RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2006; **20**: 1954–1962
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.2545

Identification of the aromatase inhibitors anastrozole and exemestane in human urine using liquid chromatography/tandem mass spectrometry

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Received 18 January 2006; Revised 13 April 2006; Accepted 14 April 2006

Anastrozole (2,2′-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1.3-phenylene]bis(2-methylpropionitrile)) and exemestane (6-methylenandrostan-1,4-diene-3,17-dione) are therapeutically used to treat hormone-sensitive breast cancer in postmenopausal women. For doping purposes they may be used to counteract adverse effects of an extensive abuse of anabolic androgenic steroids (gynaecomastia) and to increase plasma testosterone concentrations. Excretion study urine samples and spot urine samples from women suffering from metastatic breast cancer, being treated with anastrozole or exemestane, were collected and analyzed to develop/optimize a detection system for anastrozole and exemestane to allow the identification of athletes who do not comply with the internationally prohibited use of these cancer drugs. The assay was based on liquid-liquid extraction after enzymatic hydrolysis following liquid chromatography/tandem mass spectrometry (LC/MS/MS). Anastrozole, exemestane and its main metabolite (17-dihydroexemestane) were identified in urine by comparison of mass spectra and retention times with respective reference substances. An assay validation for the analysis of anastrozole and exemestane was performed regarding lower limits of detection (anastrozole: 0.02 ng/mL; exemestane: 3.1 ng/mL; dihydroexemestane: 0.5 ng/mL), interday precisions (6.6–11.1%, 4.9–9.1% and 5.6–8.3% for low [10 ng/mL], medium [50 ng/mL] and high [100 ng/mL] concentration) and recoveries (ranged from 85–97%).

Aromatase inhibitors such as anastrozole and exemestane are commonly employed for the treatment of postmenopausal women with hormone-sensitive breast cancer. In estrogen-dependent tumors estrogen deprivation causes growth arrest and possibly tumor cell death, making it a successful principle in the treatment of metastatic breast cancer in postmenopausal women.[1] A therapeutic application of anastrozole in men has been described for the treatment of male infertility,[2] but its use as an anti-ageing product has also been advertised.[3]

Anastrozole and exemestane are orally active, potent inhibitors of peripheral aromatase activity.[4,5] The drugs are known to have almost no effect on plasma follicle-stimulating hormone, luteinizing hormone, cortisol or aldosterone levels.[1,4] An increase in the serum testosterone level by approximately 58% has been observed during the course of treatment with Arimidex® and Aromasin®.[6,7] Both substances and their metabolites are eliminated mainly via hepatic metabolism and renal excretion.[4,8,9] Their pharmacological activity is primarily due to the respective parent drug.[4,8,9]

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Contract/grant sponsor: Manfred Donike Society e.V.

The non-steroidal aromatase inhibitor anastrozole inhibits aromatase activity by competitively binding to the heme of the cytochrome P450 subunit of the enzyme.[10] The recommended therapeutic dose for anastrozole is 1 mg per day,[11] and its major route of elimination occurs via N-dealkylation, hydroxylation and glucuronidation. Three metabolites (triazole and glucuronic acid conjugates of hydroxyanastrozole and anastrozole) have been identified in human plasma and urine.[4] Approximately 10% of the drug is excreted unchanged and its apparent terminal elimination half-life time is reported between 30 and 60 h.[12,13]

Exemestane is a third-generation, steroidal irreversible aromatase inactivator that is structurally related to the natural substrate androstenedione. The 'suicidal' inhibition of aromatase produced by exemestane has lead to the drug being called an aromatase 'inactivator'.[5] The recommended therapeutic dose for exemestane is 25 mg per day.[8,9] The orally administered drug is rapidly adsorbed into the systemic circulation and has a mean terminal half-life of approximately 24 h.[8,9] Exemestane is extensively metabolized. The initial steps are the reduction of the 17-keto group to give the 17β-hydroxy steroid and possibly the oxidation of

the methylene residue in position 6 with subsequent formation of several currently unidentified secondary metabolites.[5]

Underground literature advertises anastrozole (Arimidex®) to avoid gynaecomastia while (ab)using high amounts of anabolic androgenic steroids.[14] Hence, male athletes surreptitiously using performance-enhancing drugs such as anabolic androgenic steroids may feel encouraged to treat adverse effects with the above-mentioned aromatase inhibitors. Therefore, this class of compounds has been added to the 'Prohibited List' by the International Olympic Committee (IOC) and World Anti-Doping Agency (WADA) for male and female athletes in September 2001 and January 2005, respectively.[15]

For various agents with anti-estrogenic activity analytical screening procedures have been reported recently. The main metabolites of the anti-estrogenic substances clomiphene, cyclofenil and tamoxifen,[16] the aromatase inhibitor aminoglutethimide[17] and the main metabolite of letrozole[18] were determined using gas chromatography/mass spectrometry (GC/MS). In addition, methods for the determination of exemestane in human plasma using liquid chromatography/thermospray mass spectrometry[19] or LC/MS/MS employing a heated nebulizer interface[20] have been established, and an assay enabling the determination of anastrozole in human plasma by GC with electron capture detection[21–23] has been described recently. These procedures have been optimized to allow the rapid and sensitive detection of respective target analytes in human plasma, but for doping control purposes blood sampling is rather seldom, and the primary specimen collected for drug testing in sports is urine. Hence, a robust, sensitive and fast assay enabling the detection of prohibited aromatase inhibitors in human urine is required, preferably combined with existing screening procedures. As both compounds and the major metabolite of exemestane possess considerable proton affinities and good liquid chromatographic properties, the implementation of these target analytes into an established liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LC/APCI-MS/MS assay) was aimed, and various items such as detection limit, precision, recovery and specificity were validated.

EXPERIMENTAL

Aromatase inhibitors

Anastrozole (Arimidex®) was obtained from Astra Zeneca (UK Ltd., Macclesfield, UK) and exemestane (Aromasin®) from Pharmacia & Upjohn GmbH (Erlangen, Germany). Respective synthetic reference material was bought from Thinker Chemicals (Hangzhou, China).

Chemicals and reagents

All solvents and reagents were of analytical grade purity. *tert*-Butyl methyl ether (TBME) was purchased from KMF Laborchemie (St. Augustin, Germany) and distilled before use. β-Glucuronidase from *Escherichia coli* was supplied by Roche Diagnostics GmbH (Mannheim, Germany). 17α-Methyltestosterone (Serva, Heidelberg, Germany) and [2,2,3,4,4-$^2H_5$]-androsterone glucuronide (in-house synthesis, Institute of Biochemistry, German Sport University Cologne, Germany) were used as internal standards.[24,25]

All solutions and buffers were prepared using deionized water (Water Lab System, Millipore, Eschborn, Germany).

Sample preparation

The sample preparation was modified from an assay for steroid analysis using GC/MS described by Donike *et al*.[26] Conjugated and unconjugated anabolic steroids were extracted from urine at pH 9.6 with TBME following enzymatic hydrolysis at pH 7. The deglucuronidation process was controlled by addition of deuterated androsterone glucuronide as part of the internal standard.[25,27]

Urine samples (3 mL) were fortified with 1500 ng of the internal standards methyltestosterone and [2,2,3,4,4-$^2H_5$]-androsterone glucuronide each. The samples were buffered to pH 7.0 with 1 mL of a 0.8 M phosphate buffer (Na_2HPO_4/NaH_2PO_4, 1:2, w/w). Then, 25 μL of β-glucuronidase from *E. coli* were added. The mixture was incubated at 50°C for 1 h. After cooling to ambient temperature, the mixture was adjusted to pH 9.6 by addition of 0.75 mL of an aqueous solution containing potassium carbonate and potassium hydrogen carbonate (20%, 1:1, w/w). Then, 6 mL of TBME were added, the mixture was shaken for 5 min and subsequently centrifuged at 600 *g* for 5 min. From the organic layer 2 mL were transferred to a fresh glass tube and evaporated to dryness under reduced pressure at 50°C using a rotary evaporator. The dry residue was reconstituted in 60 μL of a mixture of methanol (MeOH)/ammonium acetate buffer (1:1, see below) and transferred into HPLC vials. A volume of 10 μL was injected into the LC/MS/MS system.[28]

Liquid chromatography/tandem mass spectrometry

All samples were analyzed by LC/MS/MS employing an Agilent 1100 series liquid chromatograph coupled to an Applied Biosystems API 2000 triple quadrupole mass spectrometer utilizing APCI. The liquid chromatograph was equipped with a Merck Purospher®Star RP-18 endcapped column (4 × 55 mm, 3 μm particle size), and the eluents used were A: 5 mM ammonium acetate buffer containing 0.1% of glacial acetic acid (pH 3.5), and B: acetonitrile. A gradient was employed from 15% B to 100% B within 8.25 min, and the column was re-equilibrated at 15% B for 2.25 min. The flow rate was set to 500 μL/min.

Positive ionization was accomplished by APCI at an interface temperature of 475°C. Declustering potential was set to 31 V, and collision energies were optimized individually for each ion transition. Nitrogen (obtained from a K75-72 Whatman nitrogen generator) was used as collision gas at a collision cell pressure of 2.9e-3 Pa.

For quantification purposes the following ion transitions were used: anastrozole (*m/z* 294/225), exemestane (*m/z* 297/121) and 17-dihydroexemestane (*m/z* 299/135).

Excretion study urine samples

Exemestane

An excretion study was performed by a healthy male volunteer who had given his written consent to the excretion

DOI: 10.1002/rcm

study. Exemestane (Aromasin®, 25 mg) was orally administered. Aliquots of each urine sample were collected for 96 h.

Eighteen spot urine samples from a caucasian women, suffering from metastatic breast cancer and treated with exemestane, were also collected. Aliquots of 30 mL of urine were collected and stored at −20°C until analysis.

Anastrozole

An excretion study was performed by a healthy male volunteer who had given his written consent to the excretion study. Anastrozole (1 mg) was orally administered per day over a period of 3 days. Each urine sample was collected during the first 7 days and, thereafter, morning urine samples over a time period of 17 days. In total, urine samples were collected for 24 days.

Ten untimed urine samples from women suffering from metastatic breast cancer, treated with anastrozole, were made available from Dr. Wolfgang Kauffels (Gynaecology and Obstetrics, Hannover Medical School). Aliquots of 30 mL of urine were collected and stored at −20°C until analysis.

Synthesis of 17-dihydroexemestane

17-Dihydroexemestane was prepared from exemestane by dissolving 296 mg (1 mmol) in 20 mL of methanol/water (4:1, v/v) followed by reduction of the 17-keto function by adding 75.7 mg of $NaBH_4$ (2 mmol) in 12.5 mL of methanol/water (4:1, v/v). The mixture was left at ambient temperature for 1 h, the solvent was removed *in vacuo* and the dry residue dissolved in 4 mL of 1.5 M aqueous HCl. After addition of 10 mL of 1 M aqueous KOH the mixture was extracted three times with 15 mL of TBME; then the ethereal layers were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 10 mL of TBME at 50°C and n-hexane was added until turbidity occurred. After standing for 2 days colorless crystals were collected, washed with n-hexane and dried in a vacuum desiccator over P_4O_{10}. Yield: 55.4 mg (18.7%), purity >95%, as determined by GC/MS and HPLC/UV.

Structure confirmation

Structure confirmation of exemestane and 17-dihydroexemestane was obtained by nuclear magnetic resonance (NMR) spectroscopy and high-resolution/high-accuracy mass spectrometry. NMR analyses were performed on a Bruker DPX 300 instrument. Amounts of 5 mg of each compound were dissolved in $CDCl_3$ and spectra were recorded at room temperature. Conducted experiments proving the desired structures were ^1H, H-H-COSY (homonuclear correlation spectroscopy), H,C-HMQC (heteronuclear multiple quantum coherence) and H,C-HMBC (hetero multiple bond correlation).

Electrospray ionization (ESI)/high-resolution/high-accuracy (tandem) mass spectrometry was accomplished using a Thermo LTQ Orbitrap mass spectrometer in positive ionization mode. The instrument was calibrated using the manufacturer's calibration mixture allowing for mass accuracies <3 ppm. Analytes were dissolved in acetonitrile/water (1:1, v/v) containing 0.1% formic acid at concentrations of 5 μg/mL and introduced into the mass spectrometer using a syringe pump at a flow rate of 5 μL/min. The ionization voltage was +3.5 kV, the capillary temperature was set to 300°C, and protonated precursor ions were either measured intact or dissociated using normalized collision energies between 25 and 35. Damping gas in the linear ion trap was helium 5.0, and gas supplied to the curved linear ion trap (CLT) was nitrogen obtained from a CMC nitrogen generator (CMC Instruments, Eschborn, Germany). Full-scan MS and MS/MS spectra were recorded at resolutions of 60 000 and 30 000, respectively, and the precursor ions for MS/MS experiments were selected in the linear ion trap at an isolation width of 2 Da. Thirty scans were averaged per MS or MS/MS spectrum in the Orbitrap analyzer for accurate mass analysis.

Assay validation

As the analysis of anastrozole and exemestane should be implemented into an established screening procedure, the assay validation[29] was performed according to the respective screening procedure for androgenic anabolic steroids.

Calibration curves

Calibration curves for anastrozole, exemestane and 17-dihydroexemestane were generated using ten calibration points each at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/mL.

The peak area ratios of analyte and ISTD were utilized to calculate the correlation coefficient, intercept and slope. The source of the spiked urine specimens is a pooled blank urine obtained from three healthy male volunteers.

Lower limit of detection

The lower limit of detection (LLOD) was defined as the 'lowest content that can be measured with reasonable statistical certainty'[29] at a signal-to-noise(S/N) ratio ≥3. Ten blank urine samples spiked with the internal standard (ISTD) only, and ten blank urine specimens fortified with 0.1 ng of anastrozole, 5 ng of 17-dihydroexemestane and 10 ng of exemestane per mL, were prepared and analyzed according to the established protocol providing the data necessary to estimate the LLOD.

Interday precision

On three consecutive days, ten urine samples of low (10 ng/mL), medium (50 ng/mL) and high (100 ng/mL) concentrations of anastrozol, exemestane and 17-dihydroexemestane were prepared, analyzed randomly, and the assay precision was calculated for each concentration level.

Recovery

The recovery of anastrozole, exemestane and 17-dihydroexemestane by liquid-liquid extraction (LLE) was determined at 50 ng/mL. Six urine samples were fortified with the analytes before sample preparation, and another six blank urine specimens were extracted according to the described protocol followed by addition of 50 ng/mL of the analytes to the ether extracts. To both sets of samples 1000 ng of methyltestosterone (ISTD) were spiked into the TBME layer before evaporation. Recovery was calculated by comparison of mean peak area ratios of analyte and ISTD of samples fortified prior to and after LLE.

DOI: 10.1002/rcm

Assay optimization

Experiments were performed with regard to an optimization of the analytical approach for confirmation purposes.

Specificity

Fifty different blank urine specimens were prepared as described above in order to probe for interfering peaks in the selected ion chromatograms at expected retention times of all target analytes, i.e. either the respective administered drug or a metabolite.

Hydrolysis experiments

The sample preparation was performed according to the standard operating procedure (SOP) for anabolic steroids as described above. The hydrolysis was stopped after different time periods (0, 5, 15, 30, 120 and 240 min).

Extraction at different pH values

Aliquots of 2 mL of a clinical urine were prepared according to the SOP for the screening procedure for anabolic steroids.[26–28] After hydrolysis the urine samples were adjusted to different pH values: 7, 9.6 and 14. The remaining sample preparation followed the SOP for the screening procedure for anabolic steroids.

Purification with n-pentane

Purification tests with excretion study urine samples were conducted according to the above described sample preparation procedure. Instead of 5 mL of TBME the equivalent volume of n-pentane was used for the extraction of the analytes from urine specimens.[30,31]

RESULTS AND DISCUSSION

Structure confirmation

The structural characteristics of 17-dihydroexemestane were confirmed by NMR spectroscopy and substantiated the reduction of the 17-keto function of exemestane to its corresponding 17β-hydroxyl group (data not shown). In addition, high-resolution/high-accuracy mass spectrometry provided information on the elemental composition of 17-dihydroexemestane, which was determined as the protonated species with $C_{20}H_{27}O_2$ at *m/z* 299.2006 (theor. *m/z* 299.2006, error 0.01 ppm).

Mass spectrometry

Product ion spectra of all analytes, i.e. parent compounds and target metabolite, were recorded from reference material. The analytical parameters of the investigated substances are presented in Table 1.

Product ion mass spectra generated from protonated molecules $[M+H]^+$ of anastrozole, exemestane and 17-dihydroexemestane were generated at collision offset voltages of 35 and 30 V, as depicted in Figs. 1–3. Diagnostic and abundant fragment ions were observed at *m/z* 294, 225, 210, 195, 142 and 130 for anastrozole (Fig. 1), at *m/z* 297, 149, 135 and 121 for exemestane (Fig. 2), and at *m/z* 299, 147, 135 and 121 for 17-dihydroexemestane (Fig. 3).

The base peak in the product ion spectrum of anastrozole at *m/z* 225 is suggested to result from a neutral loss of triazole ($C_2H_3N_3$, −69 u), which subsequently eliminates either a methyl radical (−15 u) or prussic acid (HCN, −27 u) yielding the product ions at *m/z* 210 and 198, respectively, as observed in MS^3 experiments. Moreover, the release of an isobutyronitrile radical (C_4H_6N, −68 u) from *m/z* 225 was detected giving rise to the product ion at *m/z* 157, which additionally loses 15 or 27 u yielding *m/z* 142 or 130, respectively, in accordance to the dissociation behavior described above.

The fragmentation pathways of exemestane and dihydroexemestane are much more complex, and substantial interpretation of product ions requires several experiments primarily based on stable deuterium labelling of selected positions. Until now, only suggestions for the origin of the base peak of the product ion spectrum of dihydroexemestane at *m/z* 135 are possible (Fig. 3) in accordance to related studies on the mass spectrometry of steroids with androstadiene structures.[32]

Assay validation

The validation results for the LLOD, recovery and interday precision of the investigated drugs are summarized in Table 2.

Calibration curves

Linear calibration curves were obtained for anastrozole, exemestane and 17-dihydroexemestane in urine over a range of 10–100 ng/mL. The obtained calibration equations were $y = 1.06x + 0.147$ with $r^2 = 0.9963$ (anastrozole), $y = 0.01 + 0.019$ with $r^2 = 0.977$ (exemestane), and $y = 0.022 + 0.068$ with $r^2 = 0.993$ (17-dihydroexemestane). Linearity was proven by the F-test and homoskedasticity by the Breusch-Pagan-Test in all cases.

Lower limit of detection

The average background noise plus a three-fold standard deviation was calculated for ten blank samples at respective retention times in specific extracted ion transition chromatograms, and a S/N ratio greater than 3 was utilized as the decisive criterion. The LLOD was determined at 0.02 ng/mL

Table 1. Analytical parameters of investigated aromatase inhibitors

No	Drug	Target compound in urine	Precursor ion (*m/z*)	Characteristic product ions (*m/z*)	CE (eV)	RT (min)
1	Anastrozole	Parent drug	294	225, 210, 195, 142, 130	35	5.8
2	Exemestane	Parent drug	297	149, 135, 121	30	7.1
3	Exemestane	17-Dihydroexemestane	299	147, 135, 121	30	6.7

DOI: 10.1002/rcm

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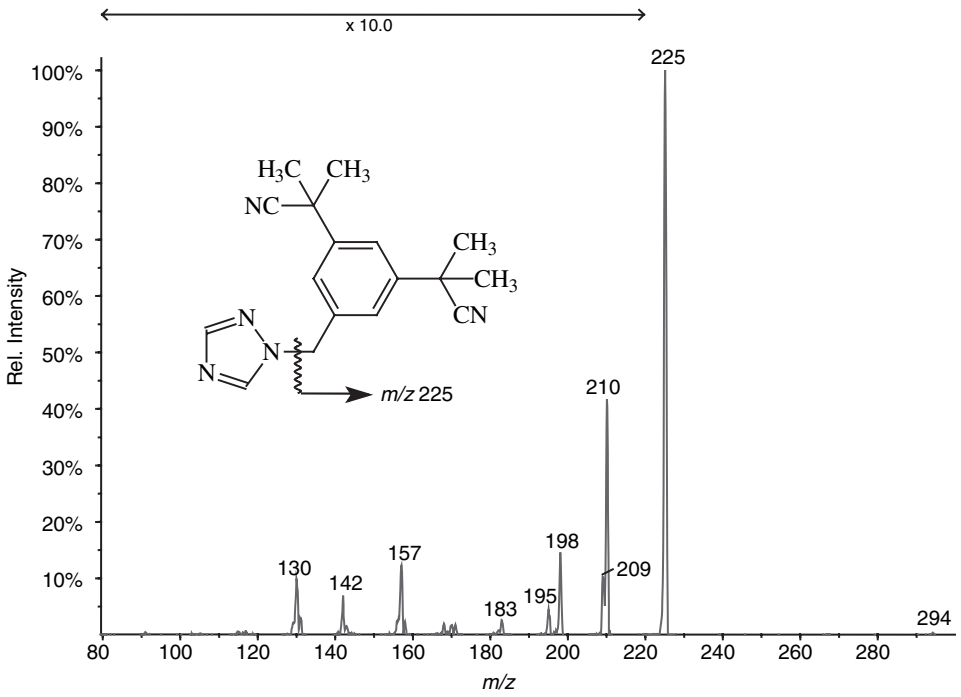

Figure 1. Product ion mass spectrum of anastrozole ($[M+H]^+ = 294$), recorded on an Applied Biosystems API 2000 triple quadrupole mass spectrometer using a CE of 35 eV.

for anastrozole, at 3.1 ng/mL for exemestane, and at 0.5 ng/mL for dihydroexemestane (Table 2).

Interday precision

The interday precision was determined at three concentrations and varied from 6.6–11.1%, 4.9–9.1%, and 5.6–8.3% for low (10 ng/mL), medium (50 ng/mL), and high (100 ng/mL) concentration, respectively (Table 2).

Recovery

The recoveries ranged from 85–97% (Table 2).

Specificity

In all 50 blank urine specimens, no interfering signals were observed at retention times (RT) of the investigated drugs or the corresponding metabolite, which were selected as target analytes.

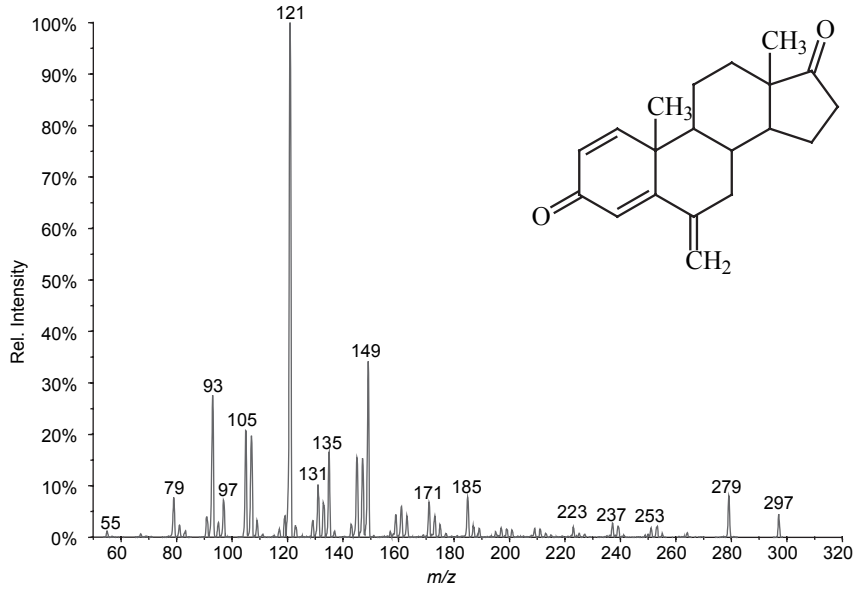

Figure 2. Product ion mass spectrum of exemestane ($[M+H]^+ = 297$), recorded on an Applied Biosystems API 2000 triple quadrupole mass spectrometer using a CE of 30 eV.

DOI: 10.1002/rcm

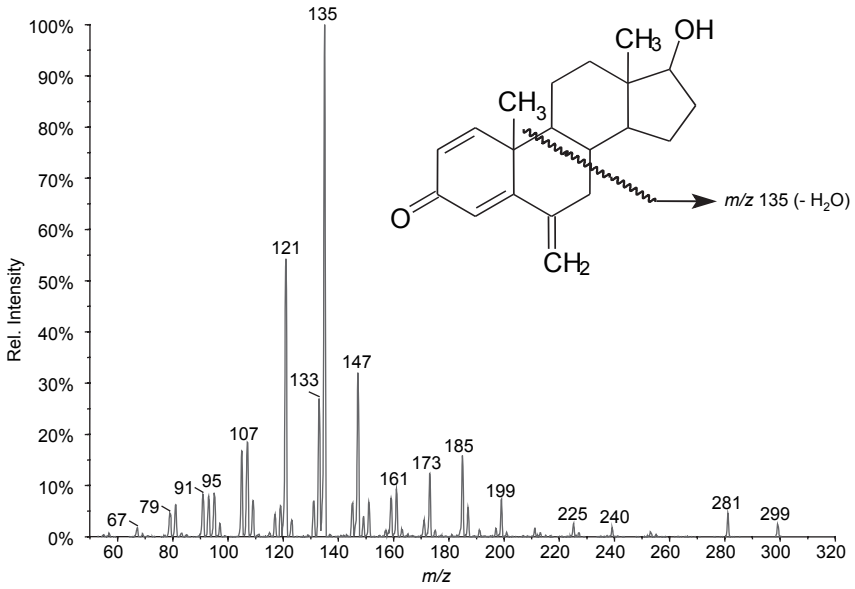

Figure 3. Product ion mass spectrum of 17-dihydroexemestane ($[M+H]^+ = 299$), recorded on an Applied Biosystems API 2000 triple quadrupole mass spectrometer using a CE of 30 eV.

Assay optimization

Hydrolysis

Approximately 50% of anastrozole is excreted unconjugated. The hydrolysis of the conjugated anastrozole with β-glucuronidase of *E. coli* is completed within 5 min. Although the presence of a glucuronide of anastrozole has been described in the literature,[4] its defined structure was not presented. Also in our study, it was not possible to clarify the conjugation site, but a gain in signal intensity of approx. 40% was obtained employing an enzymatic hydrolysis with β-glucuronidase of *E. coli*. Due to the fact that a high-purity enzyme without any putative sulfatase activity was used, the deconjugated anastrozole is suggested to originate entirely from anastrozole glucuronide.

While exemestane is excreted unchanged, its metabolite 17-dihydroexemestane is extensively conjugated.[9] Studies on the hydrolysis using β-glucuronidase of *E. coli* demonstrated completion of deconjugation within 5 min.

Extraction at different pH-values

The extraction yields for target analytes in urine samples extracted at pH 7, 9.6 and 14 show similar results of about 90%. Due to the fact that the extraction at pH 7 allowed higher yields of substances leading to disturbing background, an extraction at pH 9.6 is recommended.

Purification with n-pentane

Extraction with n-pentane instead of TBME is often used for confirmation methods of anabolic steroids (e.g. T/epiT, 19-norandrosterone) to exclude polar coeluting substances.[31] Yields of the n-pentane extraction of anastrozole accounted for approximately 3% of the corresponding TBME extraction. Hence, n-pentane purification may be used as a pre-extraction cleaning step. Biological background of low polarity will be removed from the aqueous phase, which is subsequently extracted with TBME. The ethereal layer is used for further work-up.

Extraction yields of the n-pentane extraction of exemestane and 17-dihydroexemestane are determined for approximately 80% of the corresponding TBME extraction. In this case the n-pentane extraction is recommended for an exemestane confirmation method. Biological background of high polarity will remain in the aqueous phase.

The standard operating procedure for anabolic steroids shows nearly optimal conditions for the detection of

Table 2. Assay validation parameters

No	Drug	LLOD (ng/mL)	Recovery (%) (at 50 ng/mL)	Interday precision (%)		
				Low (at 10 ng/mL)	Medium (at 50 ng/mL)	High (at 100 ng/mL)
1	Anastrozole	0.02	97	11.1	4.9	6.1
2	Exemestane	3.1	85	10.8	9.1	8.3
3	17-Dihydroexemestane	0.5	89	6.6	6.7	5.6

DOI: 10.1002/rcm

anastrozole and exemestane. Only few parameters may be taken into consideration for confirmation purposes. The hydrolysis conditions are ideal for a rapid cleavage of the conjugated anastrozole and 17-dihydroexemestane. Due to the fact that the extraction at pH 7 shows higher extraction of substances leading to disturbing background an extraction at pH 9.6 is recommended.

For confirmatory analysis of anastrozole the extraction with n-pentane may be used as an additional cleaning step (pre extraction); for the confirmation of exemestane extraction with n-pentane instead of TBME is recommended to exclude polar coeluting substances.

Screening for anastrozole and exemestane

For the detection of anastrozole and exemestane misuse in sports, the inclusion of anastrozole and 17-dihydroexemestane in the screening procedure for selected anabolic androgenic steroids by LC/MS/MS is recommended.

A suitable ion transition for anastrozole screening is *m/z* 294/225 as depicted in Fig. 4. Confirmation procedures require additional qualifier product ions, and for anastrozole complementary ion transitions at *m/z* 294/210 and 294/142 are suggested (Fig. 4). Resulting from an excellent traceability (LLOD: 0.02 ng/mL), screening for anastrozole in human urine allows a very good retrospective. Following a single application of 3 mg of Arimidex®, anastrozole was detectable for a period of 24 days with a maximum anastrozole concentration of 103 ng/mL.

The target analyte 17-dihydroexemestane is adequately detected in screening assays using the ion transition *m/z* 299/135. Confirmation of 17-dihydroexemestane is subsequently accomplished with additional ion transitions at *m/z* 299/147 and 299/121, as shown in Fig. 5. The analysis of clinical spot urine samples yielded concentrations for exemestane between 8 and 219 ng/mL and for 17-dihydroexemestane between 37 and 1229 ng/mL.

After application of 25 mg Aromasin®, exemestane and 17-dihydroexemestane were detectable for 96 h with maximum concentrations of 42 ng/mL for exemestane and of 365 ng/mL for 17-dihydroexemestane.

Since October 2004 about 15 000 authentic doping control urine samples have been analyzed for the presence of anastrozole, exemestane and 17-dihydroexemestane according to the described analytical method without detection of any adverse finding.

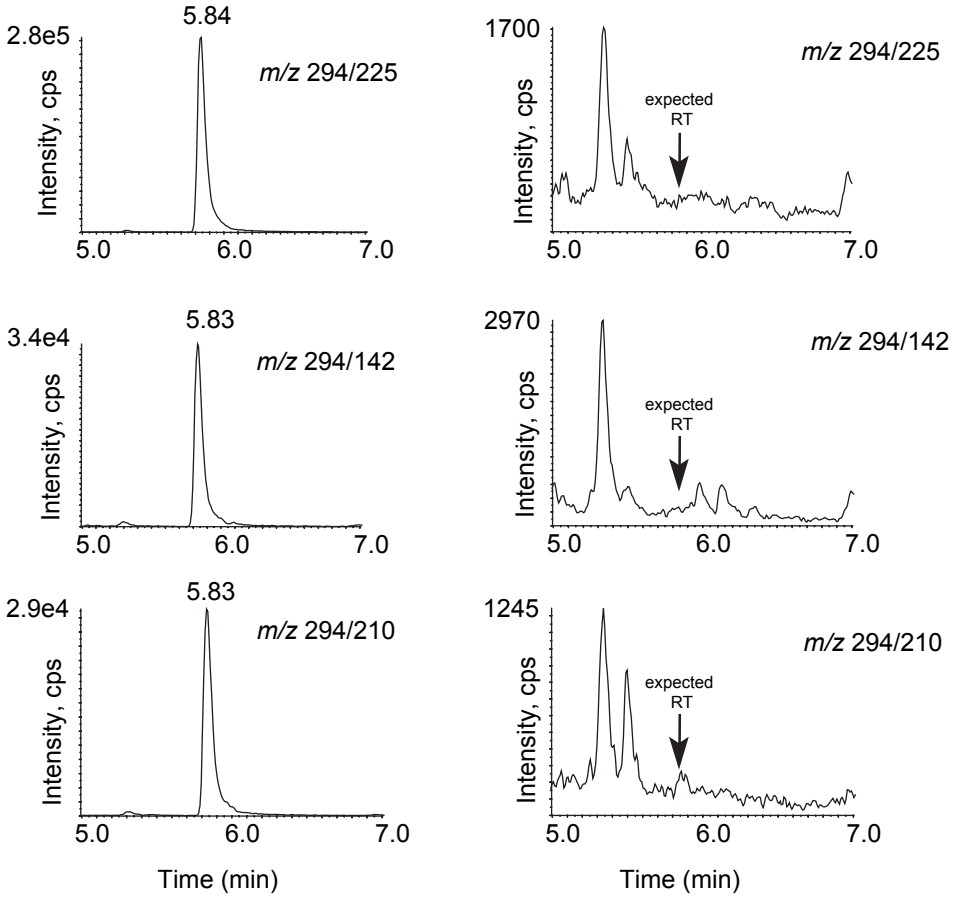

Figure 4. Screening for selected anabolic androgenic steroids. Left: clinical spot urine containing approx. 5 ng/mL anastrozole (detection at retention time 5.8 min). Right: blank urine with coextracted endogenous components with low response.

DOI: 10.1002/rcm

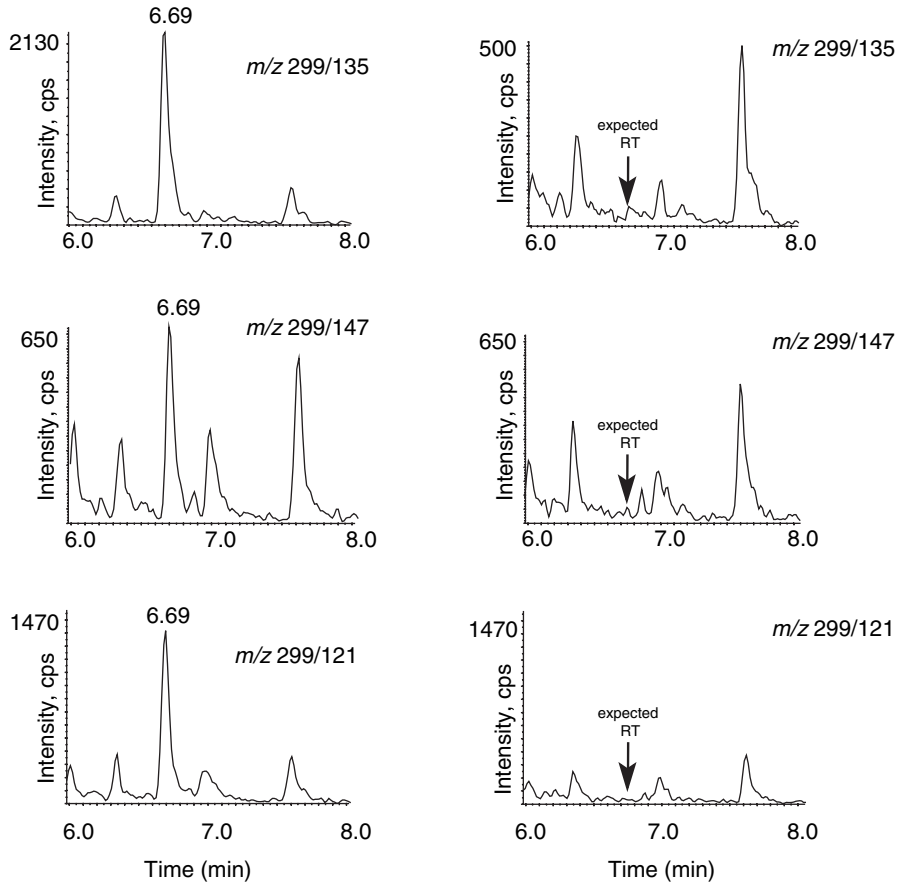

Figure 5. Screening for selected anabolic androgenic steroids. Left: urine spiked with 4 ng/mL 17-dihydroexemestane (detection at retention time 6.69 min). Right: blank urine with coextracted endogenous components with low response.

Acknowledgements

We thank Dr. Wolfgang Kauffels (Gynaecology and Obstetrics, Hannover Medical School) for providing excretion urine samples. The authors are grateful to the Manfred Donike Society e.V. for financial support. The research concerning anastrozole has been carried out with support of the World Anti-Doping Agency (WADA).

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