Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 828 (2005) 21–26
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantitative analysis of androst-4-ene-3,6,17-trione and metabolites in human urine after the administration of a food supplement by liquid chromatography/ion trap-mass spectrometry

K. Deventer [a], P. Van Eenoo [a,*], P. Mikulčíková [b], W. Van Thuyne [a], F.T. Delbeke [a]

[a] *Department of Clinical Biology, Microbiology and Immunology, Doping Control Laboratory (DoCoLab), Ghent University-UGent, Technologiepark 30, B-9052 Zwijnaarde, Belgium*
[b] *Department of Analytical Chemistry, University of Pardubice, nám. Čs. legií 565, 532 10 Pardubice, Czech Republic*

Received 30 March 2005; accepted 30 August 2005
Available online 6 October 2005

Abstract

6-OXO®, a new nutritional supplement commercially available on the internet, is sold as an aromatase-inhibitor and contains androst-4-ene-3,6,17-trione as active ingredient. This anabolic steroid is a prohibited substance in sports. Androst-4-ene-3,6,17-trione is metabolised to androst-4-ene-6α-ol-3,17-dione and androst-4-ene-6α,17β-diol-3-one. A fast, sensitive and accurate LC/MS method was developed and validated for the quantification of androst-4-ene-3,6,17-trione and its metabolites in urine. The method is capable of determining the stereochemical position of the hydroxy-group at C-6 of the metabolites and consists of a liquid–liquid extraction step with diethylether after enzymatic hydrolysis, followed by separation on a reversed phase column. Ionisation of the analytes is carried out using atmospheric pressure chemical ionisation. The limit of quantification of the method was 5 ng/mL for all compounds. The accuracy ranged from 14.8 to 1.3% for androst-4-ene-3,6,17-trione, 9.4 to 1.6% for androst-4-ene-6α-ol-3,17-dione and 4.1 to 3.2% for androst-4-ene-6α,17β-diol-3-one in the range of 5–1000 ng/mL. Using this method androst-4-ene-6α-ol-3,17-dione was identified as a major urinary metabolite, whereas androst-4-ene-6α,17β-diol-3-one as a minor metabolite. While the parent compound is predominantly excreted in conjugated form, both metabolites are solely excreted as conjugates.

Keywords: Androst-4-ene-3,6,17-trione; Doping; Urine; Androst-4-ene-6α-ol-3,17-dione

1. Introduction

Anabolic steroids are widely used by athletes to increase muscle mass [1]. In most cases anabolic steroids are intensively metabolised [2] and hence the elucidation of the metabolism of an anabolic steroid is necessary to detect its misuse.

Over the last decade a number of certain type of anabolic steroids, so-called prohormones, have become available in the United States as nutritional supplements [3]. The term "prohormone" is used because initially these substances were precursors of testosterone (T) or nortestosterone. These products are sold as over-the-counter products under the 1994 Dietary Supplement Health and Education Act, although the recently signed "Anabolic steroid Control Act of 2004" is intended to put a ban on these substances. Global distribution via the internet has resulted in a huge international commercial success for products including androst-4-ene-3,17-dione (AD), 19-norandrostenedione and dehydroepiandrosterone. The use of these substances constitutes a doping offence according to the regulations [4] of the World Anti-Doping Agency (WADA).

The target analyte of this study, androst-4-ene-3,6,17-trione, is a new anabolic steroid that has shown aromatase-inhibiting properties in several in vitro experiments [5–7]. Presently, this steroid is advertised as an anti-estrogenic agent, to be used by athletes to treat gynecomastia. A recent qualitative GC/MS method has revealed that androst-4-ene-3,6,17-trione is metabolised to androst-4-ene-6α-ol-3,17-dione and androst-4-ene- 6α, 17β-diol-3-one [8]. Using this GC/MS method an additional derivatisation step was needed to determine the position of the hydroxyl function at the C-6.

Until now, quantitative data is lacking and no method has been described for the direct detection of 6-hydroxy-steroids that

* Corresponding author. Tel.: +32 9 3313295; fax: +32 9 3313299.
E-mail address: peter.vaneenoo@ugent.be (P. Van Eenoo).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.08.024

retains the stereochemical information at the C-6 atom, although these steroids have been reported previously as metabolites of several steroids [9–12].

2. Experimental

2.1. Chemicals and reagents

Androst-4-ene-3,6,17-trione (6-oxo-AD), androst-4-ene-6α-ol-3,17-dione (6α-OH-AD), androst-4-ene-6β-ol-3,17-dione (6β-OH-AD), androst-4-ene-17β-ol-3,6-dione, androst-4-ene-6α, 17β-diol-3-one (6α-OH-T) and androst-4-ene-6β,17β-diol-3-one (6β-OH-T) were purchased from Steraloids (Newport, RI, USA). The internal standard (desoximetasone) and the β-glucuronidase preparation (type HP-2; ≥7500 Units/mL sulphatase, ≥92,500 Units/mL glucuronidase) were purchased from Sigma (Bornem, Belgium). Analytical grade sodium acetate, potassium carbonate, sodium hydrogen carbonate, glacial acetic acid and diethylether were from Merck (Darmstadt, Germany), HPLC grade acetonitrile was from Biosolve (Geel, Belgium) and HPLC grade water was from Fischer (Loughborough, UK) The nutritional supplement 6-OXO® was bought from Ergopharm (Champaign, USA) via the internet. The content of one capsule was 100 mg androst-4-ene-3,6,17-trione (6-oxo-AD). The manufacturer's recommended daily dosage was three to six capsules in cycles of 4–6 weeks.

2.2. Excretion study

The study was performed with six healthy male volunteers. The study protocol was reviewed and approved by the ethical committee of Ghent University Hospital. In agreement with the conditions of such ethical committee approval, each volunteer signed a statement of informed consent. Two volunteers took the recommended daily dose of three capsules for 3 consecutive days in the morning. Urine samples were collected before (0 h) and quantitatively 2, 4, 6, 8, 10 and 12 h after intake during the administration period. Additional samples were taken 24, 30, 36 and 48 h after the last capsule. The other four volunteers took one capsule in the morning. Samples were collected quantitatively before (0 h) and 2, 4, 6, 8, 10 and 12 h after administration. Additional samples were taken after 24, 30, 36 and 48 h.

All urine samples were either analyzed directly or stored at −20 °C awaiting analysis. Urinary pH, volume and density were measured and all samples were analyzed in duplicate. When necessary, urine samples were diluted with blank urine in order to obtain concentrations in the range of the calibration curve.

2.3. Sample treatment

Sample clean-up was kept to a minimum and a previously described method for the extraction of anabolic steroids and analysis by GC/MS was used [8].

After the addition of the internal standard solution (50 μL desoximetasone, 10 μg/mL) to 3 mL of urine, 1 mL sodium acetate buffer (pH 5.2) and 50 μl β-glucuronidase were added and the samples were hydrolysed for 2.5 h at 56 °C. After cooling, 100 mg of a solid buffer containing sodium hydrogen carbonate and potassium carbonate (2:1, w/w) was added to the hydrolysate. Liquid–liquid extraction was performed by rolling for 20 min with 5 mL diethylether. After centrifugation (2500 rpm) the organic layer was separated and evaporated under oxygen free nitrogen. The residue was dissolved in 200 μL of the initial mixture of the mobile phase.

For the determination of the percentage of nonconjugated metabolites, the samples were analysed similarly, except for the exclusion of hydrolysis step.

2.4. Method validation

A six-point calibration curve was established between 5 and 1000 ng/mL (5, 10, 50, 100, 500 and 1000 ng/mL) for 6-oxo-AD, 6α-OH-AD and 6α-OH-T. Therefore, blank urine was spiked with the appropriate amount of standards. Each concentration was analyzed in triplicate, the averages were used to construct the calibration curve. The area ratio of the protonated molecules of the compounds of interest and the protonated molecular ion m/z 377 of the internal standard were plotted versus the concentration.

The precision and accuracy of the method were tested at three levels (5, 100 and 1000 ng/mL). Precision was assessed as the percentage relative standard deviation (%R.S.D.) of both repeatability (within-day) and reproducibility (between-day and different analysts) for a selected compound and level. Maximum allowed tolerances for reproducibility and repeatability can be calculated from the Horowitz-equation $\text{R.S.D.}_{\max} = 2^{(1-0.5\log C)}$ (C = concentration (μg/mL) × 10^{-6}). The maximum allowed tolerances for repeatability and reproducibility are $2/3\text{R.S.D.}_{\max}$ and $\text{R.S.D.}_{\max}$, respectively [13].

Accuracy (expressed as mean error) was defined as the difference between the calculated amount and the specified amount for the selected compound and expressed as a percentage. The limit of quantification (LOQ) of the method was defined as the lowest concentration where acceptable reproducibility and accuracy could be guaranteed. Selectivity was tested by analysing several structurally related and other routinely screened doping agents, including corticosteroids and anabolic steroids. Concentrations of these mixtures were 1 μg/mL.

Specificity was tested during the validation procedure in which ten blank urines were extracted and analysed as described above.

Blank urine sample, system blank and a quality control sample (spiked at 100 ng/mL) were analyzed concurrently in each batch of samples.

Extraction recovery of all compounds was calculated. For this purpose negative urine samples ($n = 6$) were spiked at three levels (5, 100 and 1000 ng/mL) and extracted together with non spiked negative urine samples ($n = 6$). The extracts of the non spiked urine samples were then spiked at the three different levels simulating a 100% recovery. Both sets of samples were then evaporated and analysed with the described LC/MS method. The obtained peak areas of the two sets of samples were compared.

2.5. Instrumentation

Separation of the compounds was performed on a Nucleosil C18 column (100 mm × 3 mm, 5 μm; Chrompack, Antwerp, Belgium) using a P4000 pump and a model AS3000 autosampler (TSP, San Jose, USA). The mobile phase consisted of acetonitrile and 1% acetic acid in water. Gradient elution at a flow rate of 0.3 mL/min was as follows: 70% acetic acid (1%) for 2 min, followed by a linear decrease to 30% in 3 min, isocratic for 5 min, followed by an increase in 0.5 min to 70% acetic acid (1%) which was maintained for 8 min before the next injection (equilibration time). The total run time of the method was 18 min. The injection volume was 50 μL.

Ionization of the analytes was carried out on a LCQ-Deca instrument (Thermo, San Jose, USA) using atmospheric pressure chemical ionisation (APCI) in the positive ionisation mode. The corona discharge current was set at 5 μA. The capillary temperature and evaporator temperature were maintained at 150 and 300 °C, respectively. The drying gas (nitrogen, LASAL2001, Air Liquide, Belgium) was maintained at 80 units while the auxiliary gas (nitrogen) was set to 10 units. The capillary voltage was maintained at 10 V.

For the MS/MS experiments the collision energy (CE) was set at 35%. The isolation width was set at 3.0 and an activation q of 0.250 was applied.

3. Results and discussion

3.1. Mass spectrometry

Flow injection analysis was performed to determine (the presence of) diagnostic ions for all the compounds. For each tested compound a solution of 5 μg/mL was infused at a flow rate of 10 μL/min. In general, APCI is less susceptible to matrix effects [14,15] and hence this ionisation technique was preferred as interface. Nevertheless the presence of these effects was examined. For this purpose the blank urine samples from every volunteer collected before administration (0 h) were extracted together with a blank (aqua bidest). Analysis was performed while a dilute solution of the analytes was infused at a constant rate in the effluent flowing from the LC systems to the mass spectrometer. The obtained chromatograms did not show any fluctuation (positive or negative) in baseline at the retention times of the analytes.

Positively charged $[M + H]^+$ ions were observed for all compounds in full scan MS.

All compounds were tested in full scan MS/MS as well and similar fragmentation patterns were observed (Fig. 1). For all compounds consecutive loss of two water molecules $[M + H - H_2O]^+$ and $[M + H - 2H_2O]^+$, a very common fragmentation pattern, was observed. The intensive fragmentation in MS/MS substantially reduces the signal-to-noise ratio for the characteristic ions compared to the protonated molecular ion in full scan MS. Moreover the most abundant product ions $[M + H - H_2O]^+$ and $[M + H - 2H_2O]^+$ lack specificity in the hydrolysed samples, a phenomenon previously noticed for beclomethasone [16]. Hence better sensitivity was observed in full scan MS. Consequently quantification was done in full scan APCI+-mode and MS/MS was only used for qualitative purposes.

3.2. Extraction and chromatography

For chromatography a reversed phase column was preferred, since these columns show good selectivity for the separation of steroids [17,18]. 6α-OH-T (retention time $t_t = 4.75 \pm 0.13$, $n = 396$), 6α-OH-AD (retention time $t_t = 6.32 \pm 0.10$, $n = 396$) and 6-oxo-AD (retention time $t_t = 7.83 \pm 0.06$, $n = 396$) exhibited well separated peaks under the chromatographic conditions described.

Usually, hydrolysis of urine samples leads to an increase in matrix background. As a consequence an isocratic step of 2 min was included into the gradient program allowing non-retained and/or poorly retained matrix compounds, resulting from the hydrolysis, to elute in the early stage of the run.

3.3. Method validation

Using a least square fit, good linearity ($r^2 \geq 0.998$) was observed for all compounds in the range 5–1000 ng/mL. None of the calibration curves was forced through the origin and for the regression calculation a weighing factor of $1/x$ was used for all data points.

The results for precision and accuracy are summarised in Table 1.

As shown in Table 1, these values were never exceeded for either repeatability or reproducibility (5, 100 and 1000 ng/mL). Deviation of the mean measured concentration from the theoretical concentration (accuracy) for all compounds and were below the acceptable threshold of 15% [19] for all the levels in the range of calibration curve.

Regarding the selectivity, interference from other monitored doping agents could not be found. In addition analysis of 10

Table 1
Accuracy (between-day), repeatability, reproducibility and tolerance limits of the LC/MS method at three concentrations including the lowest point of the calibration curve for 6-oxo-androstenedione (6-oxo-AD), 6α-OH-androstenedione (6α-OH-AD), 6α-OH-testosterone (6α-OH-T)

Concentration (ng/mL)	Accuracy (%), $n = 18$			Repeatability (%), $n = 6$			Reproducibility (%), $n = 18$			R.S.D.$_{max}$ (%)	2/3R.S.D.$_{max}$ (%)
	6-oxo-AD	6α-OH-AD	6α-OH-T	6-oxo-AD	6α-OH-AD	6α-OH-T	6-oxo-AD	6α-OH-AD	6α-OH-T		
5	−14.8	9.4	−4.1	9.0	14.5	14.9	11.8	14.9	13.3	35	23
100	11.3	−5.7	2.4	3.5	6.1	4.1	7.4	8.2	7.1	22	15
1000	−1.3	−1.6	−3.2	6.1	8.5	5.2	8.4	9.5	7.4	16	10

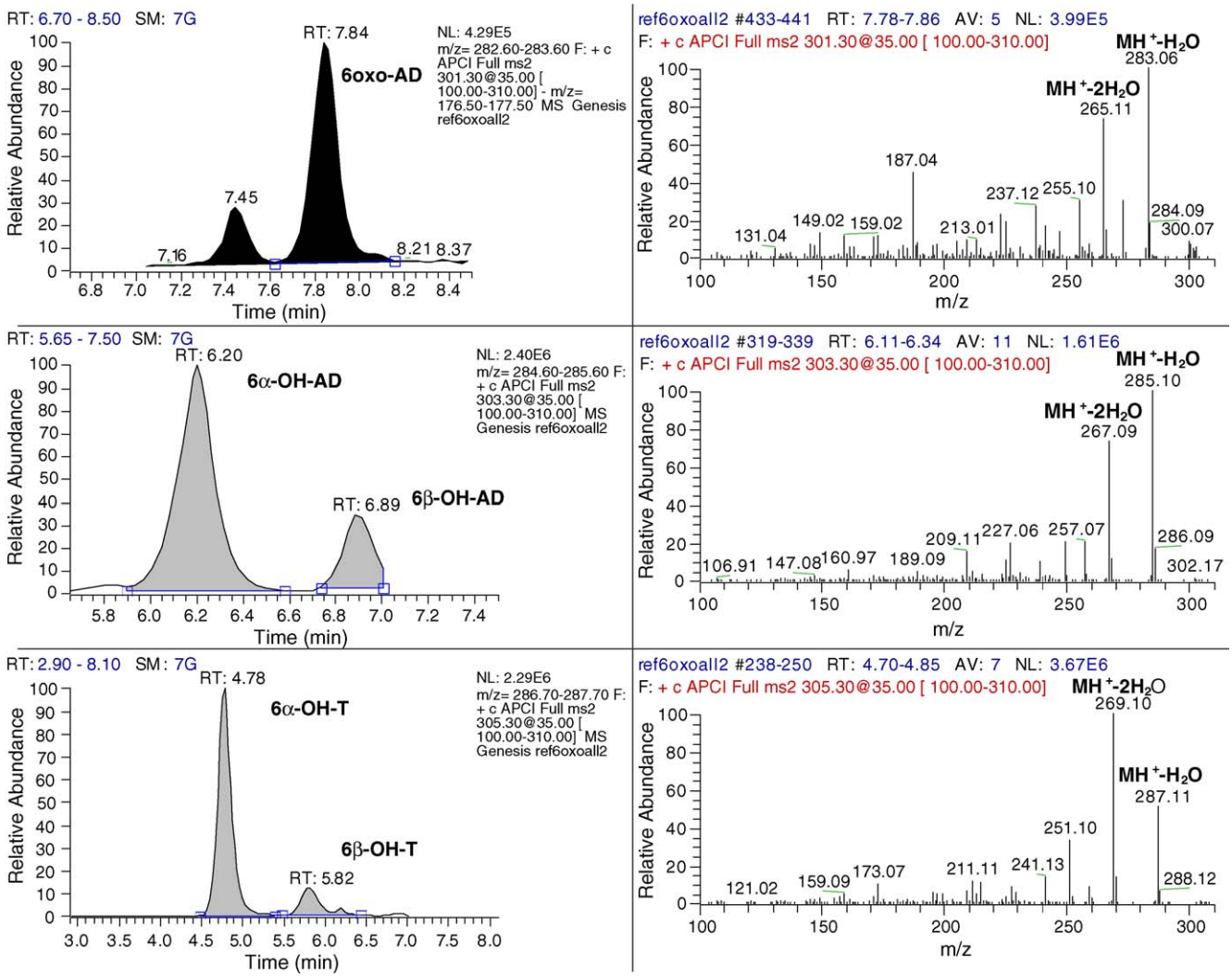

Fig. 1. Extracted ion chromatograms and mass spectra of 6oxo-AD (*m/z* 283), 6α-OH-AD (*m/z* 285) and 6α-OH-T (*m/z* 287) in a reference mixture (100 ng/mL).

different blank control urine samples did not result in the detection of background noise, proving the specificity of the method.

The limit of quantification (LOQ) of the method was 5 ng/mL. The limit of detection (2.5 ng/mL) was arbitrarily set at 1/2 of the LOQ. Extraction recoveries of the different compounds are given in Table 2. Although the original extraction method was developed as a general screening method for anabolic agents [2], moderate to good recovery was observed for all compounds.

Table 2
The recoveries of 6-oxo-androstenedione (6-oxo-AD), 6α-OH-androstenedione (6α–OH-AD), 6α-OH-testosterone (6α-OH-T)[a]

Concentration (ng/mL)	Recovery (%), $n = 6$		
	6-oxo-AD	6α-OH-AD	6α-OH-T
5	92.4 ± 16.3	83.6 ± 18.6	82.8 ± 9.6
100	97.3 ± 0.9	84.8 ± 4.8	66.0 ± 5.2
1000	78.0 ± 2.0	90.0 ± 6.4	69.9 ± 3.8

[a] Values are presented as mean ± standard deviation ($n = 6$).

3.4. Excretion studies

3.4.1. Qualitative results

Separation of isomers is unpredictable in both GC/MS and LC/MS and must be tested experimentally. Moreover comparison between both techniques is difficult because well separated compounds on GC/MS, e.g. can co-elute on LC/MS and vice versa. Derivatisation with a mixture of MSTFA/NH_4I/ethanethiol is most frequently used in doping analysis as this mixture results in the in situ formation of trimethylsilyliodide, the strongest trimethylsilylating agent, and in the formation of enol-TMS-ether-derivatives [20]. Unfortunately, for 3-keto-4-ene-steroids, trimethylsilylation by this mixture results in 3,5-dienol formation and consequently loss of stereochemical integrity at C-6 [21]. Hence, a different derivatisation procedure was applied for the GC/MS determination of the stereochemical configuration of the 6-hydroxy metabolites of 6-oxo-AD [8]. By using the described LC/MS method, the isomers of 6-OH-AD and 6-OH-T were well separated. Hence the 6-hydroxy isomers of androstenedione and testosterone, previously detected as metabolites of 6-oxo-AD [8], could be readily

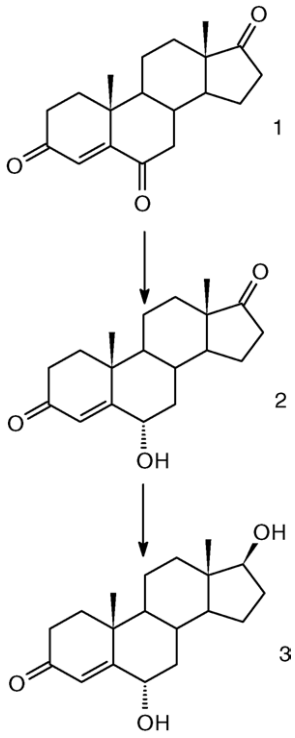

Fig. 2. Suggested in vivo metabolic pathway from androst-4-ene-3,6,17-trione (1) to androst-4-ene- 6α, 17β-diol-3-one (3) via androst-4-ene-6α-ol-3,17-dione (2).

identified as 6α-isomers. Taking into account the previously described metabolism of androst-4-ene-3,17-dione to testosterone in humans [11,22,23] and the in vivo 6α-hydroxylation of androst-4-ene-3,17-dione [11,12], the metabolic pathway shown in Fig. 2 is suggested for 6-oxo-AD. Using the described method, no 6β-hydroxy isomer of these metabolites were detected in any urine sample, similar as for androst-4-ene-3,17-dione where the 6β-hydroxy isomers were only detected as in vitro metabolites [12].

3.4.2. Quantitative results

The concentrations in the urine samples were adjusted to a standard urinary density of 1.020 kg/L.

For all volunteers the parent compound 6-oxo-AD could be detected in post administration urine samples. The urinary excretion profiles of the four volunteers who ingested 1 capsule are shown in Fig. 3a. The volunteers who took a single dose of three capsules during 3 successive days, show a maxima every time a new dose was ingested. Maximum urinary concentrations of 6-oxo-AD were obtained 2–4 h after every administration for both groups. The concentrations ranged from 470 to 1900 ng/mL. The detection time after ingestion of the last dose were similar for both test-groups and 6-oxo-AD was no longer detectable 30 h post administration.

6-Oxo-AD was excreted mainly in the conjugated fraction. Only 12.6 ± 2.8% ($n = 6$) of 6-oxo-AD was excreted nonconjugated. Cumulative excretion data for the total fraction (conjugated and free) are shown in Fig. 3b. This data shows a rapid clearance after oral intake of one capsule of 6-oxo-AD.

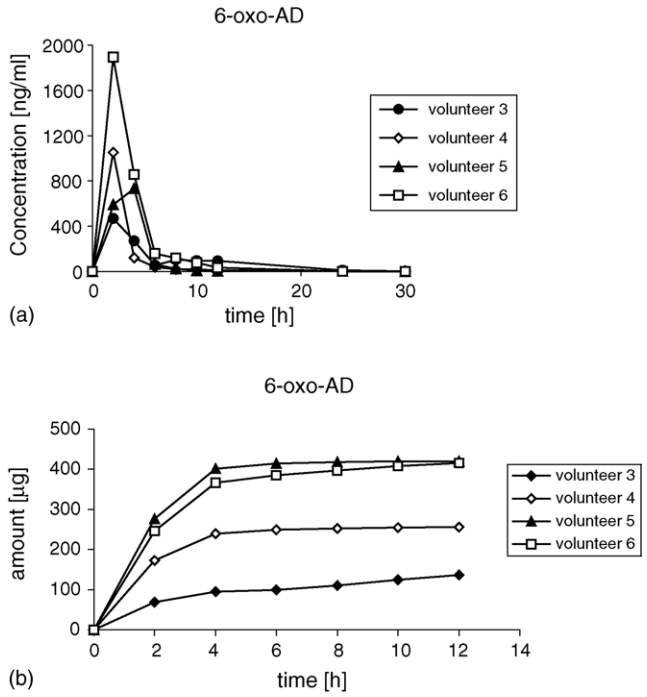

Fig. 3. Urinary concentrations (a) and cumulative excretion (b) of 6-oxo-AD after administration of one capsule of 6-OXO®.

The major metabolite of 6-oxo-AD was identified as 6α-OH-AD and maximum urinary concentrations were detected 2–4 h after every intake (Fig. 4a). The concentrations ranged from 32 to 55 μg/mL. 6α-OH-AD could only be detected in the conjugated fraction up to 30 h post administration and it was also rapidly cleared from the body.

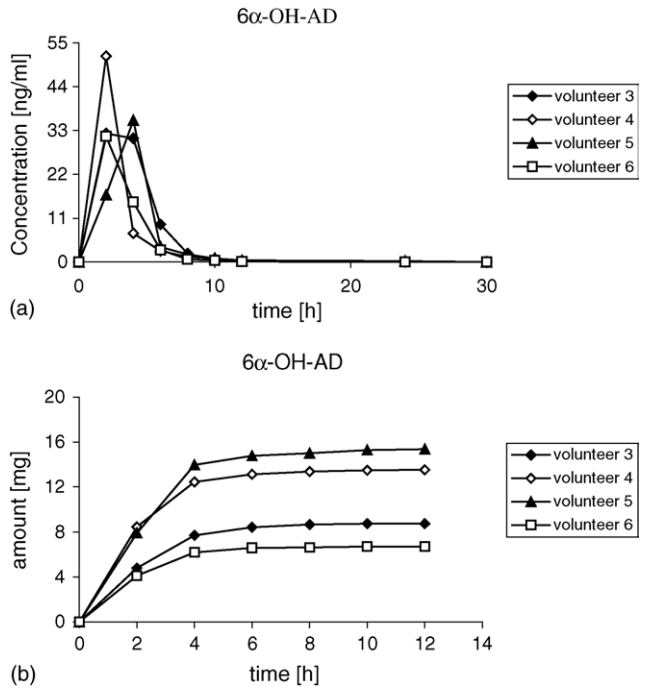

Fig. 4. Urinary concentrations (a) and cumulative excretion (b) of 6α-OH-AD after administration of one capsule of 6-OXO®.

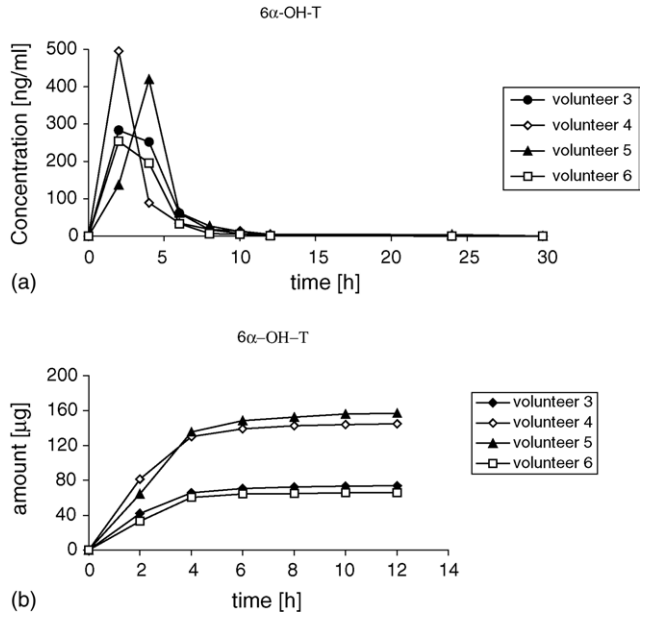

Fig. 5. Urinary concentrations (a) and cumulative excretion (b) of 6α-OH–T after administration of one capsule of 6-OXO®.

Cumulative data of 6α-OH-AD are presented in Fig. 4b. Similar excretion profiles were observed within both groups of volunteers.

6α-OH-T was identified as a minor metabolite of 6-oxo-AD and remained detectable up to 24 h after intake (Fig. 5a). Maximum urinary concentrations were reached 2–4 h after intake. Concentrations varied between 250 and 500 ng/mL and 1.2 and 1.4 μg/mL after taking one and three capsules, respectively. Cumulative excretion data of 6α-OH-T after intake of one capsule is presented in Fig. 5b. Similar to 6α-OH-AD, 6α-OH-T was only detected in the conjugated fraction. Preliminar experiments with glucuronidase from *E. coli* instead of *H. pomatia* indicate that 6-oxo-AD and metabolites are excreted as glucuronide conjugates.

4. Conclusions

A sensitive LC-APCI/MS method for the quantification of 6-oxo-AD, 6α-OH-AD and 6α-OH-T in urine was developed and validated. The method enabled the differentiation between 6α-and 6β-hydroxy isomers of 3-keto-4-ene-steroids.

Using this method, urine samples after the administration of 6-oxo-AD were analysed. Low concentrations of the parent drug 6-oxo-AD were detected in the samples up to 30 h post administration. 6α-OH-AD was identified as the major metabolite of 6-oxo-AD and 6α-OH-T was found to be a minor metabolite. 6-oxo-AD was detected predominantly in the conjugated fraction while 6α-OH-AD while 6α-OH-T were exclusively detected in the conjugated fraction.

Using the described method, misuse of 6-oxo-AD can be detected until 30 h post administration of a single dose of 100 mg.

Acknowledgements

This project has been carried out with the support of WADA. KD, PVE, WVT wish to thank the Flemish Ministry of Health for financial support. The authors are grateful to the Belgian National Lottery for the purchase of the LCQ-DECA® instrument. This work was supported by Grant Agency of the Czech Republic (grant no. 203/03/1062).

References

[1] P. Van Eenoo, F.T. Delbeke, Int. J. Sports Med. 24 (2003) 565.
[2] W. Schänzer, M. Donike, Anal. Chim. Acta 275 (1993) 23.
[3] F.T. Delbeke, P. Van Eenoo, W. Van Thuyne, J. Steroid Biochem. Mol. Biol. 83 (2003) 245.
[4] WADA (2005), The World Anti-Doping Agency, Prohibited list of substances 2005, http://www.wada-ama.org/, dd. 08/08/2005.
[5] D.F. Covey, W.F. Hood, Endocrinology 108 (1981) 1597.
[6] M. Numazawa, M. Tsuji, A. Mitumi, J. Steroid Biochem. 28 (1987) 337.
[7] M. Numazawa, A. Mitumi, M. Tachibana, Biochem. Pharmacol. 52 (1996) 1253.
[8] W. Van Thuyne, P. Van Eenoo, P. Mikulčíková, K. Deventer, F.T. Delbeke, Biomed. Chromatography, in press.
[9] O. Schaaf, K. Dettner, J. Steroid Biochem. Mol. Biol. 67 (1998) 451.
[10] J.F. Lévesque, C. Ayotte, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.), Recent Advances in Doping Analysis, vol. 7, Sport und Buch Strauß, Köln, Germany, 1999, p. 169.
[11] D.H. van de Kerkhof, Ph.D. thesis, Utrecht University, 2001, p. 237.
[12] J.F. Lévesque, M. Gaudreault, R. Houle, N. Chauret, J. Chromatogr. B 780 (2002) 145.
[13] W. Verwaal, M. van Bavel, A. Boot, J. Bravenboer, F. de Goei, C. Maas, A. Van der Putten, De Ware(n) Chemicus 26 (1996) 106.
[14] S. Souverain, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 1058 (2004) 61.
[15] M. Nelson, J. Dolan, LC-GC Europe (2002) 73.
[16] F. Guan, C. Uboh, L. Soma, A. Hess, Y. Lu, D.S. Tang, J. Mass. Spectrom. 38 (2003) 823.
[17] A. Leinonen, T. Kuuranne, T. Kotiaho, R. Kostiainen, Steroids 69 (2004) 101.
[18] K. Deventer, F.T. Delbeke, Rapid Commun. Mass Spectrom. 18 (2003) 2107.
[19] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[20] K.J.S. De Cock, F.T. Delbeke, D. De Boer, P. Van Eenoo, K. Roels, J. Anal. Toxicol. 27 (2003) 106.
[21] D.H. van de Kerkhof, J.W. Zwikker, V.E.M. Kaats-Richters, K. Bouwman, Rd. van Oijen, D. de Boer, R.A.A. Maes, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Eds.), Recent Advances in Doping Analysis, vol. 8, Sport und Buch Strauβ, Köln, Germany, 2000, p. 129.
[22] V.P. Uralets, P. Gillette, J. Anal. Toxicol. 23 (1999) 357.
[23] P. Van Eenoo, F.T. Delbeke, N. Desmet, P. De Backer, in: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (Eds.), Recent Advances in Doping Analysis, vol. 6, Sport und Buch Strauβ, Köln, Germany, 1998, p. 171.