Fast and Comprehensive Doping Agent Screening in Urine by Triple Quadrupole GC/MS

Application Note

Forensics/Doping Control

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Abstract

A rapid method was developed on the Agilent 7000 Series Triple Quadrupole GC/MS system to screen for more than 150 doping agents in seven classes of substances, at or below WADA MRPLs [1]. A short capillary column, rapid scan speed and hydrogen as carrier gas enable a run time of less than 8 minutes.

Agilent Technologies

Introduction

Drug "doping" (drug misuse and cheating in sports) is a growing global challenge, given the ongoing development of new therapeutics and "designer" drugs. Since its advent, in 2000 the World Anti-Doping Agency (WADA) has maintained and updated a list of prohibited substances, adherence to the list has been controlled by accredited doping control laboratories. WADA sets minimum required performance levels (MRPLs) for the detection of the substances on the list, which includes:

- Five categories of substances prohibited at all times (anabolic agents, hormones and related substances, beta-agonists, anti-estrogenic agents, and diuretics and other masking agents)
- Four categories of substances prohibited during competition (stimulants, narcotics, cannabinoids and glucocorticosteroids)

Although there is growing interest in samples such as blood (serum/plasma), saliva and hair, urine remains the most common sample type. In order to obtain the necessary selectivity for all of the different classes of prohibited substances at or below their MRPLs, hyphenated chromatographic mass spectrometric methods are preferred [2], and GC-MS and LC-MS are now used as complementary techniques in doping control. While several fast GC tandem mass spectrometric methods have been published, these methods normally lacked the combination of quantitative determination of the endogenous steroid profile and a qualitative analysis of a wide range of exogenous steroids and other doping agents.

This application note describes a method developed on the Agilent 7000A Triple Quadrupole GC/MS system for the detection of a wide range of endogenous and exogenous anabolic steroids and other doping agents, with a run time of less than 8 minutes.

Experimental

Standards and Reagents

The standards and reagents used were as described in reference 1.

Instruments

The method was developed on an Agilent 7890 gas chromatograph equipped with a split/splitless capillary inlet and an Agilent 7000A Triple Quadrupole GC/MS sytem, using a Gerstel MPS2 autosampler and PTV injector. The analysis parameters are listed in Tables 2–6.

Table 1. Agilent 7000A Triple Quadrupole GC/MS Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	
Analytical column	Agilent J&W HP-1 Ultra Inert 12.5 m × 0.2 mm id, 0.11 µm film (cut from a 50 m column, p/n 19091A-005)
Injection	5 µL; Injector conditions: 100 °C (0.15 min), 12 °C/sec to 280 °C
Carrier gas	Hydrogen, constant flow, 1.0 mL/min
Column temperature program	100 °C (0.4 min), 90 °C/min to 185 °C; 9 °C/min to 230 °C; 90 °C/min to 310 °C (0.95 min)
Transfer line temp	310 °C
MS conditions	
Tune	Autotune
EMV Gain	Autotune
Acquisition parameters	EI, Multiple Reaction Monitoring
Collision gas flows	N_2 Collision Gas: 1.5 mL/min
Quench gas flows	Helium, 2.25 mL/min
MS temperatures	Source 280 °C; Quad 180 °C

Sample Preparation

One mL of urine was incubated with β-Glucuronidase to effectively cleave glucuronide conjugates and produce free steroids. The urine was then extracted by liquid-liquid extraction with diethyl ether and the residue after evaporation was derivatized for GC/MS analysis.

Derivatization was achieved by dissolving the dried sample in 100 µL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)-NH_4I-ethanethiol (100:2:3, v/w/v) and heating for 60 minutes at 80 °C [3].

Analysis Parameters

The Agilent Triple Quadrupole GC/MS system parameters used in the analysis of several classes of prohibited substances are shown in Tables 2–6.

Results

Sample Preparation

Doping control laboratories need to be able to detect very low levels of a wide variety of prohibited substances in a relatively small volume of a complex, biological matrix (usually urine). From this small sample, the labs must screen for and eventually confirm (using a totally independent analysis) the presence of any prohibited substance. Due to the sensitivity and selectivity of MS/MS detection, this sample preparation method uses only 1 mL of urine for the screening of a wide range of doping agents, a volume that is 2–5 times lower than that routinely used for GC/MS anabolic steroid screening methods.

This method is also comprehensive, encompassing one or more metabolites of all prohibited narcotics, the most frequently used β2-agonists, hormone antagonists and modulators, and beta-blockers. In addition, a large number of stimulants and several substances from all other groups of prohibited substances are covered by this method (Tables 2–5). The only anabolic agents not covered are those for which GC-MS is not particularly suitable (for example tetrahydrogestrinone, methyltrienolone, stanozolol).

Several quality assurance measures are incorporated into the method to cover the three basic steps in sample preparation: hydrolysis, extraction and derivatization. Using a large excess of β-glucuronidase assures efficient hydrolysis after 1.5 h at 56 °C. The use of both glucuronidated and free steroids with similar structure (d4-A-glucuronide and d5-Et (free)) allows for an adequate evaluation of hydrolysis efficiency. The use of a diverse mixture of internal standards allows for differences in physicochemical properties that can cause differences in extraction efficiency. These internal standards also enable quantification of non-deuterated structural analogues. Finally, the inclusion of transitions for mono-TMS derivatized androsterone and etiocholanolone in the method facilitates evaluation of the derivatization efficiency. This integrated approach provides a comprehensive evaluation of the sample preparation efficiency per sample, rather than per batch or only at the time of validation, since all major sample preparation steps are monitored.

The levels of 5α-androstane-3,17-dione and 5β- androstane-3,17-dione are also monitored in this method, as elevated concentrations of these compounds can be indicative of microbial contamination, which can alter the endogenous steroid profile.

Gas Chromatography

The aim of this study was to develop a fast GC/MS method, capable of quantifying the endogenous steroids shown in Table 6 as well detecting a wide range of prohibited substances qualitatively. Sufficient resolution between compounds is a prerequisite for adequate quantification. In this method, the separation of the isomers androsterone and etiocholanolone, present at relatively high concentrations (Table 6), 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. This method enables injection of 5 µL of sample using a PTV-injector, which is substantially higher than previous methods using split/splitless injection.

Using a relatively short capillary column (12.5 meters) in combination with a high linear velocity of hydrogen as carrier gas, rather than helium, enabled a substantial reduction in the GC run time, to 7.98 minutes. However, even at high concentrations (4.8 µg/mL), androsterone and etiocholanolone are sufficiently separated to provide adequate quantification

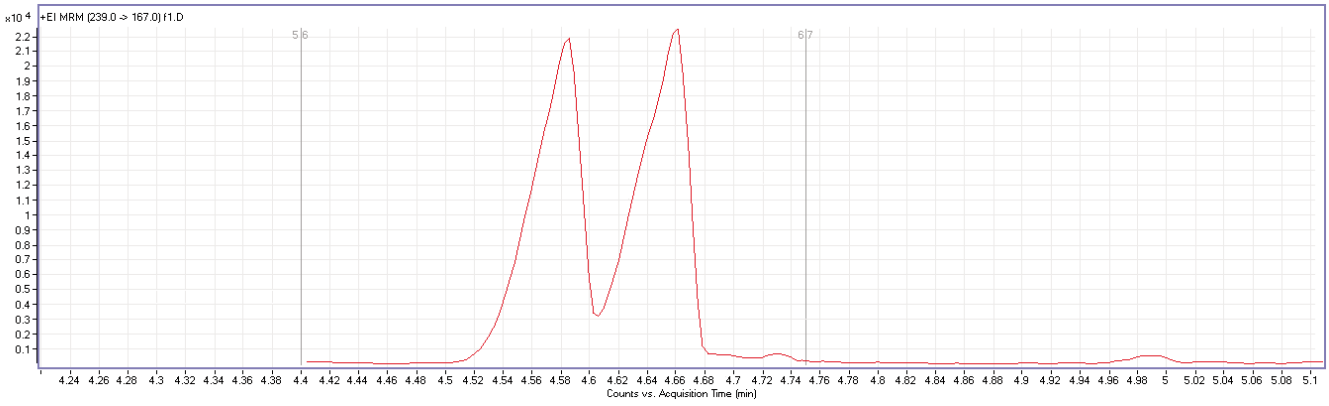

Figure 1. Extracted ion chromatogram (m/z 239 -> 167) for androsterone-bis-TMS and etiocholanolone-bis-TMS at the highest calibrator concentration (4.8 µg/mL).

(Figure 1). Shorter run time greatly improves sample turn around, which can be essential in those cases (for example Olympic competition) involving very short sample reporting times (24–48 h).

Mass Spectrometry

A multistep process was used to determine and optimize the mass spectrometric conditions. In the first step, full scan spectra were obtained for every derivatized 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 then chosen and SRM transitions 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) were then performed on both reference standards and extracts from spiked urine samples. The best signal-to-noise (S/N) ratio was used to determine the most appropriate transitions and collision energies for each analyte. Tables 2–5 list the final mass spectrometer settings for all of the analytes included in the method.

Quantitative Method Validation

The substances analyzed 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. This method also monitors 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 additional parameters can greatly assist in the evaluation process of atypical steroid profiles, due to elevated production of endogenous steroids or alteration by microbiological degradation. The method also quantifies salbutamol, the most widely used β2-agonist, norandrosterone and the major metabolite of cannabis (11-nor-Δ9- tetrahydrocannabinol.-9 carboxylic 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 (www.cipac.org/document/Guidance%20Documents/validat.pdf). Moreover, the bias at each of these points was below 15%, demonstrating acceptable accuracy as well. Therefore, in agreement with Eurachem guidelines [4], this method can be regarded as validated for quantitative purposes.

Qualitative Analysis

Method validation for the non-threshold substances was also performed in accordance with Eurachem guidelines. Selectivity was confirmed by the lack of matrix interferences in ten blank urine samples. These samples were then spiked at different concentration levels of all of the target analytes. The lowest concentration at which concurrent signals (S/N>3) for each monitored transition were obtained at the expected retention time (± 1%) in all samples was defined as the limit of detection (LOD). These LOD's for the exogenous substances are listed in Tables 2–6. The method includes 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 (Minimum Required Performance Level). 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 with an LOD at or below the MRPL. This is the case for fluoxymesterone for example: the LOD for 6β-hydroxyfluoxymesterone (Table 2) is 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. The WADA technical document does not specify which metabolites need to be monitored, with the exception of a few substances. Therefore, the method can be considered WADA compliant for the detection of fluoxymesterone.

Moreover, the use of multiple metabolites rather than 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 compound which is the major metabolite in one individual after a defined post-administration time might only be a minor metabolite in another individual which took the drug at the same time.

The current method is also capable of detecting all compounds from the class of "other anabolic agents," except for the group of selected androgen receptor modulators that are still in clinical phase trials and not included in this study.

Besides the anabolic agents, a wide variety of hormone antagonists 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 (aminoglu-thetimide, 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 [3], the only substances from this class which are not included in the method are testolactone, clomiphene and fulvestrant, due to the lack of reference standards for their metabolites.

Most prohibited narcotics also undergo extensive Phase I and Phase II metabolism. Therefore, all WADA prohibited narcotics and/or their metabolites were included in the current method. Except for fentanyl, which shows superior detection by LC-MS, all LOD's were lower than the WADA MRPL, making the methodology very well suited for monitoring the misuse of narcotics. The method also screens for codeine, since use of codeine can be detected as morphine. When the detection of morphine can be attributed to the use of codeine, a laboratory should not report such cases [5].

In general, urine is not well suited to the determination of the post-administration time of sample collection. However, the current method offers the ability to determine post-administration time for several substances by monitoring metabolites for which the excretion profile is time-dependent. This is the case for heroin, for example, since the method monitors not only the parent substance but also morphine and 6-monoacetylmorphine (MAM).

The method is also capable of simultaneously quantifying 11-nor-Δ9-tetrahydrocannabinol.9 carboxylic acid (THC-COOH), the major metabolite of cannabis and one of the most detected doping agents world-wide. Thus, this method can also be used in forensic science, toxicology, drugs of abuse and work place testing laboratories.

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

The method covers the most frequently used β2-agonists in sports. Moreover, in the case of fenoterol both the parent drug (O-TMS tetrakis derivatized) and a degradation product, the C,N-methylene fenoterol-tetrakis- TMS derivative, were monitored [6]. 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, since fenoterol can be rapidly degraded.

Although beta blockers are only prohibited in particular sports, 15 beta blockers were included in the method since their inclusion can optimize laboratory efficiency when their detection is required.

The method uses an optimized derivatization protocol [7], but the effectiveness of the derivatization step is confirmed by monitoring for the presence of mono-TMS derivatized androsterone and etiocholanolone. The formation of multiple derivatives of several other compounds (for example celiprolol, pindolol) is still possible. While one of the derivatives usually gives a better signal than the other, the inclusion of the second derivative can be regarded as a safety precaution. Given the high speed of changing SRM transitions in the Agilent 7000 Series Triple Quadrupole GC/MS system (500 transitions/sec), this addition of transitions does not decrease the overall performance of the method.

Table 2. Agilent 7890/7000A GC/MS Analysis Parameters for Endogenous Anabolic Androgenic Steroids (AAS; Prohibited Class S1a)

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 → 194.0 432.0 → 206.0	15 15	5	10
	5.32	Boldenone	430.0 → 206.0 430.0 → 191.0	10 30	10	10
	5.09	1-Androstenediol	434.0 → 195.0 434.0 → 127.0	20 20	5	10
	5.05	1-testosterone	432.0 → 194.0 432.0 → 206.0	5 10	10	10
	5.09	17α-methyl-5α-androstane-3α,17β-diol	435.0 → 255.0 435.0 → 213.0	20 20	2	2
	5.12	17α-methyl-5β-androstane-3α,17β-diol	435.0 → 255.0 435.0 → 213.0	20 20	5	2
	6.7	oxymesterone	534.0 → 389.0 534.0 → 444.0	20 20	10	10
	4.15	epimetendiol	358.0 → 301.0 358.0 → 196.0	15 5	2	2
	6.57	6β-hydroxymethandienone	517.0 → 229.0 517.0 → 337.0	20 15	5	10
	5.63	Metenolone PC	446.0 → 208.0 446.0 → 195.0	10 15	5	10
	4.92	1-Methylene-5α-androstan-3α-ol-17-one (metenolone metab)	446.0 → 341.0 446.0 → 195.0	15 5	20	10
	5.64	17α-Ethyl-5β-estrane-3α,17β-diol (norethandrolone major metab)	421.0 → 241.0 421.0 → 331.0	15 5	10	10
	5.4	17α-Ethyl-5α-estrane-3α,17β-diol (norethandrolone minor metab)	421.0 → 241.0 421.0 → 145.0	15 25	5	10
	4.77	2α-methyl-5α-androstan-3α-ol-17-one (drostanolone metab)	448.0 → 433.0 448.0 → 253.0	10 25	10	10
	6.05	Bolasterone PC	460.0 → 355.0 460.0 → 315.0	15 15	10	10
	5.62	7α,17α-dimethyl-5β-androstane-3α,17β-diol (bolasterone metab)	284.0 → 269.0 284.0 → 213.0	5 10	10	10
	6.13	Calusterone PC	460.0 → 355.0 460.0 → 315.0	15 15	10	10
	5.45	7β,17α-dimethyl-5β-androstane-3α,17β-diol (calusterone metab)	229.0 → 105.0 269.0 → 159.0	30 5	/	10
	5.07	1α-Methyl-5α-androstan-3α-ol-17- one (mesterolone metab)	448.0 → 433.0 448.0 → 253.0	10 20	5	10
	5.63	4-Chloro-4-androsten-3α-ol-17-one (clostebol metab)	466.0 → 181.0 466.0 → 431.0	20 15	10	10
	6.47	norclostebol	452.0 → 216.0 452.0 → 321.0	20 15	2	10
	6.67	fluoxymesterone PC	552.0 → 407.0 552.0 → 357.0 552.0 → 319.0	15 15 15	/	10
	6.93	6β-OH-fluoxymesterone	640.0 → 640.0 640.0 → 143.0	10 25	20	10
	5.04	9α-fluoro-17,17-dimethyl-18-nor-androstan-4,13-diene-11β-ol-3-one	462.0 → 208.0 462.0 → 337.0	15 15	5	10
	6.17	oxandrolone	363,0 → 161,0 308.0 → 117.0	15 15	10	10
	5.56	epioxandrolone	363,0 → 161,0 308.0 → 117.0	15 15	20	10
	6.68	dehydrochloromethyltestosterone PC	478.0 → 285.0 478.0 → 353.0	20 5	10	10
	6.82	6β-hydroxy-dehydrochloromethyltestosterone	315.0 → 227.0 315.0 → 241.0	20 15	20	10
	5.19	17α-trenbolone	307.0 → 291.0 307.0 → 275.0	10 20	10	10
	7.1	2-Hydroxymethyl-17α-methylandrostadiene-11α,17β-diol-3-one (formebolone metab)	444.0 → 356.0 367.0 → 257.0	25 25	/	10
	6.48	17α-methyl-4-androstene-11α,17β-diol-3-one (formebolone metab)	534.0 → 389.0 534.0 → 339.0	15 25	10	10
	5.85	mibolerone	446.0 → 431.0 446.0 → 341.0	15 20	10	10
	6.14	ethisterone	456.0 → 316.0 456.0 → 301.0	15 15	1	10
	4.76	3α,5α-tetrahydronorethisterone	431.0 → 167.0 431.0 → 193.0	20 20	2	10
	7.11	16-OH-furazabol	490.0 → 231.0 490.0 → 143.0	15 35	10	10
	5.94	methyldienolone	430.0 → 285.0 430.0 → 325.0	10 10	10	10
	5.97	13β,17α-diethyl-5α-gonane-3α, 17β-diol (norbolethone metab)	435.0 → 255.0 435.0 → 159.0	10 15	20	10
	6.14	13β,17α-diethyl-5β-gonane-3α, 17β-diol (norbolethone metab)	435.0 → 255.0 435.0 → 345.0	20 5	5	10
	3.68	madol	345.0 → 255.0 345.0 → 201.0	15 15	10	10
	6.11	2α,17α-dimethyl-17β-hydroxy-5α-androstane-3-one	462.0 → 141.0 462.0 → 143.0	15 15	10	10
	6.27	4-OH-nandrolone (oxabolone)	506.0 → 147.0 506.0 → 93.0 506.0 → 195.0	20 25 20	2	10
	6.48	4-OH-testosteron	520.0 → 225.0 520.0 → 431.0	15 15	2	10
	6.33	6-OH-androstenedione	518.0 → 319.0 518.0 → 413.0	15 15	1	10
	5.19	7β-OH-DHEA	430.0 → 325.0 430.0 → 220.0	10 10	20	10

PC = Parent Compound

Table 3. Agilent 7890/7000A Triple Quadrupole GC/MS System Analysis Parameters for Endogenous AAS when administered exogenously, Other Anabolic Agents, Beta-2 Agonists, Hormone Antagonists and Modulators, Diuretics and Other Masking Agents (Prohibited Classes S1b, S1c, S3, S4 and S5, respectively)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/mL)	MRPL (ng/mL)
S1b	4.04	19-norandrosterone	405.0 → 225.0 405.0 → 315.0	10 5	1	2
	4.12	5β-Androstane-3,17-dione	290.0 → 275.0 290.0 → 185.0	10 10		
	4.64	5α-androstane-3α,17β-diol	256.0 → 185.0 256.0 → 157.0	15 15		
	4.71	5β-androstane-3α,17β-diol	256.0 → 185.0 256.0 → 157.0	15 15		
	4.58	androsterone	239.0 → 167.0 239.0 → 117.0	35 35		
	4.63	etiocholanolone	239.0 → 167.0 239.0 → 117.0	35 35		
	5.09	5α-Androstan-3,17-dione	290.0 → 275.0 290.0 → 185.0	10 10		
	4.98	DHEA	432.0 → 327.0 432.0 → 237.0	10 10	EAAS	/
	5.14	epitestosterone	432.0 → 209.0 432.0 → 327.0	10 10		
	5.13	5α-androstane-3β,17β-diol	421.0 → 255.0 421.0 → 213.0	20 20		
	5.29	4-androstenedione	430.0 → 209.0 430.0 → 234.0	15 15		
	5.24	DHT	434.0 → 195.0 434.0 → 182.0	20 20		
	5.41	testosteron	432.0 → 209.0 432.0 → 327.0	10 10		
	5.52	11β-OH-androsterone	522.0 → 236.0 522.0 → 324.0	10 10		
	5.6	11β-OH-etiocholanolone	522.0 → 236.0 522.0 → 324.0	10 10		
	4.13	Mono TMS Androsterone	347.0 → 253.0	20	qas	/
S1c	3.37	zilpaterol	308.0 → 218.0 308.0 → 203.0 291.0 → 219.0	10 15 15	5	10
	6.43	zeranol	433.0 → 295.0 433.0 → 309.0	15 15	10	10
	2.42	clenbuterol	335.0 → 227.0 335.0 → 300.0	10 10	0.2	2
	5.37	3α-hydroxytibolone	443.0 → 193.0 443.0 → 167.0	35 30	5	10
S3	2.17	salbutamol	369.0 → 207.0 369.0 → 191.0	15 15	25	100
	1.96	terbutaline	356.0 → 267.0 356.0 → 355.0	25 25	50	100
	6.07	fenoterol	322.0 → 68.0 322.0 → 279.0	15 15	100	100
	6.6	fenoterol C,N-methylene	308.0 → 207.0 308.0 → 179.0	15 15	/	50
S4	6.73	formoterol	178.0 → 121.0 178.0 → 135.0	20 20	50	100
	7.82	salmeterol	311.0 → 149.0 311.0 → 121.0	15 25	100	100
	5.02	bambuterol	354.0 → 72.0 354.0 → 282.0	25 10	5	100
	3.63	aminogluthetimide deriv.1	361.0 → 206.0 361.0 → 221.0	30 10	5	50
	5.26	aminogluthetimide deriv.2	580.0 → 551.0 580.0 → 519.0	20 20	/	50
	3.16	anastrazole	293.0 → 70.0 293.0 → 209.0	10 15	50	50
	3.17	letrozole metabolite	291.0 → 160.0 291.0 → 217.0	15 20	2.5	50
	6.94	exemestane PC	441.0 → 307.0 441.0 → 193.0	20 20	/	50
	6.94	17β-hydroxy-6-methylene-androsta-1,4-diene-3-one	443.0 → 207.0 443.0 → 193.0	20 20	25	50
	6.43	4-OH-androstene-3,17-dione (formestane)	518.0 → 221.0 518.0 → 190.0	15 10	2	10
	6.57	toremiphene	405.0 → 58.0 405.0 → 72.0	15 5	25	50
	6.86	4-hydroxy-methoxytamoxifen 1	489.0 → 72.0 489.0 → 58.0	5 15	25	50
	7.02	4-hydroxy-methoxytamoxifen 2	489.0 → 72.0 489.0 → 58.0	5 15	25	50
	5.78	4-OH-tamoxifen	459.0 → 72.0 459.0 → 58.0	5 15	2.5	50
	7.74	raloxiphene	578.0 → 193.0 578.0 → 413.0	35 30	25	50
	6.57	4-OH-cyclofenil	512.0 → 422.0 512.0 → 343.0	10 5	2.5	50
S5	3.13	probenecid	328.0 → 103.0 328.0 → 193.0	25 15	12.5	250

PC = Parent Compound

Table 4. Agilent 7890/7000A kTriple Quadrupole GC/MS System Analysis Parameters for Stimulants, Narcotics and Cannabinoids (Prohibited Classes S6, S7 and S8, respectively)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/mL)	MRPL (ng/mL)
S6	2.16	carphedon	272.0 → 104.0 272.0 → 229.0	25 15	50	500
	4.98	6-OH-bromantan	395.0 → 91.0 393.0 → 91.0	30 30	2,5	500
	2.08	pemoline	178.0 → 104.0 392.0 → 178.0	10 10	5	500
	2.28	octopamine	174.0 → 866.0 426.0 → 206.0 426.0 → 179.0	5 15 15	100	500
	7.14	strychnine	316.0 → 144.0 316.0 → 220.0	15 10	100	200
	1.37	crotethamide	154.0 → 86.0 154.0 → 69.0	10 15	50	500
	1.97	ethamivan	295.0 → 223.0 295.0 → 265.0	25 20	50	500
	1.36	fencamfamine	215.0 → 186.0 215.0 → 98.0	5 15	50	500
	4.24	fenspiride	241.0 → 96.0 241.0 → 154.0	10 10	25	500
	2.57	3,3-dihenylpropylamine	174.0 → 86.0 174.0 → 100.0	15 15	50	500
	4.65	prenylamine	238.0 → 58.0 238.0 → 91.0	20 20	50	500
	1.94	clobenzorex	168.0 → 125.0 168.0 → 89.0	20 35	100	500
	2.51	cyclazodone	360.0 → 178.0 360.0 → 247.0	15 15	10	500
	6.57	famprofazone	286.0 → 72.0 286.0 → 214.0	20 15	50	500
	1.66	benzphetamine	148.0 → 91.0 148.0 → 65.0	20 35	10	500
	1.74	methylphenidate	156.0 → 45.0 156.0 → 84.0	35 10	100	500
	6.47	amineptine	193.0 → 115.0 193.0 → 178.0	15 15	10	500
	4.53	amineptine C5 metabolite	193.0 → 115.0 193.0 → 178.0	15 15	50	500
	2.7	cocaine	303.0 → 82.0 303.0 → 198.0	15 5	50	500
	3.07	benzoylecgonine	240.0 → 82.0 361.0 → 82.0	20 20	100	500
	3.56	prolintane metabolite14	322.0 → 293.0 322.0 → 205.0	20 20	excr	500
	2.28/2.34	prolintane metabolite 5a/b	304.0 → 142.0 304.0 → 75.0	20 20	excr	500
	2.67	prolintane metabolit e9	228.0 → 158.0 228.0 → 138.0	20 20	excr	500
	2.52	sibutramine metabolite 1	158.0 → 116.0 158.0 → 102.0	10 10	excr	500
	2.74/2.82	sibutramine metabolite 2/3	246.0 → 156.0 246.0 → 84.0	20 20	excr	500
S7	7.47	buprenorphine	554.0 → 522.0 554.0 → 450.0	15 20	0.5	10
	6.57	dextromoramide	265.0 → 166.0 265.0 → 98.0	15 10	20	200
	4.91	heroine	369.0 → 327.0 369.0 → 268.0	10 25	2.5	200
	4.66	MAM	399.0 → 287.0 399.0 → 340.0	15 10	20	200
	5.37	fentanyl	245.0 → 189.0 245.0 → 146.0	10 15	/	10
	2.19	norfentanyl	175.0 → 120.0 175.0 → 56.0	5 15	/	10
	4.32	hydromorphone	429.0 → 234.0 429.0 → 357.0	15 25	100	200
	2.73	methadon	296.0 → 191.0 296.0 → 281.0	20 10	10	200
	2.93	methadon 2	296.0 → 191.0 296.0 → 281.0	20 10	40	200
	2.37	normethadon 1	224.0 → 103.0 224.0 → 191.0	35 35	100	200
	2.73	normethadon 2	296.0 → 191.0 296.0 → 252.0	20 20	10	200
	2.14	EDDP	277.0 → 105.0 277.0 → 220.0	25 20	40	200
	4.42	morphine	429.0 → 287.0 429.0 → 220.0	20 35	10	200
	4.37	oxycodone	459.0 → 368.0 459.0 → 312.0	15 15	200	200
	4.76	oxymorphone	502.0 → 70.0 517.0 → 355.0	30 15	40	200
	3.12	pentazocine	357.0 → 246.0 357.0 → 289.0	15 15	100	200
	1.47	pethidine	247.0 → 71.0 247.0 → 173.0	5 5	4	200
	3.97	codeine	371.0 → 229.0 371.0 → 234.0	5 5	10	200
	4.21	ethylmorphine	385.0 → 214.0 385.0 → 234.0	35 10	10	200
	2.51	pipradrol	239.0 → 161.0 239.0 → 221.0	20 20	5	200
	5.25	fenbutrazate	261.0 → 103.0 261.0 → 175.0	35 15	50	200
S8	6.06	THC-COOH	371.0 → 289.0 371.0 → 265.0	15 15	<5	7,5

PC = Parent Compound

Table 5. Agilent 7890/7000A Triple Quadrupole GC/MS System Analysis Parameters for Beta Blockers Prohibited in Competition in Certain Sports (Prohibited Class P2) and the Internal Standards (ISTDs)

Class	RT (min)	Substance	Transitions	Collision energy (eV)	LOD (ng/mL)	MRPL (ng/mL)
P2	1.91	oxprenolol	150.0 → 109.0 221.0 → 72.0	15 15	50	500
	3.62	betaxolol	364.0 → 209.0 364.0 → 172.0	10 10	100	500
	2.94	bisoprolol	405.0 → 56.0 405.0 → 172.0	25 15	100	500
	3.07	pindolol 1	204.0 → 133.0 220.0 → 75.0	15 15	500	500
	3.65	pindolol 2	205.0 → 130.0 292.0 → 218.0	15 15	50	500
	3.05	esmolol	352.0 → 193.0 352.0 → 56.0	5 15	100	500
	3.02	metipranolol	366.0 → 281.0 366.0 → 239.0	5 15	25	500
	2.64	propanolol	316.0 → 231.0 316.0 → 75.0	5 15	25	500
	3.15	timolol	373.0 → 186.0 373.0 → 70.0	15 35	50	500
	4.12	carteolol	421.0 → 186.0 421.0 → 365.0	15 5	50	500
	4.12	levobunolol	234.0 → 233.0 234.0 → 217.0	5 10	25	500
	2	celiprolol 1	319.0 → 129.0 205.0 → 89.0 205.0 → 117.0	15 15 15	/	500
	3.45	celiprolol 2	200.0 → 128.0 200.0 → 144.0	15 15	500	500
	4.53	nadolol	510.0 → 70.0 510.0 → 186.0	35 20	250	500
	6.2	acebutolol 1 + 2	278.0 → 166.0 278.0 → 208.0	30 30	500	500
	1.72	alprenolol	321.0 → 72.0 306.0 → 203.0	15 15	250	500
	6.67	labetolol	383.0 → 265.0 383.0 → 251.0	15 15	100	500
ISTD	4.66	5β-Androstane-3a,17b-diol-d5	246.0 → 190.0 246.0 → 164.0	15 15		
	4.62	5α-Androstane-3a,17b-diol-d3	244.0 → 202.0 244.0 → 188.0	15 15		
	4.51	androsterone-d4	423.0 → 333.0 423.0 → 243.0	20 20		
	4.56	etiocholanolone-d5	424.0 → 334.0 424.0 → 244.0	20 20		
	5.12	epitestosterone-d3	435.0 → 330.0 435.0 → 209.0	5 20	ISTD	/
	5.38	testosterone-d3	435.0 → 330.0 435.0 → 209.0	20 20		
	5.17	DHT-d3	437.0 → 205.0 437.0 → 195.0	15 15		
	2.16	salbutamol-d3	372.0 → 210.0 372.0 → 193.0	20 20		
	5.97	17α-methyltestosterone	446.0 → 301.0 446.0 → 198.0	25 20		

PC = Parent Compound

Table 6. Target Substances for 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

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. The use of a wide range of internal standards provides an evaluation of the sample preparation efficiency to assure accuracy of the results. Using hydrogen as a carrier gas and a short (12.5 m) capillary column with the Agilent 7000A Triple Quadrupole GC/MS system, all doping agents could be detected within a single run of less than 8 minutes.

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