VacElut SPS 24 station (Varian, Harbor City, CA, USA) was used for SPE of the steroids from urine samples. A thermostatic bath (Grand Instruments, Cambridge, England) and a vortex mixer Reax 2000 (Heidolph Elektro, Kelheim, Germany) were utilized to evaporate the extraction eluent and facilitate the dissolution of the residues, respectively. HPLC was performed with a chromatograph provided with an isocratic pump and a UV-visible detector (Hewlett-Packard, Model HP 1050, Palo Alto, CA, USA). The steroids were detected at 246 nm, except dydrogesterone for which 292 nm was used. The flow-rate was 1 mL/min. The chromatographic signal was acquired through an integrator Model HP 3396A connected to a PC computer, using the softwares PEAK-96 (Hewlett-Packard, Avondale, PA, USA) and MICHROM.[15]

Procedure

C_{18} SPE cartridges are preconditioned with 2 mL of methanol, followed by 2 mL of water, at a flow-rate of 0.2 mL/min. Aliquots of 10 mL of urine sample (filtered through a glass filter) are loaded into the SPE cartridges and washed with 2 mL of 50:50 (*v/v*) methanol-water and 200 μL of 70:30 (*v/v*) methanol-water. The cartridge is dried with air and the retained steroids eluted with 2 mL of methanol. The eluate is collected in 10 mL tubes and methanol evaporated to dryness under nitrogen at 50°C. The residue is finally redissolved with 200 μL of the micellar mobile phase used in the chromatographic separation. The analyses are

performed by injection into the chromatographic column of 20 μL of the steroid extracts.

RESULTS AND DISCUSSION

Optimization of the Solid-Phase Extraction Step

Urine samples spiked with four steroidal hormones, medroxyprogesterone, medroxyprogesterone acetate, progesterone, and testosterone, were used to develop the extraction procedure. The recoveries and interferences from endogeneous compounds were studied by comparison of the chromatograms of standard aqueous solutions of the steroids before and after extraction, and the chromatograms of extracts of urine matrix and spiked urine samples. A solution of 0.12 M SDS containing 4.5% pentanol was used as mobile phase in the chromatographic analysis.

The retention of the steroids in the SPE C_{18} columns was checked to be quantitative (>90–95%). The elution protocol was optimized on the basis of previous procedures.[3,4,9,16] The following parameters were studied to achieve adequate cleaning and preconcentration of the urine sample: sample volume, nature and volume of the eluent, and composition and volume of the wash solvent.

Nature and Volume of the Eluent

The nature and volume of the eluent needed to get quantitative elution of the steroids from the SPE column, as well as adequate preconcentration, were first studied. Two procedures were considered to preconcentrate the steroids:

(i) loading of a large volume of sample into the SPE cartridge and elution with a small volume of the micellar solution used as mobile phase in the chromatographic procedure, and

(ii) elution with a volatile solvent, such as methanol, that could be evaporated, and redissolution of the dry extract with a small amount of the micellar mobile phase, before injection into the chromatograph.

The first procedure was simpler, but the results unsatisfactory. The recoveries for a 10 mL urine sample were too low (especially for medroxyprogesterone acetate and progesterone), when the volume of micellar mobile phase used as eluent was below 2 mL. The recoveries for the second procedure were above 92% and the preconcentration factor was ×50 when the steroids were eluted with 2 mL of methanol, and the dry extract redissolved with 200 μL of mobile phase.

However, the extracts obtained using 10 mL of urine were not sufficiently clean: a broad band appeared at the chromatogram front, which overlapped the

peaks of the most polar steroids (medroxyprogesterone and testosterone), making their determination difficult. The C_{18} cartridges should be consequently washed before the elution of the steroids to eliminate other compounds in the matrix.

Composition and Volume of the Wash Solvent

A washing step with a methanol-water mixture was added to the SPE procedure before the elution of the analytes. The composition and volume of the wash solvent were selected as follows: Aliquots of 10 mL of spiked urine samples were loaded into several C_{18} cartridges, washed with different volumes (2 to 8 mL) of 0:100, 20:80, 40:60, 50:50, and 60:40 (*v/v*) methanol-water, and extracted with 2 mL of methanol. The eluates were dried and the residues redissolved with 200 µL of micellar mobile phase. Aqueous solutions of the steroids not subjected to extraction, were used as reference to evaluate the recoveries.

The background of the chromatograms and recoveries scarcely changed at an increasing volume of the wash solvent, but the extracts were cleaner at a higher volume fraction of methanol. Pure water was not adequate as wash solvent. Figure 1 shows chromatograms of extracts obtained using a wash solvent of decreasing polarity. The front of the chromatogram was cleaner as the strength of the wash solvent increased, but important losses resulted for volume fractions of methanol above 50%.

The losses were in the 50 – 70% range for 60:40 (*v/v*) methanol-water. A washing step using 2 mL of 50:50 (*v/v*) methanol-water, followed by an additional wash with a small volume (200 µL) of a more apolar mixture, 70:30 (*v/v*) methanol-water, was found to yield cleaner chromatograms with lower losses of the steroids and higher reproducibility (Figure 2).

Chromatographic Analysis

The extracts of spiked urine samples collected from several volunteers in different days were chromatographed. Two micellar mobile phases containing 0.12 M SDS and different amounts of pentanol, 4.5% (mobile phase A) and 7% (mobile phase B), were used. With mobile phase A, most steroids were eluted in less than 20 min and the backpressure of the chromatographic system was lower. Mobile phase B was more convenient to elute the most hydrophobic steroids.

The retention times (min) of the steroids with mobile phases A and B were: medroxyprogesterone (4.0, 3.6), dehydrotestosterone (4.0, 3.7), methandienone (4.7, 4.2), testosterone (5.0, 4.4), medroxyprogesterone acetate (6.0, 4.9), dydrogesterone (8.6, 6.4), methyltestosterone (9.4, 5.0), progesterone (11.0, 7.2),

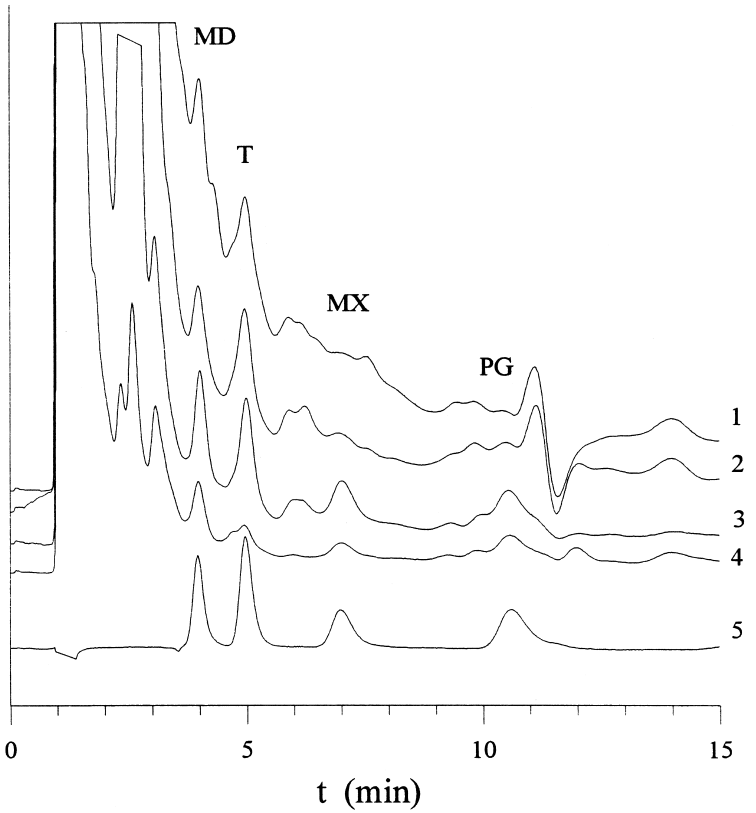

Figure 1. Chromatograms of extracts of 10 mL aliquots of urine spiked with 20 ng/mL of medroxyprogesterone, testosterone, medroxyprogesterone acetate, and progesterone. The C_{18} cartridges were washed with different methanol-water (*v/v*) mixtures: 20:80 (1), 40:60 (2), 50:50 (3), and 60:40 (4), and the steroids eluted with 2 mL of methanol. Chromatogram of a reference aqueous solution of the steroids (5). Mobile phase: 0.12 M SDS-4.5% (*v/v*) pentanol.

testosterone propionate (13.4, 9.2), nandrolone (16.4, 10.0), testosterone enanthate (17.7, 10.5), nandrolone decanoate (>20, 10.8), and methenolone enanthate (>20, 11.2).

The chromatograms of the urine extracts showed a band at their front and peaks of diverse intensity, due to endogeneous compounds found in the urine matrix, which could interfere with the determination of some steroids. The elution strength of the mobile phases used in this work is high, which means that the

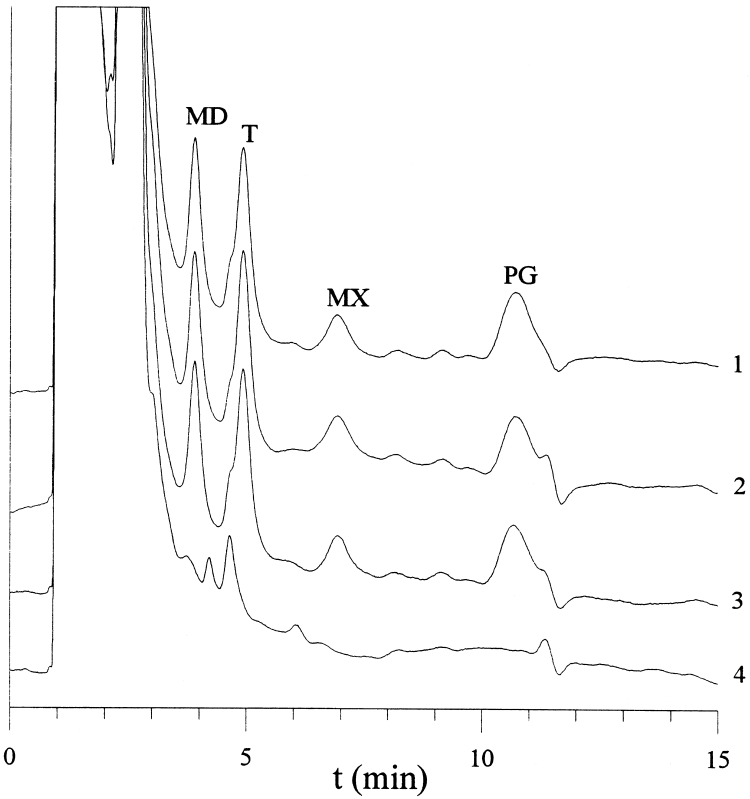

Figure 2. Chromatograms of the extracts of three 10 mL aliquots of a urine sample spiked with 20 ng/mL of medroxyprogesterone, testosterone, medroxyprogesterone acetate, and progesterone (1) – (3), and non-spiked sample (4). The SPE cartridge was washed with 2 mL of 50:50 (*v/v*) methanol-water, followed by 200 µL of 70:30 (*v/v*) methanol-water, the steroids were eluted with 2 mL of methanol, and the dried extract redissolved with 200 µL of mobile phase. Mobile phase: 0.12 M SDS-4.5% (*v/v*) pentanol.

hydrophobicity of the endogeneous compounds retained in these conditions should be high. These interferences were eliminated, in most cases, by changing the composition of the mobile phase.

The chromatograms of the extracts obtained from the same individual in different days were similar, but the width of the band at the front of the chromatograms and the intensity of the peaks of the endogeneous compounds varied for the extracts from different individuals. Also, occasionally, a sharp change in the baseline was achieved between 10.5 and 12 min with mobile phase A, and between 7.5 and 9 min with mobile phase B. The shape of this perturbation, and

the fact that its intensity varied in the chromatograms of the same extract, suggested a refractometric origin. This perturbation made the determination of progesterone with mobile phase A difficult, but the extracts of this steroid could be analyzed with mobile phase B.

Figures of Merit

Calibration curves were obtained for 13 steroidal hormones, after applying the optimized SPE procedure to spiked urine samples. In these series, an increasing volume of standard steroid solution of adequate concentration up to 2 mL, was added to 8 mL of urine sample and completed to 10 mL with water. The final concentration ranges were: 4 – 42 ng/mL for medroxyprogesterone, methyltestosterone, methenolone enanthate, testosterone, medroxyprogesterone acetate and progesterone, 12 – 120 ng/mL for testosterone propionate, and 20 – 210 ng/mL for nandrolone.

The solutions were next subjected to extraction, and injected by triplicate into the chromatograph. Areas of the chromatographic peaks were measured. The results were compared with those achieved by direct injection of reference aqueous solutions of the steroids, prepared in 0.12 M SDS medium. The concentrations of the steroids were 50-fold greater in the aqueous solutions than in the spiked urine samples, to compensate the preconcentration carried out in the SPE procedure.

The calibration curves of the reference solutions were obtained in the same day as the corresponding urine samples. Both mobile phases A (0.12 M SDS-4.5% pentanol) and B (0.12 M SDS-7% pentanol) were used.

Three solutions containing different steroids were prepared to obtain the calibration curves with mobile phase A, and four solutions for mobile phase B. For mobile phase A, the mixtures of steroids were: (i) medroxyprogesterone, medroxyprogesterone acetate, nandrolone, progesterone, and testosterone, (ii) dehydrotestosterone, methenolone enanthate, methyltestosterone and testosterone propionate, and (iii) dydrogesterone, methandienone and testosterone enanthate. For mobile phase B: (i) medroxyprogesterone, methenolone enanthate, methyltestosterone and testosterone propionate, (ii) medroxyprogesterone acetate, nandrolone, progesterone, and testosterone, (iii) dydrogesterone, methandienone and testosterone enanthate, and (iv) dehydrotestosterone and nandrolone decanoate.

The fitting parameters of the calibration curves for the extracts obtained from the spiked urine samples and reference aqueous solutions, are given in Table 1. Figure 3 depicts chromatograms of extracts of urine samples containing increasing concentration of several steroids. The chromatograms can be compared with those of urine matrix (without steroids) in the bottom of Figures 3a and 3b. The differences between the slopes of the calibration curves obtained

Table 1. Calibration Parameters for the Steroidal Hormones Eluted with SDS-Pentanol Mobile Phases ($y = c_0 + c_1 x$)[a]

Compound	Mobile Phase[b]	c_0	c_1	r
Medroxyprogesterone	Urine (A)	−0.011 ± 0.008	17.8 ± 0.4	0.9990
	Reference (A)	0.091 ± 0.004	18.13 ± 0.19	0.9995
	Urine (B)	−0.06 ± 0.02	22.5 ± 1.1	0.995
	Reference (B)	−0.09 ± 0.02	18.9 ± 1.0	0.995
Dehydrotestosterone	Urine (A)	−0.02 ± 0.02	8.1 ± 0.9	0.97
	Reference (A)	−0.01 ± 0.03	24.4 ± 1.2	0.994
	Urine (B)	−0.08 ± 0.06	18 ± 5	0.92
	Reference (B)	−0.088 ± 0.016	21.9 ± 0.7	0.998
Methandienone	Urine (A)	−0.02 ± 0.03	22.4 ± 1.0	0.997
	Reference (A)	−0.014 ± 0.011	24.5 ± 0.4	0.9995
	Urine (B)	0.07 ± 0.03	17.8 ± 1.1	0.990
	Reference (B)	0.02 ± 0.04	20.2 ± 1.7	0.98
Testosterone	Urine (A)	−0.25 ± 0.02	20.5 ± 1.0	0.995
	Reference (A)	0.018 ± 0.010	23.2 ± 0.4	0.9990
	Urine (B)	0.16 ± 0.02	14.2 ± 1.0	0.990
	Reference (B)	−0.08± 0.03	29.4 ± 1.2	0.997
Methyltestosterone	Urine (A)	0.00 ± 0.02	27.9 ± 0.9	0.998
	Reference (A)	−0.007 ± 0.03	22.9 ± 1.3	0.990
	Urine (B)	0.006 ± 0.03	27.8 ± 1.1	0.997
	Reference (B)	−0.005 ± 0.006	22.9 ± 0.3	0.9997
Medroxyprogesterone acetate	Urine (A)	−0.018 ± 0.004	12.10 ± 0.16	0.9997
	Reference (A)	−0.004 ± 0.005	15.9 ± 0.2	0.9997
	Urine (B)	−0.023 ± 0.005	19.2 ± 0.2	0.9997
	Reference (B)	−0.09 ± 0.03	21.0 ± 1.1	0.995
Dydrogesterone	Urine (A)	0.055 ± 0.013	33.4 ± 0.6	0.9990
	Reference (A)	−0.07 ± 0.03	37.7 ± 1.3	0.997
	Urine (B)	0.14 ± 0.07	73.3 ± 2.9	0.997
	Reference (B)	0.04 ± 0.15	86 ± 6	0.990
Progesterone	Urine (A)	0.002 ± 0.016	20.1 ± 0.7	0.997
	Reference (A)	0.012 ± 0.004	21.0 ± 0.2	0.9995
	Urine (B)	0.028 ± 0.015	18.4 ± 0.7	0.997
	Reference (B)	−0.11 ± 0.03	27.6 ± 1.5	0.995
Testosterone propionate	Urine (A)	0.053 ± 0.08	19.8 ± 1.8	0.98
	Reference (A)	−0.08 ± 0.03	23.4 ± 0.6	0.9990
	Urine (B)	−0.10 ± 0.08	23.2 ± 1.3	0.995
	Reference (B)	−0.31 ± 0.14	31.5 ± 2.2	0.990
Testosterone enanthate	Urine (B)	0.05 ± 0.03	8.1 ± 0.5	0.996
	Reference (B)	−0.35 ± 0.08	19.1 ± 0.8	0.997
Nandrolone	Urine (A)	−0.04 ± 0.07	7.5 ± 0.6	0.98
	Reference (A)	−0.38 ± 0.10	17.9 ± 0.9	0.990

Table 1. Continued

Compound	Mobile Phase[b]	c_0	c_1	r
	Urine (B)	0.053 ± 0.05	7.8 ± 0.5	0.995
	Reference (B)	−0.14 ± 0.06	13.4 ± 0.6	0.996
Nandrolone decanoate	Urine (B)	−0.14 ± 0.04	5.2 ± 0.3	0.990
	Reference (B)	−0.21 ± 0.07	9.5 ± 0.6	0.995
Methenolone enanthate	Urine (A)	0.05 ± 0.03	3.1 ± 0.2	0.990
	Reference (A)	−0.07 ± 0.05	4.6 ± 0.6	0.98
	Urine (B)	−0.12 ± 0.07	8.7 ± 0.6	0.990
	Reference (B)	−0.06 ± 0.06	9.4 ± 0.8	0.990

[a] The concentration of steroidal hormones (x) is given in μg/mL; y is area of the chromatographic peak.
[b] Mobile phases A (0.12 M SDS-4.5% pentanol) and B (0.12 M SDS-7% pentanol) were used. Reference calibration curves were made with aqueous solutions of the steroids not subjected to extraction.

with the spiked samples and reference solutions, for medroxyprogesterone, methandienone, methyltestosterone, medroxyprogesterone acetate, dydrogesterone, testosterone propionate, and methenolone enanthate, were similar to the variations observed in the calibration curves obtained in different days. The fitting parameters did not depend, significantly, on the composition of the mobile phase.

As commented above, the peaks of endogeneous compounds can interfere the determination of some steroids. Thus, with both mobile phases, there was a peak next to the peaks of testosterone and progesterone at shorter retention times, which affected the accuracy of the determinations (Figure 3). The results were, however, acceptable for the mobile phase of lower elution strength (mobile phase A), whereas with mobile phase B, the slopes of the calibration curves obtained with urine were significantly lower than for the aqueous solutions, although, the correlation coefficients were good (Table 1).

The peaks of the most retained steroids (methenolone enanthate, nandrolone, nandrolone decanoate, and testosterone enanthate) were very broad with mobile phase A. Better peaks were obtained with mobile phase B, but there was some overlapping with the peak of an endogeneous compound at 10 min. Moreover, the slopes of the calibration curves for nandrolone, nandrolone decanoate, and testosterone enanthate were half the slopes obtained with the reference solutions, but the correlation coefficients were, again, good. This suggests, that the elution with methanol of these steroids from the SPE C_{18} columns (where they are highly retained), although reproducible, is not quantitative. The

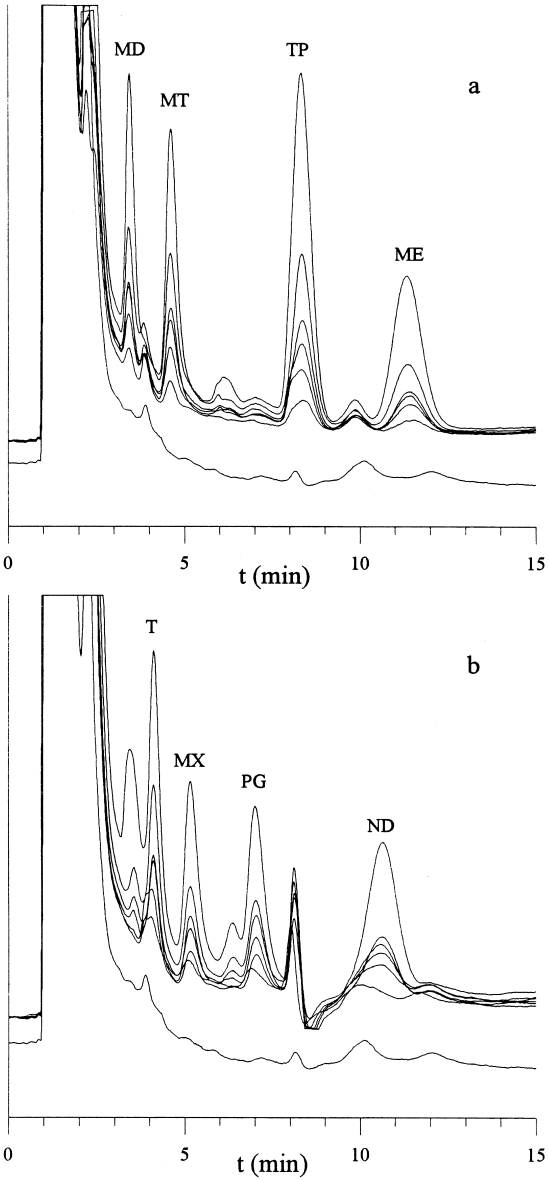

Figure 3. Chromatograms of urine samples spiked with increasing amounts of: (a) medroxyprogesterone, methyltestosterone, testosterone propionate, and methenolone enanthate, (b) testosterone, medroxyprogesterone acetate, progesterone and nandrolone. The concentrations of the steroids were 4, 8, 12, 17, 22, and 42 ng/mL, except for testosterone propionate (12, 22, 38, 50, 60, 120 ng/mL), and nandrolone (20, 45, 65, 85, 110, 210 ng/mL). Lower chromatogram in (a) and (b) belongs to urine matrix. Mobile phase: 0.12 M SDS-7% (*v/v*) pentanol.

Table 2. Limits of Detection (ng/ml, 3s Criterion) for the Steroidal Hormones Spiked in Urine Samples and Eluted with SDS-Pentanol Mobile Phases[a]

Compound	Mobile Phase A	Mobile Phase B	Compound	Mobile Phase A	Mobile Phase B
Medroxyprogesterone	1.6	2.6	Progesterone	1.2	1.2
Dehydrotestosterone	3.6	5.0	Testosterone propionate	3.4	12
Methandienone	1.7	2.4	Testosterone enanthate	-	12
Testosterone	2.7	1.9	Nandrolone	19	11
Methyltestosterone	0.8	4.1	Nandrolone decanoate	-	22
Medroxyprogesterone acetate	1.3	1.3	Methenolone enanthate	4.6	5.8
Dydrogesterone	1.0	0.7			

[a]Mobile phases A (0.12 M SDS-4.5% pentanol) and B (0.12 M SDS-7% pentanol).

use of an eluent of lower polarity or a more polar column in the extraction step will likelyimprove the recoveries.

Calculation of the LODs was made, according to the 3s criterion, by measuring the areas of the chromatographic peaks in five independent extracts (triplicate injections) of spiked urine samples at very low concentrations of the steroids (close to the LODs) . LODs (see Table 2) were below 5 ng/mL for medroxyprogesterone, dehydrotestosterone, methandienone, testosterone, methyltestosterone, medroxyprogesterone acetate, dydrogesterone, and progesterone, eluted with both mobile phases A and B, and also for testosterone propionate and methenolone enanthate with mobile phase A. The proposed procedure permits, therefore, the detection and quantification of ten steroids at the levels found in urine samples. The recoveries for nandrolone, nandrolone decanoate, and testosterone enanthate are not adequate due to excessive retention in the C_{18} SPE columns. Although, the direct injection of the sample is not possible, the proposed procedure is simpler than other reported procedures.

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