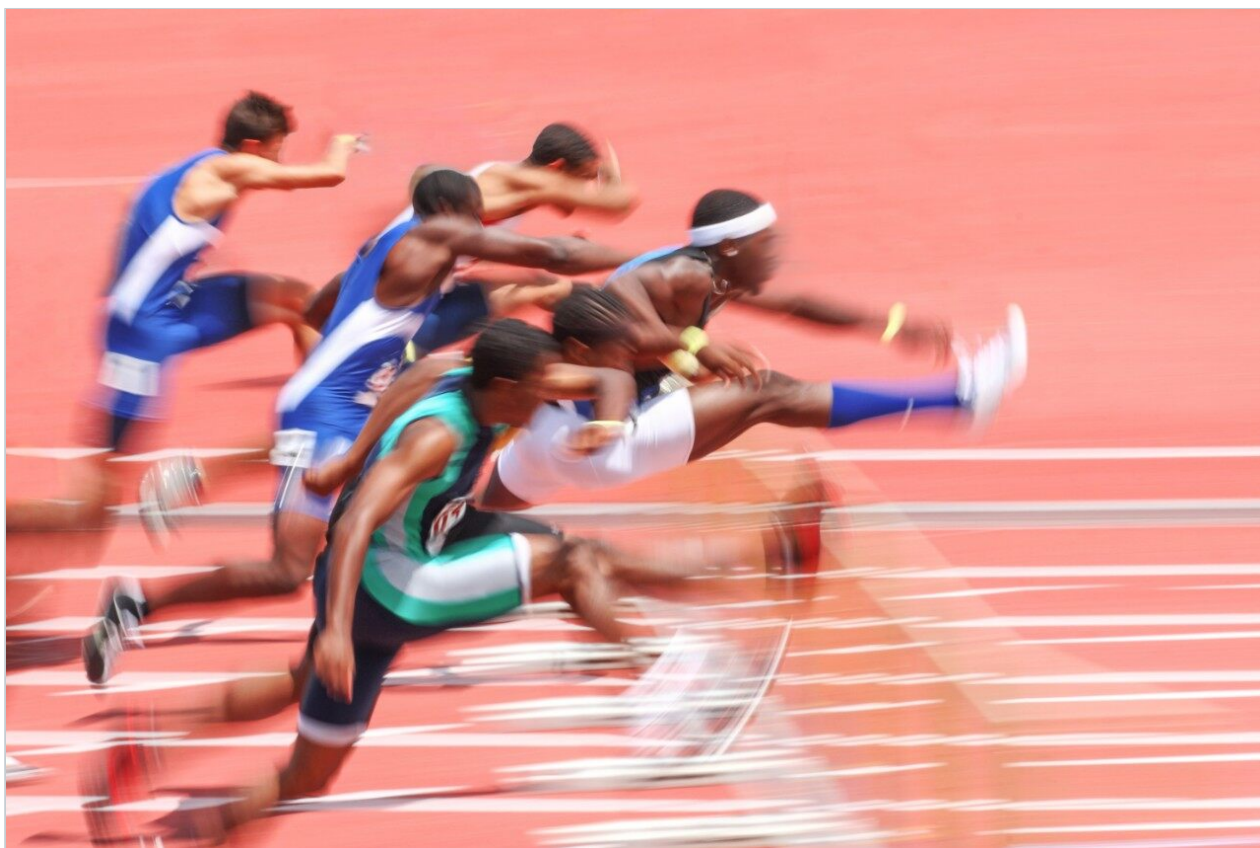


Nota de aplicación

Improving Detection of Anabolic Steroids in Sports: Simultaneous Detection of Intact Phase I and Phase II Urinary Metabolites by UPLC-MS/MS

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Abstract

This application note presents a novel LC-MS/MS screening method for the simultaneous detection of intact phase I and phase II (glucuronides and sulfates) urinary metabolites of anabolic androgenic steroids (AAS).

A UPLC-MS/MS screening method for the simultaneous detection of steroid metabolites is a feasible alternative compared to the conventional procedures. Incorporation of new phase II metabolites as described is straightforward and can significantly enhance the screening and detection capabilities of steroids in sport.

Benefits

- Simultaneous detection of metabolites irrespective of their nature by UPLC-MS/MS
- Simple and fast SPE sample preparation prior to chromatographic analysis
- An extended window of detection through the monitoring of long term excreted metabolites
- Elimination of hydrolysis and derivatization steps
- High recovery efficiency for all types of metabolites (unconjugated, glucuronides, sulfates)

Introduction

Anabolic androgenic steroids (AAS) are prohibited in sports. They are the most frequently detected substances in doping controls reflecting their wide misuse among athletes.¹ Screening of AAS is currently performed using a combination of both gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), after hydrolysis of the urine using β -glucuronidase enzymes.² Therefore, only unconjugated metabolites and hydrolyzable glucuronic acid conjugates are detectable under these conditions. Other phase II metabolites such as sulfates or glucuroconjugates stable to this hydrolysis cannot be detected using the current screening methods.

LC-MS/MS technology allows for the intact detection of all types of conjugates, and this application note presents a novel LC-MS/MS screening method for the simultaneous detection of intact phase I and phase II (glucuronides and sulfates) urinary metabolites of AAS.³

The proposed analytical strategy has the advantage of simplified sample preparation as hydrolysis and derivatization steps, which are required in conventional screening methods, are not needed. In addition, the method improves the detection capabilities through the monitoring of long term excreted phase II metabolites not detectable using the current screening strategy.⁴⁻⁷ Moreover, new long term phase II metabolites described in the future could be incorporated irrespective of their urinary nature. Finally, automation of the procedure, with on-line solid phase extraction system, could be easily implemented.

Experimental

LC conditions

System:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C ₁₈ 2.1 x 100 mm, 1.7 μm
Column temp.:	45 °C
Flow rate:	0.3 mL/min
Mobile phase A:	1 mM ammonium formate in water, containing 0.01% formic acid
Mobile phase B:	1 mM ammonium formate in acetonitrile:water (95:5, v/v), containing 0.01% formic acid
Gradient:	0 min, 20% B; 2 min, 20% B; 15 min, 40% B; 16 min, 70% B; 17 min, 95% B; 18 min, 95% B; 18.5 min, 20% B; 20 min, 20% B
Injection volume:	10 μL
Analysis time:	20 min

Strong wash:	Acetonitrile
Weak wash:	water/acetonitrile (95:5, v/v)

MS conditions

System:	Xevo TQ MS
Ionization modes:	ESI+ and ESICapillary
voltage:	3.5 kV (ESI+) and 2.5 kV (ESI-)
Source temp.:	120 °C
Desolvation temp.:	450 °C
Desolvation gas:	1200 L/h
Cone gas:	50 L/h
Acquisition mode:	Multiple reaction monitoring (MRM) shown in Table 1

Sample description

Drug-free urine samples were collected from healthy volunteers and used for method development.

Excretion study samples were obtained following methyltestosterone and stanozolol administration. A single oral dose of methyltestosterone (10 mg) or stanozolol (6 mg) was administered to three and four healthy male volunteers, respectively. Samples were collected before administration and up to 31 days thereafter. Routine doping samples positive for stanozolol were also analyzed.

Sample preparation

After the addition of 20 µL of the internal standard (IS) (a methanolic solution containing methyltestosterone, nandrolone-d3 sulfate and testosterone-d3 glucuronide at 1 µg/mL, and androsterone-d4 glucuronide at 5 µ

g/mL), 2 mL of urine samples were vortex-mixed and passed through a C₁₈ cartridge previously conditioned with 2 mL of methanol and 2 mL of water. The cartridge was then washed with 2 mL of water, and the analytes eluted with 2 mL of methanol. The samples were evaporated to dryness under nitrogen stream in a bath at 40 °C. The extract was redissolved into 200 µL of a solution of ACN:water (10:90, v/v).

Results and Discussion

Optimization of the mass spectrometric detection conditions

All steroid metabolites showed to be readily ionizable.² In positive mode, unconjugated and glucuronide metabolites with a 3-keto function ionized as $[M+H]^+$. Glucuronides lacking this feature showed the adduct $[M+NH_4]^+$ as the major ion. In negative mode, all glucuronides formed the ion $[M-H]^-$ resulting from deprotonation of the acidic group. The sulfate conjugates also yielded the $[M-H]^-$ ion; in all cases, this species gave the most intense signals. Collision-induced dissociation (CID) gave common ions or losses depending on the analytes chemical features (Table 1 and Figure 1).

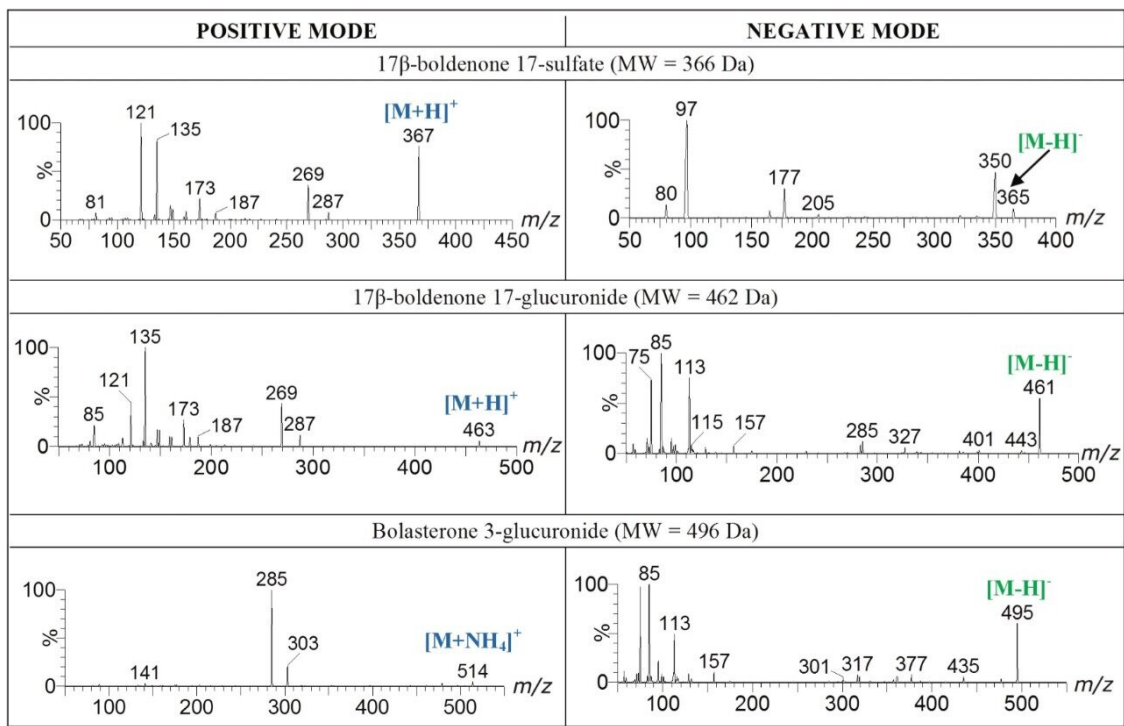


Figure 1. Product ion mass spectra of representative sulfate and glucuronide metabolites in positive and negative electrospray ionization modes.

Precurso	Metabolite *	RT (min)	Mode (ESI)	Precursor ion		CV (V)	CE (eV)	Product ion (m/z)
				(m/z)	Adduct			
Bolasterone	bolasterone 3-G	10.55	NEG	495	[M-H] ⁻	50	45	85
			POS	514	[M+NH ₄] ⁺	15	40	175
Boldenone/boldione	17β-boldenone 17-G	6.16	POS	463	[M+H] ⁺	20	25	121
			POS	463	[M+H] ⁺	20	20	135
	5β-androst-1-ene-17β-ol-3-one 17-G	9.65	NEG	463	[M-H] ⁻	30	40	85
			POS	465	[M+H] ⁺	25	25	187
	17β-boldenone 17-S	6.61	NEG	365	[M-H] ⁻	55	30	350
			NEG	365	[M-H] ⁻	55	40	177
epiboldenone 17-S	7.32	NEG	365	[M-H] ⁻	55	30	350	
		NEG	365	[M-H] ⁻	55	40	177	
Calusterone	5β-androstan-7β,17α-dimethyl-3α,17β-diol 3-G	11.80	NEG	495	[M-H] ⁻	50	45	75
			NEG	495	[M-H] ⁻	50	45	85
Dehydrochloromethyltestosterone	6β-hydroxy-4-chloro-metandienone	9.00	POS	351	[M+H] ⁺	15	20	147
			POS	351	[M+H] ⁺	15	50	91
Drostanolone	2α-methyl-5α-androstan-3α-ol-17-one 3-G	14.79	NEG	479	[M-H] ⁻	55	35	85
			NEG	479	[M-H] ⁻	50	35	75
Fluoxymesterone	9-fluoro-18-nor-17,17-dimethyl-4,13-diene-11-ol-3-one	17.02	POS	319	[M+H] ⁺	35	25	225
			POS	319	[M+H] ⁺	35	25	281
Mesterolone	1α-methyl-5α-androstan-3α-ol-17-one 3-G	12.95	NEG	479	[M-H] ⁻	55	35	85
			NEG	479	[M-H] ⁻	55	35	75
	1α-methyl-5α-androstan-3α-,17β-diol 3-G	10.28	NEG	481	[M-H] ⁻	50	35	75
			NEG	481	[M-H] ⁻	50	35	85
	6β-hydroxy -metandienone	5.58	POS	281	[M+H-2H ₂ O] ⁺	30	30	171
			POS	299	[M+H-H ₂ O] ⁺	25	25	121
Metandienone	Epimetandienone	16.42	POS	301	[M+H] ⁺	20	15	149
			POS	301	[M+H] ⁺	20	10	283
	18-nor-17β-hydroxymethyl-17α-methylandro-1,4,13-triene-3-one 18-S	7.52	NEG	377	[M-H] ⁻	60	40	80
			NEG	377	[M-H] ⁻	60	30	362
Metenolone	1-methylen-5α-androstan-3α-ol-17-one 3-G	11.47	NEG	477	[M-H] ⁻	50	35	75
			POS	496	[M+NH ₄] ⁺	15	15	285
Methyltestosterone	17α-hydroxy-17β-methylandro-4,6-dien-3-one	16.57	POS	301	[M+H] ⁺	25	20	225
			POS	301	[M+H] ⁺	25	30	210
	methyl-5β-androstan-3α,17β-diol 3-G	9.91	POS	500	[M+NH ₄] ⁺	15	40	161
			POS	500	[M+NH ₄] ⁺	15	20	271
	17α-methyl-5α-androstan-3α,17β-diol 3/17-G	10.01	NEG	481	[M-H] ⁻	50	35	75
			NEG	481	[M-H] ⁻	50	35	85
	17α-methyl-5β-androstan-3α,17β-diol 3-S	9.47	NEG	385	[M-H] ⁻	60	40	97
			NEG	385	[M-H] ⁻	60	40	97
	17β-methyl-5α-androstan-3α,17α-diol 3-S	15.16	NEG	385	[M-H] ⁻	60	40	97
			NEG	385	[M-H] ⁻	60	40	97
17β-methyl-5β-androstan-3α,17α-diol 3-S	15.56	NEG	385	[M-H] ⁻	60	40	97	
		POS	470	[M+NH ₄] ⁺	20	25	241	
Nandrolone	19-noretiocholanolone 3-G	10.20	POS	470	[M+NH ₄] ⁺	20	20	259
			NEG	451	[M-H] ⁻	50	35	85
	19-norandrosterone 3-G	10.68	NEG	451	[M-H] ⁻	50	35	75
			POS	451	[M+H] ⁺	30	30	85
	17β-nandrolone 17-G	5.91	NEG	449	[M-H] ⁻	45	30	113
			NEG	353	[M-H] ⁻	55	35	97
	17α-nandrolone 17-S	6.88	NEG	353	[M-H] ⁻	55	35	97
			NEG	353	[M-H] ⁻	55	35	97
	17β-nandrolone 17-S	6.04	NEG	353	[M-H] ⁻	55	35	97
			NEG	355	[M-H] ⁻	55	35	97
19-noretiocholanolone 3-S	9.70	NEG	355	[M-H] ⁻	55	35	97	
		NEG	355	[M-H] ⁻	55	35	97	
Oxandrolone	Oxandrolone	12.68	POS	307	[M+H] ⁺	20	30	93
			POS	307	[M+H] ⁺	25	10	289
	Epioxandrolone	16.22	POS	307	[M+H] ⁺	20	30	93
			POS	307	[M+H] ⁺	20	10	289
Stanozolol	3'-hydroxystanozolol 3'-G	9.22	POS	345	[M+H-gluc] ⁺	60	45	97
			NEG	519	[M-H] ⁻	45	30	343
	4β-hydroxy-stanozolol 4-G	7.08	POS	521	[M+H] ⁺	25	25	309
			NEG	519	[M-H] ⁻	45	25	193
	16β-hydroxy-stanozolol 16-G	4.64	POS	521	[M+H] ⁺	25	65	81
			POS	521	[M+H] ⁺	25	40	345
	stanozolol-O-G	7.74	POS	505	[M+H] ⁺	25	45	329
			POS	505	[M+H] ⁺	25	65	81
	stanozolol-N-G	7.55	POS	505	[M+H] ⁺	25	45	329
			POS	505	[M+H] ⁺	25	65	81
17-epistanozolol-N-G	10.90	POS	505	[M+H] ⁺	25	45	329	
		POS	505	[M+H] ⁺	25	65	81	
d3-NAN-S (IS)	nandrolone-d3 17-S	6.11	NEG	356	[M-H] ⁻	55	40	98
d3-T-G (IS)	testosterone-d3 17-G	7.30	POS	468	[M+H] ⁺	35	25	97
d4-And-G (IS)	androsterone-d4 3-G	12.08	NEG	466	[M-H] ⁻	45	30	85
			POS	488	[M+NH ₄] ⁺	15	40	95
MET (IS)	Methyltestosterone	14.81	POS	303	[M+H] ⁺	30	25	109

Table 1. Compounds and internal standards included in the screening method: retention time (RT), ionization mode (POS, positive; NEG, negative), precursor ion, cone voltages (CV), collision energies (CE) and product ions.

* S: sulfate; G: glucuronide

An MRM method was set up including one or more ion transitions for each steroid metabolite (Table 1). To select the ion transitions to monitor each analyte, ten different urine samples spiked with the analytes at different concentrations were analyzed, and the selection was based on the signal to noise ratio (>3:1) and the signal intensity of each metabolite. In the case of sulfate conjugates, the ion transitions that yielded the highest signal were those to the product negative ion m/z 97 and, for most sulfate metabolites, only these transitions were monitored (Table 1). However, for other sulfates with molecular masses closer to steroidal endogenous compounds such as 17β -boldenone 17-sulfate, the ion transition $[M-H]^-$ to m/z 97 showed low specificity and negative ion transitions resulting from characteristic fragmentations (m/z 365>350, m/z 365>177) had higher selectivity (Figure 2).⁴

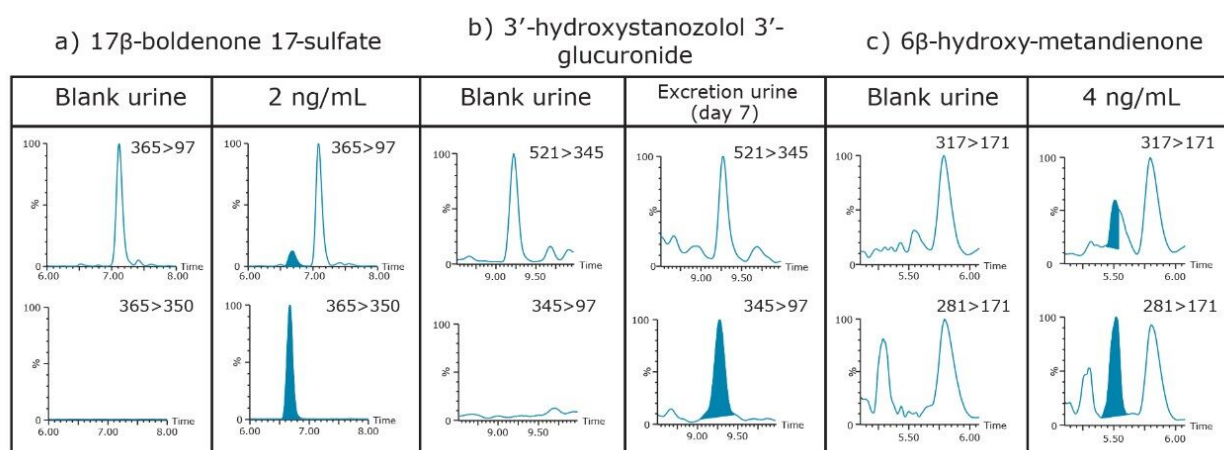


Figure 2. Differences in the detection of (a) 17β -boldenone 17-sulfate, (b) 3'-hydroxystanozolol 3'-glucuronide, and (c) 6β -hydroxy-metandienone in urine using the ion transitions involving common ions/losses (top) and using specific transitions or in-source fragments (bottom).

For the majority of glucuronides, the ion transitions were related to the glucuronide moiety and the best signal intensity was generally obtained in negative ionization mode (product ions m/z 75, 85, 113) (Figure 1). Some ion transitions demonstrated significant background interferences, therefore a strategy based on the CID of in-source fragments was used to have more selective transitions. As shown in Figure 2, the MRM transition $[M+H]^+$ (m/z 521) to m/z 345 for 3'-hydroxystanozolol 3'-glucuronide had a big interfering peak that was not present when monitoring the ion transition $[M+H-gluc]^+$ (m/z 345) to m/z 97.

The same strategy was used for some unconjugated compounds, such as 6 β -hydroxy-metandienone, for which the use of the in-source fragment $[M+H-2H_2O]^+$ (m/z 281) as precursor ion improved its detectability compared to the use of $[M+H]^+$ (m/z 317) (Figure 2).

Validation of the method

The method was validated for qualitative purposes. Results are listed in Table 2. The limit of detection (LOD) was defined as the lowest concentration at which an analyte was detected in ten different urine samples with a signal to noise ratio of at least 3:1. The LODs were in the range 0.25–4.00 ng/mL for 18 of the 23 analytes. Extraction recovery was evaluated by spiking six urines with the analytes before and after sample preparation, and was calculated by comparing the areas obtained in the samples spiked before sample preparation with the mean of the areas obtained in samples spiked after sample preparation. Extraction recoveries were above 77% for all 23 validated analytes.

Intraday precision was assessed by analysis, on the same day, of six replicates of a urine sample spiked at low and upper concentrations levels. Results were expressed as % relative standard deviation (RSD) of the measured area ratios between the analyte and the IS. Intraday precision was better than 21% for all analytes.

Matrix effect was evaluated using post-column infusion and ranged from 92 to 147%.

Compound*	LOD (ng/mL)	Extraction recovery (%). mean \pm SD	Intraday precision				%Matrix effect (RSD %)
			ng/mL	RSD%	ng/mL	RSD%	
9-fluoro-18-nor-17,17-dimethyl-4,13-diene-11-ol-3-one	0.25	77.8 \pm 12.70	0.25	9.1	2.5	2.3	92 (2.35)
epimetandienone	0.5	85.2 \pm 10.7	0.5	11.1	5	1.6	116 (4.31)
oxandrolone	1	123.9 \pm 1.9	1	20.2	5	13.7	116 (4.95)
epioxandrolone	1	109.9 \pm 6.3	1	15.7	5	8.2	109 (1.48)
6 β -hydroxy-4-chloro-metandienone	4	92.2 \pm 4.6	4	10.6	20	6.1	100 (8.77)
6 β -hydroxy-metandienone	4	95.7 \pm 4.9	4	16.2	20	7.3	112 (8.02)
17 β -boldenone 17-S	0.25	93.2 \pm 7.7	0.25	4.5	2.5	9.6	118 (4.35)
17 β -nandrolone 17-S	0.25	96.2 \pm 1.4	0.25	12.9	2.5	7.2	125 (7.81)
17 α -nandrolone 17-S	0.5	87.1 \pm 2.1	0.5	8.8	5	4.0	138 (3.07)
19-norandrosterone 3-S	0.5	93.8 \pm 1.5	0.5	10.8	5	3.8	141 (6.69)
19-noretiocholanolone 3-S	0.5	92.30 \pm 9.6	0.5	8.3	5	12.7	114 (6.81)
19-noretiocholanolone 3-G	0.5	77.1 \pm 13.5	0.5	19.8	5	18.9	141 (8.50)
17 β -boldenone 17-G	0.5	93.6 \pm 5.2	0.5	21.4	5	7.7	123 (10.09)
3'-hydroxystanozolol 3'-G	0.5	87.6 \pm 7.1	0.5	9.9	5	5.7	120 (2.13)
2 α -methyl-5 α -androstan-3 α -ol-17-one 3-G	2	86.8 \pm 4.0	2	7.8	10	1.6	104 (2.55)
1 α -methyl-5 α -androstan-3 α -ol-17 β -diol 3-G	4	98.3 \pm 3.8	4	5.2	20	12.1	119 (5.30)
19-norandrosterone 3-G	4	95.0 \pm 6.7	4	10.7	20	9.1	147 (9.19)
1-methylen-5 α -androstan-3 α -ol-17-one 3-G	4	82.8 \pm 3.5	4	18.0	20	2.1	144 (5.36)
1 α -methyl-5 α -androstan-3 α -ol-17-one 3-G	8	88.7 \pm 4.4	8	13.1	20	3.8	132 (2.19)
17 β -nandrolone 17-G	10	96.0 \pm 4.4	10	17.8	50	5.8	115 (5.03)
5 β -androst-1-ene-17 β -ol-3-one 17-G	16	94.9 \pm 4.7	16	10.6	40	8.5	112 (4.31)
5 β -androstan-7 β ,17 α -dimethyl-3 α ,17 β -diol 3-G	20	93.5 \pm 2.0	20	18.8	50	6.0	119 (1.78)
bolasterone 3-G	>20	92.9 \pm 1.4	20	12.6	50	1.9	130 (2.61)

Table 2. LOD, extraction recovery, intraday precision, and matrix effect results of the validation for qualitative purposes.

*S: sulfate; G: glucuronide

Analysis of excretion study samples and positive samples

The method was applied to samples obtained after administration of different anabolic steroids. As examples, results obtained for methyltestosterone and stanozolol are presented.

Samples obtained after administration of methyltestosterone were analysed using the MRM method to determine the detection times of the six described metabolites. The developed methodology allowed for the detection of 17 β -methyl-5 α -androstan-3 α ,17 α -diol 3-sulfate (METm3-S) up to 23 days (Figure 3), whereas the current screening methods (analysis by GC-MS/MS after hydrolysis and derivatization step) detect 17 α -methyl-5 α -androstan-3 α ,17 β -diol and 17 α -methyl-5 β -androstan-3 α ,17 β -diol (METm1 and METm2) metabolites up to 4 and 6 days respectively. Therefore the detection capabilities for methyltestosterone have been significantly improved. Unaltered glucuronide METm1-G and METm2-3-G metabolites were detected in urine up to 1 and 5 days, respectively. Detection of the unconjugated metabolite 17 α -hydroxy-17 β -methylandrostan-4,6-dien-3-one (METm5) was around one week. Other recently reported sulfate metabolites (METm2-S, 17 α -methyl-5 β -androstan-3 α ,17 β -diol 3-sulfate, and METm4-S, 17 β -methyl-5 β -androstan-3 α ,17 α -

diol 3-sulfate) were detected up to 8 and 13 days, respectively (Figure 3).⁵

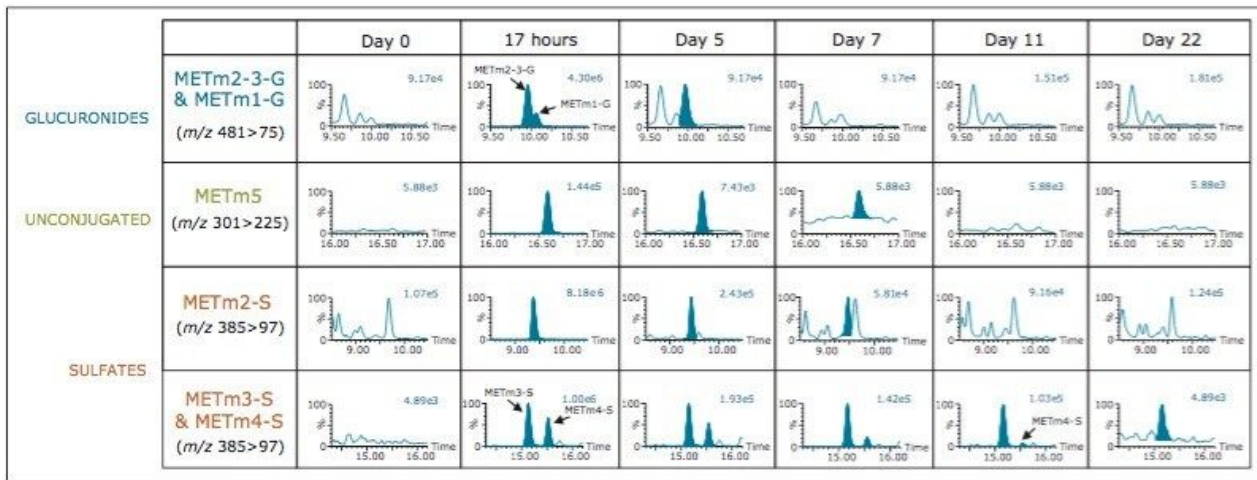


Figure 3. Results of methyltestosterone excretion study: chromatograms of the characteristic ion transitions of the metabolites 17 α -methyl-5 β -androstan-3 α ,17 β -diol 3-G (METm2-3-G), 17 α -methyl-5 α -androstan-3 α ,17 β -diol 3/17-G (METm1-G), 17 α -hydroxy-17 β -methylandrostan-4,6-dien-3-one (METm5), 17 α -methyl-5 β -androstan-3 α ,17 β -diol 3-S (METm2-S), 17 β -methyl-5 α -androstan-3 α ,17 α -diol 3-S (METm3-S) and 17 β -methyl-5 β -androstan-3 α ,17 α -diol 3-S (METm4-S), obtained after analysis of a pre-administration sample and samples collected at 1, 5, 7, 11 and 22 days after methyltestosterone administration.

Regarding stanozolol, as shown in Figure 4, six different unaltered glucuronides: 3'-hydroxystanozolol 3'-glucuronide (3STAN-G), 4 β -hydroxy-stanozolol 4-glucuronide (4STAN-G), 16 β hydroxy stanozolol 16-glucuronide (16STAN-G), stanozolol-N-glucuronide (STAN-N-G), stanozolol-O-glucuronide (STAN-O-G) and 17-epistanozolol-N-glucuronide (eSTAN-N-G); were simultaneously monitored. One of them, eSTAN-N-G, has been recently described as a long-term metabolite and it is not detectable in the current screening conditions because it is stable to enzymatic hydrolysis.⁷ Our method allows the detection of this metabolite up to 21 days after administration and, therefore, it improves the detection capabilities for stanozolol misuse.

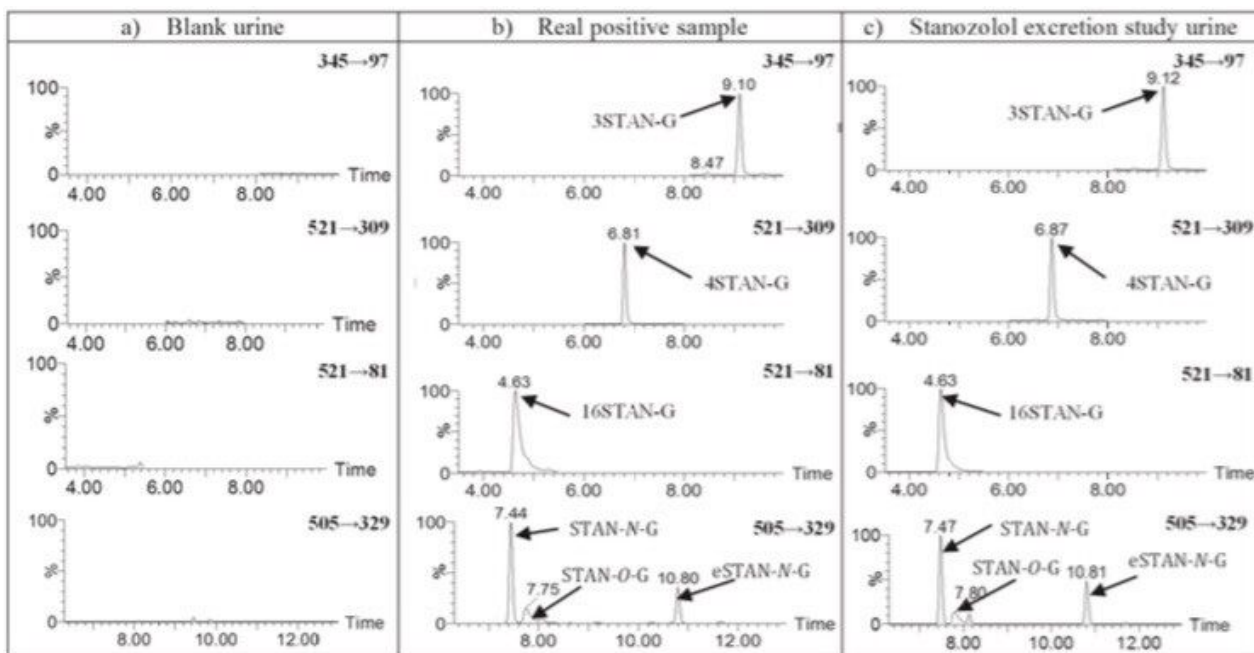


Figure 4. Results of the analysis of a blank urine (a) authentic positive sample (b) and stanozolol excretion study urine (c); chromatograms of the characteristic ion transitions of stanozolol metabolites: 3'-hydroxystanozolol 3' glucuronide (3STAN-G), 4 β -hydroxy-stanozolol 4-glucuronide (4STAN-G), 16 β -hydroxy-stanozolol 16-glucuronide (16STAN-G), stanozolol-N-glucuronide (STAN-N-G), stanozolol-O-glucuronide (STAN-O-G) and 17-epistanozolol-N glucuronide (eSTAN-N-G).

Conclusion

A UPLC-MS/MS screening method for the simultaneous detection of steroid metabolites is a feasible alternative compared to the conventional procedures. Incorporation of new phase II metabolites as described, is straightforward and can significantly enhance the screening and detection capabilities of steroids in sport.

References

1. World Antidoping Agency (WADA). 2013 Adverse Analytical Findings and Atypical Findings Reported by Accredited Laboratories. Available from: <https://www.wada-ama.org/en/resources/laboratories/2013-anti-doping-testing-figureslaboratoryreport#.VBmQFhZNRzB>. (accessed April, 2015).
2. C. Gomez, A. Fabregat, O.J. Pozo, J. Marcos, J. Segura, R. Ventura. Analytical strategies based on mass spectrometric techniques for the study of steroid metabolism. *TrAC-Trend Anal. Chem.* 53 (2013) 106.
3. G. Balcells, O.J. Pozo, A. Esquivel, A. Kotronoulas, J. Joglar, J. Segura, R. Ventura. Screening for anabolic steroids in sports: analytical strategy based on the detection of phase I and phase II intact urinary metabolites by liquid chromatography tandem mass spectrometry. *J Chromatogr A.* 1389 (2015) 65.
4. C. Gomez, O.J. Pozo, H. Geyer, J. Marcos, M. Thevis, W. Schanzer, J. Segura, R. Ventura. New potential markers for the detection of boldenone misuse. *J. Steroid Biochem. Mol. Biol.* 132 (2012) 239.
5. C. Gomez, O.J. Pozo, J. Marcos, J. Segura, R. Ventura. Alternative long-term markers for the detection of methyltestosterone misuse. *Steroids.* 78 (2013) 44.
6. C. Gomez, O.J. Pozo, L. Garrostas, J. Segura, R. Ventura. A new sulphate metabolite as a long-term marker of metandienone misuse. *Steroids.* 78 (2013) 1245.
7. W. Schanzer, S. Guddat, A. Thomas, G. Opfermann, H. Geyer, M. Thevis. Expanding analytical possibilities concerning the detection of stanozolol misuse by means of high resolution/ high accuracy mass spectrometric detection of stanozolol glucuronides in human sports drug testing. *Drug Test Anal.* 5 (2013) 810.

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