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A fast, comprehensive screening method for doping agents in urine by gas chromatography–triple quadrupole mass spectrometry

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ABSTRACT

The use of performance enhancing drugs in sports is prohibited. For the detection of misuse of such substances gas chromatography or liquid chromatography coupled to mass spectrometry are the most frequently used detection techniques. In this work the development and validation of a fast gas chromatography tandem mass spectrometric method for the detection of a wide range of doping agents is described. The method can determine 13 endogenous steroids (the steroid profile), 19-norandrosterone, salbutamol and 11-nor-Δ9-tetrahydrocannabinol.9carboxylic acid in the applicable ranges and to detect qualitatively over 140 substances in accordance with the minimum required performance levels of the World Anti-Doping Agency in 1 ml of urine. The classes of substances included in the method are anabolic steroids, β2-agonists, stimulants, narcotics, hormone antagonists and modulators and beta-blockers. Moreover, using a short capillary column and hydrogen as a carrier gas the run time of the method is less than 8 min.

1. Introduction

The use of performance enhancing drugs in sports is prohibited by the World Anti-Doping Agency and a wide range of pharmacological classes of drugs figures on the Prohibited List [1]. In the framework of the fight against doping, mostly urine samples are collected and analyzed for the presence of prohibited drugs or metabolites. In general, except for peptide hormones, screening for the misuse of doping substances is performed using chromatography. Moreover, to combine the necessary selectivity with the required sensitivity to detect the different classes of prohibited substances at or below the minimum required performance limit (MRPL), hyphenated chromatographic mass spectrometric methods are preferred [2].

Within the group of low molecular weight doping agents (MW < 1000 Da), the anabolic steroids are probably the most challenging and important class. They are the most misused substances in doping control [3], they are intensively metabolized in humans and have the lowest MRPL [2]. Adequate screening for misuse of these substances therefore relies on the detection of metabolites in urine samples collected from athletes [4].

Most of the studies investigating the metabolism of pharmaceutically available steroids were performed in the 1980s and early 1990s [5,6]. This research resulted in the selection of appropriate metabolites for the detection of steroid misuse. Over the years the selection of metabolites was further elaborated to include several metabolites that can result in prolonged detection times [7]. Gas chromatography–mass spectrometry (GC–MS) was essential as a technique for these findings and was therefore also employed to screen doping control samples routinely for the misuse of anabolic steroids. GC–MS remained the technology of choice for the detection of anabolic steroids until the appearance of tetrahydrogestrinone (THG) as a designer steroid on the underground market, which was almost undetectable by the GC–MS screening methods used at that time. THG lead to the introduction of liquid chromatography tandem mass spectrometry (LC–MS^n) as a screening technology in doping control laboratories. As a result GC–MS and LC–MS are now used as compatible techniques in doping control [8–10].

Besides anabolic steroids, GC–MS is also routinely applied for many other groups of doping agents. Particularly for stimulants and narcotics, GC–MS is still the most employed technology [11,12].

For routine purposes quadrupole GC–MS methods are normally used in the selected ion monitoring mode (SIM) for anabolic steroids and in full scan or a combination of SIM/scan for narcotics and stimulants. SIM-methods monitor a limited number of *m*/*z*-values (typical for a substance) rather than a range of *m*/*z*-values (full scan mode). Although less structural information is obtained in the SIM mode, in this way higher sensitivity is achieved, which

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is typically required for anabolic agents. Most screening methods for the detection of anabolic steroids have a run time of 20–35 min [13,14]. In the past several fast tandem mass spectrometric methods, using ion trap technology, have been published. However, these methods normally lacked the combination of a quantitative determination of the steroid profile (a range of endogenous steroids monitored to detect use of natural steroids) and a qualitative analysis of a wide range of exogenous steroids and other doping agents [16,17].

In this paper the use of a gas chromatograph coupled to a triple quadrupole mass spectrometer for the detection of a wide range of exogenous anabolic steroids and other doping agents as well as the determination of a full steroid profile within a single run of less than 10 min was investigated.

2. Materials and methods

2.1. Reagents

N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was purchased from Chem. Fabrik Karl Bucher (Waldstedt, Germany) and the enzyme preparation β-glucuronidase from *E. coli* K12 was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Diethyl ether was purchased from Acros (Acros, Geel, Belgium), methanol and sodium hydrogen carbonate ($NaHCO_3$) from Fisher Scientific (Loughborough, UK), potassium carbonate (K_2CO_3), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), and sodium sulphate (Na_2SO_4) were all from Merck (Darmstadt, Germany).

The phosphate buffer (pH 7) was prepared by dissolving 7.1 g $Na_2HPO_4{\cdot}2H_2O$ and 1.4 g $NaH_2PO_4{\cdot}H_2O$ in 100 ml water. The carbonate buffer (pH = 9.5) was prepared by dissolving 135 g K_2CO_3 and 111 g $NaHCO_3$ in 900 ml aqua bidest.

2.2. Reference standards

2.2.1. Internal standards

D3-testosterone glucuronide (d3-T), d3-epitestosterone glucuronide (d3-E), d4-androsterone glucuronide (d4-A), d5-etiocholanolone (d5-Et), d3-dihydrotestosterone glucuronide (d3-DHT), d3-5α-androstane-3α,17β-diol (d3-aab), d5-5β-androstane-3α,17β-diol(d5-bab) and d3-salbutamol (d3-sal) were from NMI (Pymble, Australia). 17α-methyltestosterone (MT) was a kind gift from Organon (Oss, The Netherlands). A mixture was made in methanol containing 2 μg/ml d4-A, d5-Et, DP and d3-sal, 1 μg/ml d3-aab, d5-bab and MT, 1.2 μg/ml d3-T, 0.3 μg/ml d3-E and 0.4 μg/ml d3-DHT.

2.2.2. Natural steroids

Testosterone, epitestosterone, androsterone, etiocholanolone, 11β-hydroxyandrosterone (11β-OH-androsterone), 11β-hydroxyetiocholanolone (11β-OH-etiocholanolone), 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5β-androstane-3α,17β-diol, 4-androstene-3,17-dione, 5β-androstane-3,17-dione and 5α-androstane-3,17-dione were obtained from Sigma (Bornem, Belgium), 19-norandrosterone from NMI (Pymble, Australia), dehydroepiandrosterone (DHEA) from Serva (Heidelberg, Germany) and dihydrotestosterone (DHT) from Piette International Laboratories (Drogenbos, Belgium).

2.3. Exogenous steroids

3α-hydroxytibolone (tibolone metabolite) was a kind gift from Akzo Nobel (Oss, The Netherlands), fluoxymesterone was obtained from Ciba-Geigy, boldenone and oxymesterone were purchased from the Institut für Biochemie of the Deutsche Sporthochschule (Cologne, Germany). 3α,5α-tetrahydronorethisterone, was a kind gift from the Institute of Organic Chemistry and Biochemistry (Academy of Sciences of the Czech Republic, Prague, Czech Republic) and metenolone from the Drug Control Centre of King's College (London, UK).

16α-OH-furazabol, 16α-OH-stanozolol,17α-ethyl-5α-estrane-3α,17β-diol, 17α-ethyl-5β-estrane-3α,17β-diol, 17α-methyl-5α-androstane-3α,17β-diol, 17α-methyl-5β-androstane-3α,17β-diol, 17α-trenbolone, 17β-hydroxy-17α-methyl-5α-androst-1-ene-3-one, 1α-methyl-5α-androstan-3α-ol-17-one, 1-methylene-5α-androstan-3α-ol-17-one, 1-testosterone, 2α-hydroxymethyl-17α-methyl-1,4-androstadiene-11α,17β-diol-3-one, 2α-hydroxymethylethisterone, 2α-methyl-5α-androstan-3α-ol-17-one, 4-chloro-4-androsten-3α-ol-17-one, 4-OH-testosterone, 5β-androst-1-en-17b-ol-3-one, 5α-Androst-1-ene-3β,17β-diol, 6β-hydroxy-dehydrochloromethyltestosterone, 6β-hydroxyfluoxymesterone, 6β-hydroxymethandienone, epimetendiol, 7α,17α-dimethyl-5β-androstane-3α,17β-diol, 7β,17α-dimethyl-5β-androstane-3α,17β-diol, 9α-fluoro-17,17-dimethyl-18-norandrostan-4,13-diene-11β-ol-3-one, 9α-fluoro-17α-methyl-4-androsten-3α, 6β,11β,17β-tetra-ol, dehydrochloromethyltestosterone, epioxandrolone, methyldienolone, 13β,17α-diethyl-5β-gonane-3α,17β-diol, 13β,17α-diethyl-5α-gonane-3α, 17β-diol, oxabolone were purchased from NMI (Pymble, Australia). Oxandrolone was a gift from Searle & Co (Chicago, IL, USA), 1-androstene-3,17-dione, 4-androstene-17α-methyl-11α,17β-diol-3-one, 7β-OH-DHEA, calusterone and mibolerone were from Steraloids (Newport, RI, USA). Madol and 2α,17α-dimethyl-17β-hydroxy-5α-androstan-3-one were bought from TRC (Toronto, Canada). Bolasterone was a gift from Upjohn and danazol from Whintrop.

2.4. Narcotics

11-nor-Δ9-tetrahydrocannabinol.9carboxylic acid, normethadone, (±)-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), fentanyl, norfentanyl, fenbutrazate, buprenorphine, morphine, codeine, ethylmorphine, 6-monoacetylmorphine (6-MAM), oxymorphone were purchased from Cerriliant. Pethidine, hydromorphone, oxycodone, heroine, dextromoramide and methadone were bought from Sigma (Bornem, Belgium). Pentazocine was a gift from Whintrop Laboratories (Newcastle, United Kingdom).

2.5. Stimulants

Pipradrol and pemoline were gifts from Merrell-Dow (Cincinnati, OH, USA) and Boehringer-Ingelheim (Brussels, Belgium), respectively. Cocaine, benzoylecgonine were purchased from Cerriliant. Fenethylline was a gift from Chemiwerk Hamburg (Germany) and methylphenidate from Ciba-Geigy (Groot-Bijgaarden, Belgium). Fencamine was obtained from Laboratoires Miquel S.A. (Barcelona, Spain), fenspiride from Laboratoires Servier (Orléans, France) and amineptine and amineptine C5 metabolite from Laboratoires Servier (Orleans, France). Strychnine and fencamfamine were donated by Merck. Carphedon, 6-OH-bromantaan, crotethamide, cropropamide, cyclazodone and famprofazone were bought from NMI. Dimefline was from Recordate Industria Chemica & Farmaceutica (Milan, Italy) and furfenorex and clobenzorex from Roussel Uclaf (Romainville, France). Amiphenazole and octopamine were purchased from Sigma (Bornem, Belgium), while ethamivan, chloorphentermine and benzphetamine were gifts from Sinclair Pharmaceuticals (Godalmings, UK), Tropon Werke (Cologne) and Upjohn (Kalamazoo, USA), respectively. 3,3-diphenylamine and prenylamine were gifts from the World Association of Anti-Doping Scientists (WAADS).

2.6. Beta-blockers

Beta-blockers obtained as reference substances were: acebutolol from Rhone-Poulenc (Brussels, Belgium), alprenolol from Astra Chemicals (Holstein, Germany), propranolol from ICI (Kortenberg, Belgium), betaxolol from Synthelabo (Brussels, Belgium), labetolol from Glaxo (Brussels, Belgium), metoprolol from Ciba-Geigy, nadolol from Squibb (Braine l'Alleud, Belgium), oxprenolol from CIBA (Dilbeek, Belgium), pindolol from Sandoz (Vilvoorde, Belgium), sotalol from Pfizer, timolol from MSD (Brussels, Belgium), bisoprolol from Merck (Overijse, Belgium), carvedilol from Roche (Mannheim, Germany). Levobunolol (l-bunolol), esmolol were a kind gift from the South African Doping Control Laboratory. Carteolol was a gift from the Portuguese Doping Control Laboratory. The following products were extracted from therapeutical preparations: celiprolol (Selectol, Pharmacia, Brussels, Belgium) and metipranolol (Beta-Ophtiole, Tramedic, Sint-Niklaas, Belgium).

2.7. Beta-2-agonists

Salbutamol, terbutaline and clenbuterol were purchased from RIVM. Salmeterol xinafoate was a gift from GlaxoSmithKline (Philadelphia, PA, USA). Fenoterol was a gift from Boehringer & Sohn (Ingelheim am Rhein) and formoterol from Novartis (Arnhem, The Netherlands). Bambuterol was donated by the Instituto Nacional do Desporto (Lisbon, Portugal).

2.8. Hormone antagonists and modulators

Anastrazole, toremiphene, exemestane, 17β-hydroxy-6-methylene-androsta-1,4-diene-3-one (exemestane metabolite), were generous gifts from Astra Zeneca (Macclesfield, UK), WAADS, Pfizer (Groton, UK) and the Faculty of Pharmacy of the Helsinki University (Helsinki, Finland), respectively. 6α-OH-androstenedione, 4-OH-androstenedione (formestane), 4-hydroxycyclofenil, 3-hydroxy-4-methoxytamoxifen and bis-(4-cyanophenyl)methanol (Letrozole metabolite) were purchased from NMI. 4-OH-tamoxifen was bought from Sigma–Aldrich.

2.9. Other substances

Zilpaterol and zeranol were purchased from NMI, probenecid from Federa (Brussels, Belgium) and 5 hydroxypentoxyfylline (5-OH-pentoxyfylline) from Hoechst (Frankfurt, Germany).

2.10. Excretion urines

Excretion urines from the stimulants prolintane (catovit®) and sibutramine (Reductil®) were obtained after the controlled administration of an oral therapeutic dose (10 mg each) to healthy volunteers, which had given written consent, and were provided by other doping control laboratories. The samples were stored at −20 °C awaiting analysis.

2.11. Sample preparation

To 1 ml of urine, 50 μl of the internal standard mixture, 1 ml phosphate buffer (pH = 7.0) and 50 μl of β-glucuronidase from *E. coli* were added. The samples were incubated for 1.5 h at 56 °C. 1 ml of a liquid mixture $NaHCO_3/K_2CO_3$ (pH = 9.5) and 5 ml of diethyl ether were added to hydrolyzed urine samples and extracted for 20 min by rolling. The organic layer was evaporated to dryness under oxygen free nitrogen (OFN) at room temperature.

To the dried residues, 20 μl of acetonitrile was added, followed by derivatisation for 1 h at 80 °C with 100 μl MSTFA/ethanethiol/NH_4I (500:4:2).

2.12. Instrumentation

An Agilent (Agilent Technologies, Palo Alto, USA) model GC 7890 gas chromatograph coupled with an Agilent 7000A triple quadrupole mass spectrometer (Agilent Technologies) and a MPS2 autosampler and PTV-injector from Gerstel (Mülheim an der Ruhr, Germany) were used.

The GC was equipped with a 12.5 m capillary column (HP-Ultra 1, column length 12.5 m × 0.2 mm with a 0.11 μm film thickness) from J&W Scientific (Agilent Technologies, USA).

The column temperature was programmed as follows: the initial temperature was 100 °C (0.2 min), 90 °C/min → 185 °C, 9 °C/min → 230 °C, 90 °C/min → 310 °C (0.95 min). The transfer line was maintained at 310 °C. Hydrogen (α-gas1, Airliquide, Desteldonk, Belgium) was used as a carrier gas at a flow rate of 1 ml/min.

5 μl was injected and the PTV-injector settings were 100 °C (0.15 min), 12 °C/s → 280 °C.

Helium was used as a quench gas at a flow of 2.25 ml/min and nitrogen as a collision gas at a flow of 1.5 ml/min.

3. Method validation

3.1. Quantitative

Six point calibration curves were made by spiking steroid stripped urine samples (3 replicates per concentration). Unweighed least squares regression was used to construct the calibration curves.

Accuracy and precision (repeatability) of the method were subsequently tested at every level ($n = 6$). Acceptable tolerances (%) for precision were calculated from $2/3RSD_{max} = 2^{(1-0.5\log C)}$ [18]. Tolerances for the accuracy – expressed as bias – were set at a maximum 15% [19].

Additionally, 50 urine samples were analyzed using the traditional method on a single quadrupole instrument and on the triple quadrupole instrument.

3.2. Qualitative analysis

The method validation was performed according the Eurachem guidelines [20] on 10 different, randomly chosen urine samples.

To determine the limits of detection (LOD), 10 different urine samples were spiked with reference mixtures at different levels in the concentration range of 0.05, 0.1, 0.2, 0.5, 1 and 2 times the MRPL level. The LOD was defined as the lowest concentration where a substance can be detected in all samples analysed ($n = 10$). Repeatability was assessed through the analysis of multiple samples spiked at different levels during the determination of the LOD. Selectivity and specificity were tested by the analysis of blank urine samples.

4. Results and discussion

4.1. Sample preparation

One of the main difficulties for doping control laboratories is that the methods used need to be able to detect very low levels of a wide variety of prohibited substances in a small volume of a dirty matrix (predominantly urine). Indeed, the volume of urine delivered to laboratories is currently 60 ml [21]. This amount needs to be sufficient for laboratories to screen for and eventually confirm (using a totally independent analysis) the presence of any prohibited substance. Therefore methods used in doping control laboratories need to be able to detect a wide variety of substances in as little urine as possible. The method in this work only uses 1 ml of urine for the screening of a wide range of doping agents. In general the volume is 2–5 times lower than the volume normally used for screening

Table 1
Target substances for a quantitative analysis.

Substance	Internal standard	Calibrators (ng/ml)	Correlation coefficient (r^2)
Testosterone	d3-T	2–5–20–50–100–200	0.9918
Epitestosterone	d3-E	2–5–20–50–100–200	0.9933
Androsterone	d4-A	48–120–600–1200–2400–4800	0.9903
Etiocholanolone	d5-E	48–120–600–1200–2400–4800	0.9716
11β-OH-androsterone	d4-A	40–100–500–1000–2000–4000	0.9769
11β-OH-etiocholanolone	d5-E	40–100–500–1000–2000–4000	0.9877
Dihydrotestosterone	d3-DHT	4–10–40–100–200–400	0.9755
Dehydroepiandrosterone	d3-DHT	4–10–40–100–200–400	0.9927
4-androstene-3,17-dione	d3-DHT	4–10–40–100–200–400	0.9908
5α-androstane-3α,17β-diol	d3-aab	4–10–40–100–200–400	0.9841
5β-androstane-3α,17β-diol	d5-bab	4–10–40–100–200–400	0.9603
5α-androstane-3β,17β-diol	d3-aab	4–10–40–100–200–400	0.9933
5α-androstane-3,17-dione	MT	4–10–40–100–200–400	0.9975
5β-androstane-3,17-dione	MT	4–10–40–100–200–400	0.9853
19-norandrosterone	MT	1–3–5–10–15–20	0.9902
Salbutamol	d3-sal	100–300–500–1000–1500–2000	0.9807
THC-COOH	MT	5–15–25–50–75–100	0.9862

methods for anabolic steroids [13–17] and therefore constitutes a drastic reduction thanks to the PTV-injection. This is useful since in doping control a limited amount of urine is available for screening and confirmation of a wide range of substances.

The developed method is very comprehensive. Only a few anabolic agents for which GC–MS is not particularly suitable (e.g. tetrahydrogestrinone, methyltrienolone, stanozolol) and for which LC–MS offers a valuable alternative [22] are not included in the current method. The method also includes one or more metabolites of all prohibited narcotics, the most frequently used β2-agonists and hormone antagonists and modulators and beta-blockers. Additionally, the method contains a high number of stimulants and several substances from all other groups of prohibited substances (except peptide hormones and glucocorticosteroids).

The method incorporates a high number of quality assurance measures which cover the three basic steps in sample preparation: hydrolysis, extraction and derivatisation.

The use of a high amount of β-glucuronidase allows for an efficient hydrolysis after 1.5 h at 56 °C. Moreover, the use of both glucuronidated and free steroids with similar structure (d4-A, d5-Et) allows for an adequate evaluation of hydrolysis efficiency.

Using a diverse mixture of internal standards the differences in physico-chemical properties, possibly leading to changes in extraction efficiency of the broad spectrum of target compounds in the current method is covered. This has several important benefits, besides the obvious advantages for the quantitation of the non-deuterated structural analogues.

The inclusion of transitions for mono-TMS derivatised androsterone and etiocholanolone in the method additionally allows for the evaluation of the derivatisation efficiency.

Hence, this integrated approach allows for a comprehensive evaluation of the sample preparation efficiency per sample (rather than per batch or solely at the time of validation) since all major sample preparation steps are monitored.

To evaluate possible microbiological sample degradation, 5α-androstane-3,17-dione and 5β- androstane-3,17-dione are monitored. This is important since microbiological degradation can affect the steroid profile (the combination of endogenous steroids quantified to detect misuse with natural steroids). In cases where elevated concentrations of 5α-androstane-3,17-dione or 5β- androstane-3,17-dione are detected, particular care must be taken in the evaluation of the steroid profile.

4.2. Gas chromatography

The aim of this study was to develop a fast GC–MS method, capable of quantifying the endogenous steroids given in Table 1 and at the same time detecting a wide range of other substances qualitatively. Sufficient resolution between compounds is a prerequisite for adequate quantitation. In this method, the separation of the isomers androsterone and etiocholanolone, present at relatively large concentrations (Table 1), and to a minor extent the other isomers (11β-OH-A and 11β-OH-Et and 5aab and 5bab) put restrictions on chromatographic speed and injected volumes. Nevertheless, in this method 5 μl of sample could be injected using a PTV-injector. This is substantially higher than in previous methods using split/splitless injection.

The use of a short capillary column compared to those used in previous methods [13–17], in combination with a high flow rate of hydrogen (instead of helium which is used traditionally as a carrier gas) resulted in a substantial reduction of the GC run time. Indeed, the current method has a run time of 7.98 min. However, even at high concentrations of 4.8 μg/ml, androsterone and etiocholanolone are sufficiently separated to allow for an adequate quantification (Fig. 1). This significant reduction in GC run time greatly improves sample turn around, which can be essential in those cases (e.g. Olympics) where stringent demands are put on sample reporting times (24–48 h).

4.3. Mass spectrometry

Determination and optimization of the mass spectrometric conditions were done in a multistep process. In the first step, full scan spectra were obtained for every (derivatised) compound. After selection of a suitable precursor ion, full product scan mass spectra were acquired at different collision energies (10 and 25 eV). Suitable product ions were selected and SRM transitions were set up. Selection of the final product ions (at least two transitions per substance) and optimization of the collision energy (5, 10, 15, 20, 30, 35 eV) was then performed both on reference standards and extracts from spiked urine samples. Per substance, the best *S*/*N* ratio was used for the final selection of the most appropriate transitions and collision energies.

Table 2 shows the final instrumental settings of the mass spectrometer for the investigated target analytes.

5. Method validation

5.1. Quantitative

The substances included in the quantitative part of the method include those steroids traditionally used in doping control to establish the use of a prohibited substance (T, E, A, Et, DHT, DHEA, androstenedione, 5aab, 5bab). Additionally the method moni-

Table 2
Substances (as TMS-enol-TMS ether derivatives) included in the method according to WADA prohibited class, instrument settings, limit of detection and applicable minimum required performance limit (MRPL).

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/ml)	MRPL (ng/ml)
S1a	4.14	5β-androst-1-en-17β-ol-3-one	432.0 → 194.0	15	5	10
			432.0 → 206.0	15		
	5.32	Boldenone	430.0 → 206.0	10	10	10
			430.0 → 191.0	30		
	5.09	1-Androstenediol	434.0 → 195.0	20	5	10
			434.0 → 127.0	20		
	5.05	1-testosterone	432.0 → 194.0	5	10	10
			432.0 → 206.0	10		
	5.09	17α-methyl-5α-androstane-3α,17β-diol	435.0 → 255.0	20	2	2
			435.0 → 213.0	20		
	5.12	17α-methyl-5β-androstane-3α,17β-diol	435.0 → 255.0	20	5	2
			435.0 → 213.0	20		
	6.7	Oxymesterone	534.0 → 389.0	20	10	10
			534.0 → 444.0	20		
	4.15	Epimetendiol	358.0 → 301.0	15	2	2
			358.0 → 196.0	5		
	6.57	6β-hydroxymethandienone	517.0 → 229.0	20	5	10
			517.0 → 337.0	15		
	5.63	Metenolone PC	446.0 → 208.0	10	5	10
			446.0 → 195.0	15		
	4.92	1-Methylene-5α-androstan-3α-ol-17-one (metenolone metab)	446.0 → 341.0	15	20	10
			446.0 → 195.0	5		
	5.64	17α-ethyl-5β-estrane-3α,17β-diol (norethandrolone major metab)	421.0 → 241.0	15	10	10
			421.0 → 331.0	5		
	5.4	17α-ethyl-5α-estrane-3α,17β-diol (norethandrolone minor metab)	421.0 → 241.0	15	5	10
			421.0 → 145.0	25		
	4.77	2α-methyl-5α-androstan-3α-ol-17-one (drostanolone metab)	448.0 → 433.0	10	10	10
			448.0 → 253.0	25		
	6.05	Bolasterone PC	460.0 → 355.0	15	10	10
			460.0 → 315.0	15		
	5.62	7α,17α-dimethyl-5β-androstane-3α,17β-diol (bolasterone metab)	284.0 → 269.0	5	10	10
			284.0 → 213.0	10		
	6.13	Calusterone PC	460.0 → 355.0	15	10	10
			460.0 → 315.0	15		
	5.45	7β,17α-dimethyl-5β-androstane-3α,17β-diol (calusterone metab)	229.0 → 105.0	30	/	10
			269.0 → 159.0	5		
	5.07	1α-methyl-5α-androstan-3α-ol-17- one (mesterolone metab)	448.0 → 433.0	10	5	10
			448.0 → 253.0	20		
	5.63	4-chloro-4-androsten-3α-ol-17-one (clostebol metab)	466.0 → 181.0	20	10	10
			466.0 → 431.0	15		
	6.47	Norclostebol	452.0 → 216.0	20	2	10
			452.0 → 321.0	15		
	6.67	Fluoxymesterone PC	552.0 → 407.0	15	/	10
			552.0 → 357.0	15		
			552.0 → 319.0	15		
	6.93	6β-OH-fluoxymesterone	640.0 → 640.0	10	20	10
			640.0 → 143.0	25		
	5.04	9α-fluoro-17,17-dimethyl-18-nor-androstan-4,13-diene-11β-ol-3-one	462.0 → 208.0	15	5	10
			462.0 → 337.0	15		
	6.17	Oxandrolone	363,0 → 161,0	15	10	10
			308.0 → 117.0	15		
	5.56	Epioxandrolone	363,0 → 161,0	15	20	10
			308.0 → 117.0	15		
	6.68	Dehydrochloromethyltestosterone PC	478.0 → 285.0	20	10	10
			478.0 → 353.0	5		
	6.82	6β-hydroxy-dehydrochloromethyltestosterone	315.0 → 227.0	20	20	10
			315.0 → 241.0	15		
	5.19	17α-trenbolone	307.0 → 291.0	10	10	10
			307.0 → 275.0	20		
	7.1	2-hydroxymethyl-17α-methylandrostadiene-11α,17β-diol-3-one (formebolone metab)	444.0 → 356.0	25	/	10
			367.0 → 257.0	25		
	6.48	17α-methyl-4-androstene-11α,17β-diol-3-one (formebolone metab)	534.0 → 389.0	15	10	10
			534.0 → 339.0	25		
	5.85	Mibolerone	446.0 → 431.0	15	10	10
			446.0 → 341.0	20		
	6.14	Ethisterone	456.0 → 316.0	15	1	10
			456.0 → 301.0	15		
	4.76	3α,5α-tetrahydronorethisterone	431.0 → 167.0	20	2	10
			431.0 → 193.0	20		
	7.11	16-OH-furazabol	490.0 → 231.0	15	10	10
			490.0 → 143.0	35		
	5.94	Methyldienolone	430.0 → 285.0	10	10	10
			430.0 → 325.0	10		
	5.97	13β,17α-diethyl-5α-gonane-3α, 17β-diol (norbolethone metab)	435.0 → 255.0	10	20	10

Table 2 (*Continued*)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/ml)	MRPL (ng/ml)
			435.0 → 159.0	15		
	6.14	13β,17α-diethyl-5β-gonane-3α, 17β-diol (norbolethone metab)	435.0 → 255.0	20	5	10
			435.0 → 345.0	5		
	3.68	Madol	345.0 → 255.0	15	10	10
			345.0 → 201.0	15		
	6.11	2α,17α-dimethyl-17β-hydroxy-5α-androstane-3-one	462.0 → 141.0	15	10	10
			462.0 → 143.0	15		
	6.27	4-OH-nandrolone (oxabolone)	506.0 → 147.0	20	2	10
			506.0 → 93.0	25		
			506.0 → 195.0	20		
	6.48	4-OH-testosteron	520.0 → 225.0	15	2	10
			520.0 → 431.0	15		
	6.33	6-OH-androstenedione	518.0 → 319.0	15	1	10
			518.0 → 413.0	15		
	5.19	7β-OH-DHEA	430.0 → 325.0	10	20	10
			430.0 → 220.0	10		
s1b	4.04	19-norandrosterone	405.0 → 225.0	10	1	2
			405.0 → 315.0	5		
	4.12	5β-androstane-3,17-dione	290.0 → 275.0	10	EAAS	/
			290.0 → 185.0	10		
	4.64	5α-androstane-3α,17β-diol	256.0 → 185.0	15		
			256.0 → 157.0	15		
	4.71	5β-androstane-3α,17β-diol	256.0 → 185.0	15		
			256.0 → 157.0	15		
	4.58	Androsterone	239.0 → 167.0	35		
			239.0 → 117.0	35		
	4.63	Etiocholanolone	239.0 → 167.0	35		
			239.0 → 117.0	35		
	5.09	5α-androstan-3,17-dione	290.0 → 275.0	10		
			290.0 → 185.0	10		
	4.98	DHEA	432.0 → 327.0	10		
			432.0 → 237.0	10		
	5.14	Epitestosterone	432.0 → 209.0	10		
			432.0 → 327.0	10		
	5.13	5α-androstane-3β,17β-diol	421.0 → 255.0	20		
			421.0 → 213.0	20		
	5.29	4-androstenedione	430.0 → 209.0	15		
			430.0 → 234.0	15		
	5.24	DHT	434.0 → 195.0	20		
			434.0 → 182.0	20		
	5.41	Testosteron	432.0 → 209.0	10		
			432.0 → 327.0	10		
	5.52	11β-OH-androsterone	522.0 → 236.0	10		
			522.0 → 324.0	10		
	5.6	11β-OH-etiocholanolone	522.0 → 236.0	10		
			522.0 → 324.0	10		
	4.13	Mono TMS Androsterone	347.0 → 253.0	20	qas	/
S1c	3.37	Zilpaterol	308.0 → 218.0	10	5	10
			308.0 → 203.0	15		
			291.0 → 219.0	15		
	6.43	Zeranol	433.0 → 295.0	15	10	10
			433.0 → 309.0	15		
	2.42	Clenbuterol	335.0 → 227.0	10	0.2	2
			335.0 → 300.0	10		
	5.37	3α-hydroxytibolone	443.0 → 193.0	35	5	10
			443.0 → 167.0	30		
S3	2.17	Salbutamol	369.0 → 207.0	15	25	100
			369.0 → 191.0	15		
	1.96	Terbutaline	356.0 → 267.0	25	50	100
			356.0 → 355.0	25		
	6.07	Fenoterol	322.0 → 68.0	15	100	100
			322.0 → 279.0	15		
	6.6	Fenoterol C,N-methylene	308.0 → 207.0	15	/	50
			308.0 → 179.0	15		
	6.73	Formoterol	178.0 → 121.0	20	50	100
			178.0 → 135.0	20		
	7.82	Salmeterol	311.0 → 149.0	15	100	100
			311.0 → 121.0	25		
	5.02	Bambuterol	354.0 → 72.0	25	5	100
			354.0 → 282.0	10		
S4	3.63	Aminogluthetimide deriv.1	361.0 → 206.0	30	5	50
			361.0 → 221.0	10		
	5.26	Aminogluthetimide deriv.2	580.0 → 551.0	20	/	50
			580.0 → 519.0	20		

Table 2 (*Continued*)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/ml)	MRPL (ng/ml)
	3.16	Anastrazole	293.0 → 70.0	10	50	50
			293.0 → 209.0	15		
	3.17	Letrozole metabolite	291.0 → 160.0	15	2.5	50
			291.0 → 217.0	20		
	6.94	Exemestane PC	441.0 → 307.0	20	/	50
			441.0 → 193.0	20		
	6.94	17β-hydroxy-6-methylene-androsta-1,4-diene-3-one	443.0 → 207.0	20	25	50
			443.0 → 193.0	20		
	6.43	4-OH-androstene-3,17-dione (formestane)	518.0 → 221.0	15	2	10
			518.0 → 190.0	10		
	6.57	Toremiphene	405.0 → 58.0	15	25	50
			405.0 → 72.0	5		
	6.86	4-hydroxy-methoxytamoxifen 1	489.0 → 72.0	5	25	50
			489.0 → 58.0	15		
	7.02	4-hydroxy-methoxytamoxifen 2	489.0 → 72.0	5	25	50
			489.0 → 58.0	15		
	5.78	4-OH-tamoxifen	459.0 → 72.0	5	2.5	50
			459.0 → 58.0	15		
	7.74	Raloxiphene	578.0 → 193.0	35	25	50
			578.0 → 413.0	30		
	6.57	4-OH-cyclofenil	512.0 → 422.0	10	2.5	50
			512.0 → 343.0	5		
S5	3.13	Probenecid	328.0 → 103.0	25	12.5	250
			328.0 → 193.0	15		
S6	2.16	Carphedon	272.0 → 104.0	25	50	500
			272.0 → 229.0	15		
	4.98	6-OH-bromantan	395.0 → 91.0	30	2.5	500
			393.0 → 91.0	30		
	2.08	Pemoline	178.0 → 104.0	10	5	500
			392.0 → 178.0	10		
	2.28	Octopamine	174.0 → 866.0	5	100	500
			426.0 → 206.0	15		
			426.0 → 179.0	15		
	7.14	Strychnine	316.0 → 144.0	15	100	200
			316.0 → 220.0	10		
	1.37	Crotethamide	154.0 → 86.0	10	50	500
			154.0 → 69.0	15		
	1.97	Ethamivan	295.0 → 223.0	25	50	500
			295.0 → 265.0	20		
	1.36	Fencamfamine	215.0 → 186.0	5	50	500
			215.0 → 98.0	15		
	4.24	Fenspiride	241.0 → 96.0	10	25	500
			241.0 → 154.0	10		
	2.57	3,3-dihenylpropylamine	174.0 → 86.0	15	50	500
			174.0 → 100.0	15		
	4.65	Prenylamine	238.0 → 58.0	20	50	500
			238.0 → 91.0	20		
	1.94	Clobenzorex	168.0 → 125.0	20	100	500
			168.0 → 89.0	35		
	2.51	Cyclazodone	360.0 → 178.0	15	10	500
			360.0 → 247.0	15		
	6.57	Famprofazone	286.0 → 72.0	20	50	500
			286.0 → 214.0	15		
	1.66	Benzphetamine	148.0 → 91.0	20	10	500
			148.0 → 65.0	35		
	1.74	Methylphenidate	156.0 → 45.0	35	100	500
			156.0 → 84.0	10		
	6.47	Amineptine	193.0 → 115.0	15	10	500
			193.0 → 178.0	15		
	4.53	Amineptine C5 metabolite	193.0 → 115.0	15	50	500
			193.0 → 178.0	15		
	2.7	Cocaine	303.0 → 82.0	15	50	500
			303.0 → 198.0	5		
	3.07	Benzoylecgonine	240.0 → 82.0	20	100	500
			361.0 → 82.0	20		
	3.56	Prolintane metabolite14	322.0 → 293.0	20	excr	500
			322.0 → 205.0	20		
	2.28/2.34	Prolintane metabolite 5a/b	304.0 → 142.0	20	excr	500
			304.0 → 75.0	20		
	2.67	Prolintane metabolit e9	228.0 → 158.0	20	excr	500
			228.0 → 138.0	20		
	2.52	Sibutramine metabolite 1	158.0 → 116.0	10	excr	500
			158.0 → 102.0	10		
	2.74/2.82	Sibutramine metabolite 2/3	246.0 → 156.0	20	excr	500
			246.0 → 84.0	20		

Table 2 (*Continued*)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/ml)	MRPL (ng/ml)
S7	7.47	Buprenorphine	554.0 → 522.0	15	0.5	10
			554.0 → 450.0	20		
	6.57	Dextromoramide	265.0 → 166.0	15	20	200
			265.0 → 98.0	10		
	4.91	Heroine	369.0 → 327.0	10	2.5	200
			369.0 → 268.0	25		
	4.66	MAM	399.0 → 287.0	15	20	200
			399.0 → 340.0	10		
	5.37	Fentanyl	245.0 → 189.0	10	/	10
			245.0 → 146.0	15		
	2.19	Norfentanyl	175.0 → 120.0	5	/	10
			175.0 → 56.0	15		
	4.32	Hydromorphone	429.0 → 234.0	15	100	200
			429.0 → 357.0	25		
	2.73	Methadon	296.0 → 191.0	20	10	200
			296.0 → 281.0	10		
	2.93	Methadon 2	296.0 → 191.0	20	40	200
			296.0 → 281.0	10		
	2.37	Normethadon 1	224.0 → 103.0	35	100	200
			224.0 → 191.0	35		
	2.73	Normethadon 2	296.0 → 191.0	20	10	200
			296.0 → 252.0	20		
	2.14	EDDP	277.0 → 105.0	25	40	200
			277.0 → 220.0	20		
	4.42	Morphine	429.0 → 287.0	20	10	200
			429.0 → 220.0	35		
	4.37	Oxycodone	459.0 → 368.0	15	200	200
			459.0 → 312.0	15		
	4.76	Oxymorphone	502.0 → 70.0	30	40	200
			517.0 → 355.0	15		
	3.12	Pentazocine	357.0 → 246.0	15	100	200
			357.0 → 289.0	15		
	1.47	Pethidine	247.0 → 71.0	5	4	200
			247.0 → 173.0	5		
	3.97	Codeine	371.0 → 229.0	5	10	200
			371.0 → 234.0	5		
	4.21	Ethylmorphine	385.0 → 214.0	35	10	200
			385.0 → 234.0	10		
	2.51	Pipradrol	239.0 → 161.0	20	5	200
			239.0 → 221.0	20		
	5.25	Fenbutrazate	261.0 → 103.0	35	50	200
			261.0 → 175.0	15		
S8	6.06	THC-COOH	371.0 → 289.0	15	<5	7.5
			371.0 → 265.0	15		
P2	1.91	Oxprenolol	150.0 → 109.0	15	50	500
			221.0 → 72.0	15		
	3.62	Betaxolol	364.0 → 209.0	10	100	500
			364.0 → 172.0	10		
	2.94	Bisoprolol	405.0 → 56.0	25	100	500
			405.0 → 172.0	15		
	3.07	Pindolol 1	204.0 → 133.0	15	500	500
			220.0 → 75.0	15		
	3.65	Pindolol 2	205.0 → 130.0	15	50	500
			292.0 → 218.0	15		
	3.05	Esmolol	352.0 → 193.0	5	100	500
			352.0 → 56.0	15		
	3.02	Metipranolol	366.0 → 281.0	5	25	500
			366.0 → 239.0	15		
	2.64	Propanolol	316.0 → 231.0	5	25	500
			316.0 → 75.0	15		
	3.15	Timolol	373.0 → 186.0	15	50	500
			373.0 → 70.0	35		
	4.12	Carteolol	421.0 → 186.0	15	50	500
			421.0 → 365.0	5		
	4.12	Levobunolol	234.0 → 233.0	5	25	500
			234.0 → 217.0	10		
	2	Celiprolol 1	319.0 → 129.0	15	/	500
			205.0 → 89.0	15		
			205.0 → 117.0	15		
	3.45	Celiprolol 2	200.0 → 128.0	15	500	500
			200.0 → 144.0	15		
	4.53	Nadolol	510.0 → 70.0	35	250	500
			510.0 → 186.0	20		
	6.2	Acebutolol 1 + 2	278.0 → 166.0	30	500	500
			278.0 → 208.0	30		

Table 2 (*Continued*)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/ml)	MRPL (ng/ml)
	1.72	Alprenolol	321.0 → 72.0	15	250	500
			306.0 → 203.0	15		
	6.67	Labetolol	383.0 → 265.0	15	100	500
			383.0 → 251.0	15		
ISTD	4.66	5β-androstane-3a,17b-diol-d5	246.0 → 190.0	15	ISTD	/
			246.0 → 164.0	15		
	4.62	5α-androstane-3a,17b-diol-d3	244.0 → 202.0	15		
			244.0 → 188.0	15		
	4.51	Androsterone-d4	423.0 → 333.0	20		
			423.0 → 243.0	20		
	4.56	Etiocholanolone-d5	424.0 → 334.0	20		
			424.0 → 244.0	20		
	5.12	Epitestosterone-d3	435.0 → 330.0	5		
			435.0 → 209.0	20		
	5.38	Testosterone-d3	435.0 → 330.0	20		
			435.0 → 209.0	20		
	5.17	DHT-d3	437.0 → 205.0	15		
			437.0 → 195.0	15		
	2.16	Salbutamol-d3	372.0 → 210.0	20		
			372.0 → 193.0	20		
	5.97	17α-methyltestosterone	446.0 → 301.0	25		
			446.0 → 198.0	20		

PC: parent compound.

tors other endogenous steroids which are not affected by the intake of natural anabolics (11bOH-A and 11b-OH-Et) as well as markers of microbiological degradation (5α-androstanedione and 5β-androstanedione). The inclusion of these parameters can greatly assist in the evaluation process of atypical steroid profiles due to increased production of endogenous steroids or alteration by microbiological degradation. Besides these steroids the method also quantifies salbutamol, the most widely used β2-agonist, norandrosterone and the major metabolite of cannabis (11-nor-Δ9-tetrahydrocannabinol.9carboxylic acid, THC-COOH).

Although large differences in calibration ranges exist between the monitored compounds, correlation coefficients of 6-point calibration curves (3 replicates per calibrator) made in steroid stripped urine were acceptable. Additional analysis revealed that the residual standard deviations at every point of the calibration curves were lower than 2/3 of the maximum residual standard deviation as calculated by Horwitz. Moreover, the bias at each of these points was below 15%, showing acceptable accuracy as well. Therefore, in agreement with Eurachem guidelines, the method can be regarded as validated for quantitative purposes.

A comparison between the traditionally used GC–MS method in selected ion monitoring and the new methodology using selected reaction monitoring (SRM) for the testosterone to epitestosterone ratio is shown in Fig. 2. These results indicate an excellent correlataion ($r^2 = 0.95$) and comparability (slope is almost equal to 1) between both methodologies.

5.2. Qualitative analysis

The method validation for the non-threshold substances was also performed in accordance with Eurachem guidelines. Selectivity was tested by analyzing 10 blank urine samples and verifying that there were no matrix interferences. Additionally these samples were spiked at different concentration levels. The lowest concentration where concurrent signals ($S/N > 3$) for each monitored transition were obtained at the expected retention time (±1%) in all samples was labeled as the lower limit of detection (LOD). These LODs for the exogenous substances are given in Table 2. The method comprises 41 (metabolites) of anabolic steroids, 4 other anabolic agents, 6 β2-agonists, 11 hormone antagonists and modulators, 19 narcotics and 16 stimulants.

It should be noted that in some cases, the observed LOD for a metabolite exceeds WADA's MRPL. For these substances, the method was regarded as non-validated, although they remained part of the method. For all such cases, the method includes another metabolite (of the same parent drug) that has an LOD at or below the MRPL. This is the case for e.g. fluoxymesterone: the LOD for 6β-hydroxyfluoxymesterone (Table 2) is

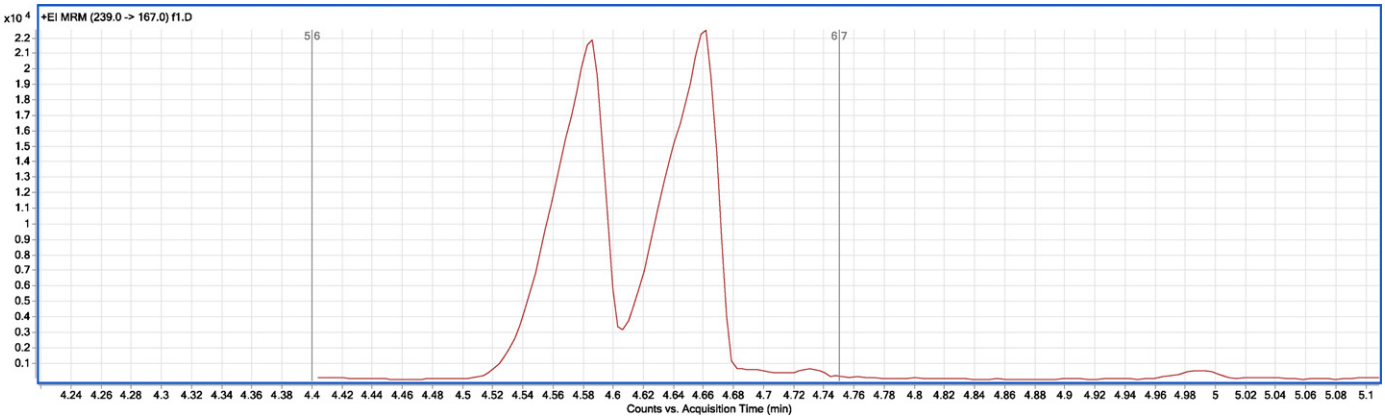

Fig. 1. Extracted ion chromatogram (m/z 239 → 167) for androsterone-bis-TMS and etiocholanolone-bis-TMS at the highest calibrator concentration (4.8 μg/ml).

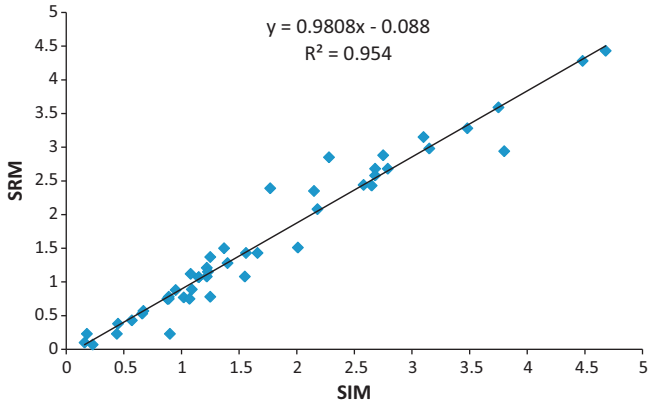

Fig. 2. Testosterone to epitestosterone ratio (T/E) in 50 urine samples using a single quadrupole mass spectrometer in selected ion monitoring (SIM) and using a triple quadrupole mass spectrometer in selected reaction monitoring (SRM).

20 ng/ml, while WADA's MRPL is set at 10 ng/ml. However, the LOD of 9α-fluoro-17,17-dimethyl-18-nor-androstan-4,13-diene-11β-ol-3-one, another fluoxymesterone metabolite, is compliant with the MRPL. Because – except for a few substances – WADA's technical document does not specify which metabolites needs to be monitored, the method can therefore still be considered as WADA-compliant for the detection of fluoxymesterone. Moreover the detection of multiple metabolites instead of a single metabolite to determine misuse of a doping agent has multiple advantages. Firstly, it can provide additional supporting evidence for misuse since in most cases the concentration in a "positive" sample will be clearly above the MRPL. Additionally, the inclusion of multiple metabolites can assist in the detection of a prohibited substance at different time points after use. Indeed, it is widely known that the excretion profile of metabolites is time and inter-individual dependent. Therefore, a metabolite which is the major metabolite in one individual after a definite post-administration time, might only be a minor metabolite in another individual which took the drug at another point in time. The current method is also capable of detecting all compounds from the class of "other anabolic agents", except the group of selected androgen receptor modulators for which it was not tested, as these compounds are still in clinical phase trials.

Besides the anabolic agents, a wide variety of hormone antagonist and modulators can be detected at or below the MRPL. This list includes substances with a steroidal structure (formestane, 6α-OH-androstenedione and the metabolite of exemestane: 17β-hydroxy-6-methylene-androsta-1,4-diene-3-one) as well as non-steroidal compounds (aminogluthetimide, anastrazole, letrozole metabolite, raloxiphene, toremiphene, 4-OH-cyclofenil, 4-OH-tamoxifen and the isomers of 4-OH-methoxytamoxifen). Moreover, as androsta-1,4,6-triene-3,17-dione also metabolizes to boldenone and its metabolites [23], the only substances from this class which are not included in the method are testolactone, clomiphene and fulvestrant, due to lack of reference standards for the metabolites of these substances.

Similar as for the previous groups, most prohibited narcotics undergo extensive Phase I and Phase II metabolism. Therefore all WADA prohibited narcotics and/or a metabolite were included in the current method. Except for fentanyl, which shows superior detection by LC–MS, all LODs were lower than WADA's MRPL and therefore the methodology is very well suited for monitoring the misuse of narcotics. In addition to the prohibited narcotics, the method also screens for codeine, because the use of codeine can result in the detection of morphine. In cases where the detection of morphine can be attributed to the use of codeine however, a laboratory should not report such cases [2]. Although in general, urine is not well suited to determine the post-administration time of sample collection, the current method offers some possibilities for several substances by monitoring multiple metabolites for which the excretion profile is time dependent. This is e.g. the case for heroine for which not only the parent substance but also morphine and 6-monoacetylmorphine (MAM) are monitored. Besides these substances, which are also important in forensic science, toxicology and laboratories working in the field of drugs of abuse and work place testing, the method is also capable of simultaneously quantifying 11-nor-Δ9-tetrahydrocannabinol.9carboxylic acid (THC-COOH), the major metabolite of cannabis and one of the most detected doping agents world-wide.

In contrast to the narcotics, most stimulants are not excreted as conjugates [24]. Therefore, the inclusion of these substances was not the focus of this research. Nevertheless, a wide range of stimulants (or metabolites), including cocaine and its metabolite benzoylecgonine are included in the method.

The method covers the most frequently used β-agonists in sports. Moreover, in the case of fenoterol both the parent drug (O-TMS tetrakis derivatised) and a degradation product, C,N-methylene fenoterol-tetrakis-TMS derivatives were monitored [26]. Although the degradation product was not detected in the validation study, its inclusion in the method will increase the detection capability of the method for real samples substantially since fenoterol can be rapidly degraded.

Although beta blockers are only prohibited in particular sports, many drugs belonging to this group of substances are excreted as glucuronide conjugates. Therefore 15 beta blockers were included in the method. The inclusion of this group has the advantage, that in case their detection is requested, no additional analysis needs to be performed and hence this leads to an optimized laboratory efficiency.

Although the derivatisation procedure uses an optimized protocol [25] and the method monitors the effectiveness by the detection of mono-TMS derivatised androsterone and etiocholanolone, the formation of multiple derivatives of several compounds (e.g. celiprolol, pindolol) is still possible. Although in general one of the derivatives gives a better signal than the other, the inclusion of the second derivative can be regarded as a safety precaution. Taking into account the scan speed of the instrument (500 scans/s), this addition of transitions does not decrease the overall performance of the method.

6. Conclusion

A fast GC–MS/MS method for the quantitative determination of the steroid profile, salbutamol, THC-COOH and norandrosterone as well as the qualitative detection of 142 doping agents (or their metabolites) was developed and validated. Using hydrogen as a carrier gas and a short (12.5 m) capillary column all doping agents could be detected within a single run of less than 8 min. The use of a wide range of internal standards allows for an evaluation of the sample preparation efficiency.

The current method shows that the combination of triple quadrupole technology and large volume injection can greatly improve the detection capabilities of target substances in complex matrices as biological fluids.

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