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RESEARCH ARTICLE

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Comparison of gas chromatography/quadrupole time-of-flight and quadrupole Orbitrap mass spectrometry in anti-doping analysis: I. Detection of anabolic-androgenic steroids

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Rationale: The World Anti-Doping Agency (WADA) encourages drug-testing laboratories to develop screening methods that can detect as many doping substances as possible in urine. The use of full-scan high-resolution acquisition (FS/HR) with gas chromatography/mass spectrometry (GC/MS) for the detection of known and unknown trimethylsilyl (TMS) derivatives of anabolic-androgenic steroids (AAS) provides anti-doping testing bodies with a new analytical tool.

Methods: The AAS were extracted from urine samples by generic liquid–liquid extraction, after enzymatic hydrolysis, and TMS derivatization. The extracted urine was analyzed by GC/Q-TOF and GC/Q-Orbitrap to compare the performance of the two instrument types for the detection of 46 AAS in human urine. The quantitation of endogenous anabolic steroids and the ability of the two analytical platforms to comply with the requirements for testing as part of the WADA Athlete Biological Passport (ABP) were also assessed.

Results: The data presented show that the analytical performance for both instruments complies with the WADA specifications. The limits of detection (LODs) for both instruments are well below the WADA 50% Minimum Required Performance Levels. The mass errors in the current study for the GC/Q-Orbitrap platform are lower than those obtained for the GC/Q-TOF instrument.

Conclusions: The data presented herein proved that both molecular profiling platforms can be used for antidoping screening. The mass accuracies are excellent in both instruments; however, the GC/Q-Orbitrap performs better as it provides higher resolution than the GC/Q-TOF platform.

1 | INTRODUCTION

The World Anti-Doping Agency (WADA) Prohibited List[1] comprises prohibited classes of drugs with pharmacological activity. The small molecules included on the list are detected using mass spectrometry (MS) coupled with either liquid chromatography (LC) or gas chromatography (GC). The criteria used to select the method of analysis are determined mainly by the sensitivity, specificity and matrix effects that fulfil the specifications of the WADA International Standard for Laboratories (ISL)[2] and the WADA Minimum Required Performance Levels (MRPL) technical document TD MRPL.[3] The molecules analyzed by GC/MS are usually those with chemical structures that result in low ionization efficiency with LC/MS.[4]

In the sports anti-doping field, GC/MS screening typically utilizes low-resolution GC triple quadrupole technology (GC/QQQ);[5] however, a few methods have been published that utilize full-scan (FS) and

high-resolution (HR) acquisition modes. In 2007, a time-of-flight (TOF) screening method was published[6] that could be used for the analysis of synthetic anabolic-androgenic steroids (AAS). However, the WADA specifications in 2007 were less demanding than the contemporary regulations. In another study, a limited screening method based on two-dimensional (2D) GC coupled to TOF-MS was published that could be used for selected anabolic agents.[7] In another, more comprehensive study from the same research group, a screening method based on quadrupole-Orbitrap GC/MS was published for use in the detection of a large number of substances in urine, such as synthetic AAS, β-agonists, stimulants, narcotics, metabolic modulators and diuretics, that fulfilled the WADA sensitivity specifications.[8] Finally, in another recent study,[9] a routine screening method that used GC/MS with a triple quadrupole instrument was adapted for use with a Q-TOF GC/MS instrument and subjected to full validation, in which the FS/HR MS acquisition mode was applied to detect and quantify exogenous and endogenous steroids.[10]

However, FS/HR GC/MS technologies are more widely used in other analytical fields than in anti-doping analysis. A recent review that focused on the use of GC/MS with TOF mass analyzers describes their use for the analysis of a large number of organic contaminants and residues present at trace levels for environmental, food safety and biological applications.[11] A recent example of the use of FS/HR GC/MS in the food analysis field combined atmospheric pressure chemical ionization (APCI) with the use of a Q-TOF mass analyzer for the analysis of the volatile components of olive oil.[12]

The use of GC/MS coupled with TOF and Q-TOF mass analyzer technologies and two-dimensional GC (GC×GC) has been recently reviewed.[13] The authors observed that the popularity of GC×GC is increasing and the number of components that can be simultaneously detected is limited by characteristics of the MS system such as the dynamic range, resolution and scanning speed, and co-eluting GC × GC peaks. Moreover, several recent applications of this method to toxicology, clinical chemistry, food and environmental analysis have recently been reviewed.[13] In toxicology, the volatile organic compounds (VOCs) produced during the early stages of bodily decomposition can be detected using thermal desorption coupled to GC×GC/TOF-MS, and this approach could be used for a wide variety of forensic or epidemiologic purposes, such as during natural catastrophes with large numbers of collapsed buildings.[14] In clinical chemistry, the quantitative analysis of organic acids in urine has been performed using GC×GC/TOF-MS to determine abnormal patterns that can indicate the presence of inherited disorders of organic acid metabolism.[15] In food analysis, GC×GC/TOF-MS has been applied to the investigation of dioxin-like micropollutants in animal-derived food matrices.[16] In environmental analysis, the profiling of short- and medium-chain chlorinated paraffins in sediment and fish samples using GC×GC/TOF-MS with negative ionization has been demonstrated.[17]

Along with molecular profiling using TOF mass analyzers, the use of the GC/Q-Orbitrap has been recently developed. The use of FS/HR GC/Orbitrap MS with electron ionization (EI) was evaluated for use in pesticide residue analysis in fruit and vegetables.[18] In food analysis, GC and LC coupled to Orbitrap MS have been applied to the identification of substances that have migrated from nano-films in food packaging.[19]

The advantages of the FS/HR-MS method for anti-doping screening analysis have been described by Friedmann et al.[20] The HR mass filter results in enhanced specificity and sensitivity and the reduction of background noise, and FS acquisition allows for the detection of a virtually unlimited number of analytes, which can be identified by reprocessing of the acquired data files. Using FS/HR-GC/MS for the identification of designer drugs,[21,22] and of novel metabolites that thus allow for the prolonged detection of substances with "zero tolerance" such as AAS,[23,24] has been demonstrated.

Synthetic AAS are prohibited by WADA[1] and present a challenge[3] for anti-doping screening laboratories, since their presence should still be detected at low concentrations for a long period of time after administration of the banned substances. Moreover, endogenous AAS,[10] such as testosterone and prohormones, are administered exogenously to enhance endogenous AAS levels. Because there is no difference in the mass spectra produced by exogenously administered AAS and their endogenously produced counterparts, an indirect methodology based on the ratio of AAS metabolites is currently used to identify possible abuse of substances. To alleviate these challenges, WADA introduced the steroid profile and WADA Steroidal Athlete Biological Passport (ABP), which contains the quantified results of endogenous AAS screening analyses conducted during the career of a professional athlete for the following parameters: testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstan-3α,17β-diol (5αadiol) and 5β-androstan-3α,17β-diol (5βadiol) and the following ratios: T/E, A/Etio, 5αadiol/5βadiol, A/T and 5αadiol/E, for each analyzed sample.[10] The concentrations of the markers, in addition to the ratios of AAS metabolites, are also used in the identification of possible abuse.[25]

The goal of this study is the comparison of two FS/HR-GC/MS molecular screening technologies, which are based on the Agilent GC/Q-TOF and the Thermo GC/Q-Orbitrap instruments, using the same sample aliquots and the same GC parameters and conditions for both instruments. Both the Q-TOF and Orbitrap mass analyzers allow for high mass resolution, but the Orbitrap mass analyzer generally produces higher-resolution spectra at a lower scanning speed and higher dynamic range, depending on the applied acquisition parameters, due its increased ion trapping ability. We present an analytical platform comparison study that uses these two MS analyzers for the detection of anabolic steroids and that includes the qualitative screening of synthetic AAS, the quantitative profiling of endogenous AAS and the reprocessing of the electronic data files for preventive analysis. In this article, the performance of the GC/Q-TOF and GC/Q-Orbitrap in the detection and quantitation of exogenous and endogenous AAS in the same urine samples is compared. The analysis comprises a limited number of analytes, including representative exogenous AAS and all of the endogenous AAS that are present in the steroidal ABP.

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

Sodium hydrogen carbonate and diethyl ether were supplied by Merck (Darmstadt, Germany). Methanol (HPLC grade), 2-propanethiol,

dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ammonium iodide and sodium bicarbonate were supplied by Sigma Aldrich (Darmstadt, Germany). β-Glucuronidase derived from *Escherichia coli* (*E. coli*) was supplied by Roche (Mannheim, Germany). MSTFA (*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide) was supplied by Carl Bucher (Waldstetten, Germany).

2.2 | Reference materials

The following internal standards (ISTD) were purchased from LGC (Wesel, Germany): D5-etiocholanolone (d5 Etio), D4-androsterone glucuronide (d4A Glu), D3-testosterone (d3T), D3-epitestosterone (d3E), and D5-5β-androstane-3α-17β-diol (D5-5βDiol). The remaining reference standard materials were purchased from LGC, TRC (Toronto, Canada), Sigma Aldrich, Steraloids (Newport, RI, USA), and Cerilliant (Round Rock, TX, USA). Standard stock solutions of the analytes were individually prepared in methanol. For validation purposes, standard working solutions containing the analytes were prepared in methanol by subsequent dilution of the stock solutions. The analytes from the endogenous steroid profile were diluted in a different working solution. All the solutions were stored at −20°C in amber vials.

2.3 | Sample preparation

The sample preparation has been described in detail for a previous study.[9] In brief, it included a liquid–liquid extraction with diethyl ether at pH 9–10 and a desalting step, which is an approach that is commonly used to extract doping substances from the urine matrix. A clean extract was obtained after the separation of the organic layer from the aqueous phase following centrifugation and the cooling of the extraction tubes in an ethanol basin at −80°C. Prior to the extraction step, deconjugation of the phase II steroid glucuronide conjugates was performed by enzymatic hydrolysis using the *E. coli*-derived β-glucuronidase as indicated by the WADA.[10] The final step of the sample preparation was the formation of the trimethylsilyl (TMS) derivatives of the extracts which was performed in a mixture of MSTFA, ammonium iodide and propanethiol; under these conditions, both the hydroxyl and the keto steroidal groups were derivatized[26] by the TMS group.

2.4 | Instruments and analytical conditions

2.4.1 | GC/EI-Q-TOF analysis conditions

The GC/MS system used in the current study was a model 7890 gas chromatograph coupled with a model 7200 Q-TOF mass spectrometer (G3850–64101; both Agilent Technologies, Wilmington, DE, USA) equipped with a BPX5 5% phenyl polysilylphenylene-siloxane capillary column (30 m length, 0.25 mm ID, 0.1 µm film thickness; SGE, Melbourne Australia) and a back-flush system. The combination of the quadrupole device with the TOF MS analyzer provides the capability to conduct tandem mass spectrometry (MS/MS) experiments. Helium was used as a carrier gas at a constant flow rate of 1.1 mL/min. The injection port temperature was set to 280°C, and the injection volume was set to 2 µL with a split ratio of 20:1. The oven temperature was initially set at 160°C, increased at 10°C/min to 200°C, then increased at 2°C/min to 220°C, followed by an increase at 6°C/min to 292°C and finally 50°C/min to 310°C, where it was held for 3 min. The analysis run time was 29.36 min. The interface temperature was set to 280°C and the ion source was set to 250°C. An electron energy of 70 eV was used to acquire all the EI mass spectra. A 2 GHz extended dynamic range (EDR) acquisition mode was used for the TOF data acquisition. The acquisition rate was 5 spectra per second (200 ms per spectrum), and the number of transients per spectrum was set to 2718. The GC/QTOF instrument has the capacity to acquire MS data in FS/HR mode with a mass accuracy of <5 ppm mass error in EI mode, depending on the concentration of the analytes. The applied MS range (*m/z* 80–670) allows for the measurement of small molecules typically analyzed with GC/Q-TOF. Automated mass calibration was performed after every three injections using perfluorotributylamine (PFTBA, Agilent).

2.4.2 | GC/EI-Q-Orbitrap analysis conditions

The second GC/MS system used in the current study was a GC/Q-Orbitrap instrument (Q Exactive GC; Thermo Scientific, Bremen, Germany) equipped with an SGE BPX5 column (30 m length, 0.25 mm ID, 0.1 µm film thickness). This system consisted of a TriPlus RSH autosampler, a TRACE 1310 gas chromatograph with a hot split/splitless injector, an EI source, and a hybrid quadrupole Orbitrap (Q Exactive) mass spectrometer with HCD (higher energy collision-induced dissociation). The sample introduction was performed using the TriPlus RSH autosampler. Helium was used as carrier gas with a constant flow rate set at 1.1 mL/min. The same GC parameters as described in section 2.4.1 were used. EI at 70 eV was used with the source temperature set at 250°C. Full-scan (FS) acquisition mode was applied with a mass range of *m/z* 80–670 with 1 µs scans, a resolving power of 60,000 at *m/z* 200 and the AGC target set at $3{\cdot}10^6$. The mass calibration procedure was performed before each acquisition batch using PFTBA. The internal mass calibration during the measurement was conducted using three different background ions that originated from the column bleed (*m/z* 207.03236/$C_2H_{15}O_3Si_3^+$, 281.05115/$C_7H_{21}O_4Si_4^+$, and 355.06994/$C_9H_{27}O_5Si_5^+$) with a search window of ±2 ppm.

2.4.3 | Qualitative analysis

The validation of the screening method on both instruments was performed according to ISL guidelines.[2] In this procedure the following parameters were evaluated and validated: the identification capability, the specificity and the limit of detection (LOD). To assess the retention time for each compound, a mixture of standards at a high concentration (10-fold of the MRPL) was injected into both instruments. The evaluation of the compound identification capability based on the retention time was performed using 10 different blank urine samples that had been spiked with a standard mixture of 46 synthetic anabolic steroids at concentrations of 50% of the MRPL.[3] To evaluate the specificity of the developed methods, 10 blank urine samples were analyzed in order to demonstrate the absence of any interference. The LOD was determined by spiking the urine samples with a mixture of standards at 10%, 25%, 50% and 100% of the MRPL. The LOD is

the lowest concentration at which a compound can be detected with sufficient data points and the elimination of background interference. The validation results showed that the analytical methods that were used in this study to detect AAS in urine are in compliance with the criteria laid out in the WADA ISL guidelines.

2.4.4 | Quantitative analysis

The method used for quantitative compound profiling was validated on both instruments. The validation process was carried out over a period of 3 days. The linearity, precision, accuracy and combined measurement uncertainty were evaluated. The calibration curves for quantification purposes were generated from stripped urine samples, which were blank urine samples collected from female children and depleted of endogenous steroids using C18 solid-phase extraction (SPE) and collection of the eluent to avoid any interference with the endogenous steroids in the urine matrix that had been spiked with the standards. The calibration curves were obtained by measuring the levels of endogenous AAS included in the steroid profile of the ABP at seven different concentrations of standards; concentrations of 2–400 ng/mL were used for T and E, 100–8000 ng/mL for A and Etio, and 4–800 ng/mL for 5αadiol and 5βadiol. The ratio of the peak height (at the appropriate *m/z* value) that was obtained for the analyte to the peak height of the internal standard was calculated and plotted against the concentration of the added analyte. Linearity was determined by using the weighted linear regression model (W = 1/X, where X is the concentration of the analyte). The precision and accuracy of the method were determined using a level of spiked quality control (QC) samples that corresponded to level 5 of the calibration curve, which were prepared in four different aliquots. The analysis was performed using 4 replicates for each level for each day ($n = 4$) over a period of 3 days ($n = 12$). The intermediate precision was determined from the data obtained from the QC samples collected during the 3 days of the experiments. Both the intermediate precision and the accuracy (% bias) that were calculated based on the QC samples were used to estimate the combined measurement uncertainty (MU) for each steroid according to the following equation:

$$\text{Combined MU} = \sqrt{\text{intermediate precision}^2 + \text{Bias}^2}$$

3 | RESULTS AND DISCUSSION

The current study was conducted using the same samples and chromatographic conditions in two different laboratories: Anti-Doping Lab Qatar (ADLQ) in Qatar (GC/Q-TOF) and RIKILT-Institute of Food Safety (GC/Q-Orbitrap) in The Netherlands. The same sample set was analyzed on both analytical platforms within a 2-week period in order to reduce possible variability due to sample instability. The methods applied herein were based on that of a previous study,[9] with the exception of the GC/Q-Orbitrap MS method, which is described in section 2. The entire sample preparation procedure was conducted at ADLQ, where the aliquots were subsequently analyzed using the Q-TOF instrument. Afterwards, the aliquots were safely sent to RIKILT at room temperature and reanalyzed using the GC/Q-Orbitrap instrument with the same chromatographic conditions, including identical injection port liner, chromatographic column and temperature parameters. The only difference in the two chromatographic systems was the inclusion of a back-flush device installed on the Q-TOF instrument. In this study, representative AAS were selected for analysis. AAS are considered to be drugs with anabolic pharmacological activity that is highly beneficial for the enhancement of athletic performance, even long after administration. Consequently, AAS are required to be detected by anti-doping laboratories at the lowest possible concentrations, even below the values specified by WADA.[3] To this end, the AAS were analyzed at concentrations close to their LOD. The measured AAS analytes included endogenous AAS that are part of the ABP SP, AAS analytes that are easily detected by GC analysis, as well as analytes that are problematic for GC analysis due to matrix interferences.

The MS tuning and calibration procedures were applied to both instruments according to the manufacturers' specifications. The full width at half maximum (FWHM) resolution was set to 60,000 (specified at *m/z* 200) during the tuning procedure for the Q/Orbitrap and to 12,000 (specified at *m/z* 272) for the Q-TOF. These conditions, in addition to those described in section 2.4, provided sufficient data points to identify and quantify the analyte peaks in the FS/HR-GC/MS data; there were 20–30 and 30–50 data points across the chromatographic peaks for the Q-Orbitrap and the Q-TOF data, respectively. For the GC/Q-Orbitrap, the mass accuracy reached 2 ppm error due to the use of lock mass recalibration with ions (*m/z* 207.03236, 281.05115, and 355.06994) that originated from the column bleed. The GC/Q-TOF method did not include a lock mass procedure during acquisition; however, the mass calibration procedure was repeated after three sample injections.

3.1 | Qualitative analysis results

Table 1 summarizes the qualitative parameters used to validate the AAS screening conducted using both instruments. The GC/MS method provided sufficient data quality to discriminate between the MS signals derived from each of the investigated analytes using both instruments. Figure 1 shows the comparison of the mass accuracies for the molecular ions and fragment ions produced by the analyzed AAS from Table 1, which were spiked at 2.5 ng/mL, as a function of the *m/z* value. Table 2 summarizes the reproducibility of the mass accuracy for the GC/Q-TOF and GC/Q-Orbitrap analyses of 10 different urine samples based on the base peaks of the selected AAS. Overall, the mass errors for the GC/Q-Orbitrap platform in the current study are lower than those for the GC/Q-TOF system.

The identification capability parameters,[2] which are based on the detectability of the AAS within the different urine matrices at 50% of the MRPL concentration,[3] are shown in Table 1. The LOD for each AAS is also shown in Table 1.

Figure 2 shows the extracted ion chromatograms for the main fragment ions derived from the following AAS compounds: 1-testosterone, 5β-androst-1-ene-17β-ol-3-one (boldenone m1), 3'OH stanozolol, epimetendiol, bolasterone, 9α-fluoro-18-nor-17,17-dimethyl-4,13-diene-11β-ol-3-one (flouxymesterone-18nor), 13β,17α-diethyl-5β-gonane-3α,17β-diol (norbolethone m2), 19-norandrosterone (nandrolone

TABLE 1 Summary of the results of the qualitative analysis

	Analytes (TMS derivatives)	Chemical formula	RT (min)	Ions (*m/z*)			Spiked conc* (ng/mL)	LOD (ng/mL)		Detection in 10 urine samples	
								GC/Q-TOF	GC/Q-Orbitrap	GC/Q-TOF	GC/Q-Orbitrap
1	18-Normetenol	$C_{23}H_{38}O_1Si_1$	8.7	358.2686	253.1951	216.1873	2.5	1.25	1.25	10	10
2	1-Androstene 3β,17β diol	$C_{25}H_{46}O_2Si_2$	14.49	405.2637			2.5	1.25	0.63	9	10
3	1-Testosterone	$C_{25}H_{44}O_2Si_2$	15.45	206.1121	194.1121		2.5	1.25	1.25	9	10
4	3αOH-Tibolone	$C_{27}H_{46}O_2Si_2$	16.31	443.2796	353.2295		2.5	0.63	0.63	10	10
5	3βOH-Tibolone	$C_{27}H_{46}O_2Si_2$	15.19	443.2796	353.2295		2.5	0.63	0.63	10	10
6	3'OH Stanozolol m1	$C_{30}H_{56}N_2O_2Si_3$	24.17	560.3644	545.3409		1	1.00	0.50	7	10
7	17α-Methyl-5α-androstane-3α,17β-diol	$C_{26}H_{50}O_2Si_2$	15.69	450.3344	270.2342	255.2107	1	1.00	1.00	8	10
8	17α-Methyl-5β-androstane-3α,17β-diol	$C_{26}H_{50}O_2Si_2$	15.87	450.3344	270.2342	255.2107	1	1.00	1.00	8	10
9	6-Oxo-androstenedine	$C_{28}H_{48}O_3Si_3$	18.34	516.2906	501.2671		2.5	0.63	0.63	10	10
10	Bolasterone met (7α,17α-dimethyl-5β-androstane-3α,17β-diol)	$C_{27}H_{52}O_2Si_2$	17.11	374.2999	284.2499	269.2264	2.5	1.25	1.25	10	10
11	Boldenone met (5β-androst-1-ene-17β-ol-3-one)	$C_{25}H_{44}O_2Si_2$	12.43	194.1121	206.1121		2.5	1.25	0.30	10	10
12	Calusterone met (7β,17α-dimethyl-5β-androstane-3α,17β-diol)	$C_{27}H_{52}O_2Si_2$	16.8	374.2999	284.2499	269.2264	2.5	2.50	2.50	10	9
13	Calusterone	$C_{27}H_{48}O_2Si_2$	18.56	460.3187	445.2953		2.5			10	10
14	Clenbuterol	$C_{18}H_{34}N_2Cl_2OSi_2$	6.15	300.1007	335.06890		0.1	0.10	0.05	7	10
15	Clostebol met (4-chloroandrost-4-en-3α-ol-17-one)	$C_{25}H_{43}O_2ClSi_2$	17.49	466.2485	468.2587		2.5	0.63	0.63	10	10
16	Danazol met (Ethisterone)	$C_{27}H_{44}O_2Si_2$	18.64	456.2874	441.2640		2.5	1.25	0.63	9	10
17	Drostanolone PC	$C_{26}H_{48}O_2Si_2$	16.75	448.3187			2.5	0.63	0.63	10	10
18	Drostanolone met (Drostanolone 3ol17one)	$C_{26}H_{48}O_2Si_2$	14.26	448.3187			2.5	0.63	0.63	9	10
19	Epimetendiol	$C_{26}H_{48}O_2Si_2$	12.55	358.2686	343.2452	448.3187	1	1.00	0.25	9	10
20	Fluoxymesterone met (9α-fluoro-18-nor-17,17-dimethyl-4,13-diene-11β-ol-3-one)	$C_{26}H_{43}O_2F_1Si_2$	15.35	462.2780	447.2545	357.2076	2.5	1.25	1.25	10	10
21	Formebolone met (dealdehyde-formebolone)	$C_{29}H_{54}O_3Si_3$	19.14	534.3375	339.2170		2.5	0.63	0.63	10	10
22	Formestane		19.25	518.3062	503.2828		20			10	10
23	Furazabol	$C_{23}H_{38}N_2O_2Si_1$	21.54	402.2697	387.2462		2.5	2.50	0.63	9	10
24	Furazanol met(16β-hydroxyfurazabol)	$C_{26}H_{46}N_2O_3Si_2$	24.35	490.3042	218.1153		2.5	1.25	0.63	10	10
25	Mesterolone met (1α-methyl-5α-androstane-3α-ol-17-one)	$C_{26}H_{48}O_2Si_2$	15.47	448.3187	433.2953		2.5	0.63	0.63	10	10
26	Mesterolone PC	$C_{26}H_{48}O_2Si_2$	16.3	141.0730	433.2953		2.5	1.25	1.25	10	10
27	Methasterone met (2α,17α-dimethyl-5α-androstane-3α,17β-diol)	$C_{27}H_{52}O_2Si2$	16.24	449.3266	374.2999		2.5	2.50	2.50	10	10
28	Methasterone PC	$C_{27}H_{50}O_2Si_2$	18.3	462.3343	419.2796	332.2530	2.5		0.63	9	9
29	Methenolone met (3α-hydroxy-1-methylen-5α-androstan-17-one)	$C_{26}H_{46}O_2Si_2$	15.06	446.3031	431.2796	251.1826	2.5	1.25	0.63	8	9
30	Mibolerone	$C_{26}H_{46}O_2Si_2$	17.78	446.3031	431.2796	301.1982	2.5	1.25	0.63	10	10
31	19-Norandrosterone	$C_{24}H_{44}O_2Si_2$	11.73	405.2640	315.2139		1	0.25	0.25	9	10
32	19-Noretiocholanolone	$C_{24}H_{44}O_2Si_2$	13.13	405.2640	315.2139		2.5	0.63	0.63	10	10
33	Norbolethone m1 (13β,17α-diethyl-5α-gonane-3α, 17β-diol)	$C_{27}H_{52}O_2Si_2$	18.28	144.0965	157.1034		2.5	1.25		8	10
34	Norbolethone m2 (13β,17α-diethyl-5β-gonane-3α,17β-diol)	$C_{27}H_{52}O_2Si_2$	19	144.0965	157.1034		2.5	1.25		10	10

(Continues)

TABLE 1 (Continued)

	Analytes (TMS derivatives)	Chemical formula	RT (min)	Ions (*m/z*)			Spiked conc* (ng/mL)	LOD (ng/mL) GC/Q-TOF	LOD (ng/mL) GC/Q-Orbitrap	Detection in 10 urine samples GC/Q-TOF	Detection in 10 urine samples GC/Q-Orbitrap
35	Norclostebol	$C_{18}H_{25}ClO_2$	20.15	452.2328	417.2640		2.5	0.63	0.63	10	10
36	Norethandrolone m2 (17α-ethyl-5β-estrane-3α,17β-diol)	$C_{26}H_{50}O_2Si_2$	17.52	241.1951	331.2452		2.5		0.63	10	10
37	Oxabolone PC	$C_{27}H_{48}O_3Si_3$	18.67	506.3062	507.31405		2.5	0.63	0.63	9	10
38	Oxymesterone	$C_{29}H_{54}O_3Si_3$	20.81	534.3375	519.31405	389.2327	2.5	0.63	0.63	10	10
39	Stenbolone	$C_{26}H_{46}O_2Si_2$	16.23	220.1278	208.1278		2.5	1.25	0.63	9	10
40	Testosterone	$C_{25}H_{44}O_2Si_2$	16.83	432.2874	209.1357	417.2640	EAAS				
41	5α-Androstan-3α,17β-diol	$C_{25}H_{48}O_2Si_2$	14.21	241.1951	256.2186		EAAS				
42	5B-Androstan-3α,17β-diol	$C_{25}H_{48}O_2Si_2$	14.55	241.1951	256.2186		EAAS				
43	5B-Androstan-3α,17β-diol d5, ISTD	$C_{25}H_{43}D_5O_2Si_2$	14.15	246.2265	261.2499		ISTD				
44	Androsterone	$C_{25}H_{46}O_2Si_2$	13.8	434.3031	329.2295	419.2796	EAAS				
45	Androsterone d4, ISTD	$C_{25}H_{42}D_4O_2Si_2$	13.5	438.3282	333.25463	423.3047	ISTD				
46	Epitestosterone	$C_{25}H_{44}O_2Si_2$	15.99	432.2874	209.1356	417.2640	EAAS				
47	Epitestosterone-D3, ISTD	$C_{25}H_{41}D_3O_2Si_2$	15.86	435.3063	420.2828		ISTD				
48	Etiocholanolone	$C_{25}H_{46}O_2Si_2$	14.11	434.3031	329.2295	419.2796	EAAS				
49	Etiocholanolone d5, ISTD	$C_{25}H_{41}D_5O_2Si_2$	13.78	439.3345	334.2609	424.3109	ISTD				

Spiked concentration at 50% of the MRPL.

EAAS: endogenous androgenic-anabolic steroids

ISTD: internal standard

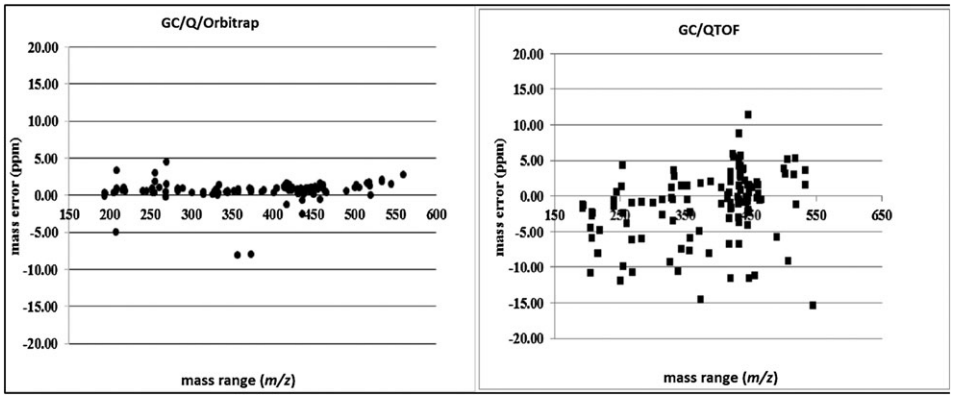

FIGURE 1 Mass errors of representative diagnostic ions of AAS over the entire mass range in both instruments

TABLE 2 Reproducibility of the mass accuracy in different urine matrices for both instruments

Instrument	Analytes	Exact mass (*m/z*)	Average (ppm)	STD	Max	Min
Q-TOF	5β-androst-1-ene-17β-ol-3-one	194.1121	3.15	2.96	9.27	0.00
	6-oxo-androstenedine	516.2906	7.62	3.73	13.17	2.32
	1α-methyl-5α-androstane-3α-ol-17-one	433.2953	2.67	1.73	5.77	0.69
	17α-ethyl-5β-estrane-3α,17β-diol	331.2458	5.30	5.72	15.09	0.30
Q-Orbitrap	5β-androst-1-ene-17β-ol-3-one	194.1121	0.40	0.23	0.52	0.00
	6-oxo- androstenedine	516.2906	1.01	0.09	1.16	0.97
	1α-methyl-5α-androstane-3α-ol-17-one	433.2953	0.18	0.22	0.46	0.00
	17α-ethyl-5β-estrane-3α,17β-diol	331.2458	1.81	0.48	2.72	1.21

m1), oxymesterone and clenbuterol. An improvement in the detection of 3'OH stanozolol using the GC/Q-Orbitrap was noted, which was due to the lower level of matrix interference than with the GC/Q-TOF. Moreover, clenbuterol was detected unambiguously using the GC/Q-Orbitrap in the FS/HR mode at 0.1 ng/mL; it was only possible to detect this compound using the GC/Q-TOF in MS/MS mode.[9] The detection of other compounds, such as boldenone, bolasterone and 13β,17α-diethyl-5α-gonane-3α17β-diol, was subject to interference using both instruments.

Generally, the chromatographic data obtained from routine screening using GC/MS and LC/MS is independently evaluated by two analysts in order to identify suspicious ions that may be the result of a prohibited substances abuse. These suspicious signals activate specific confirmatory procedures that include testing of a new aliquot

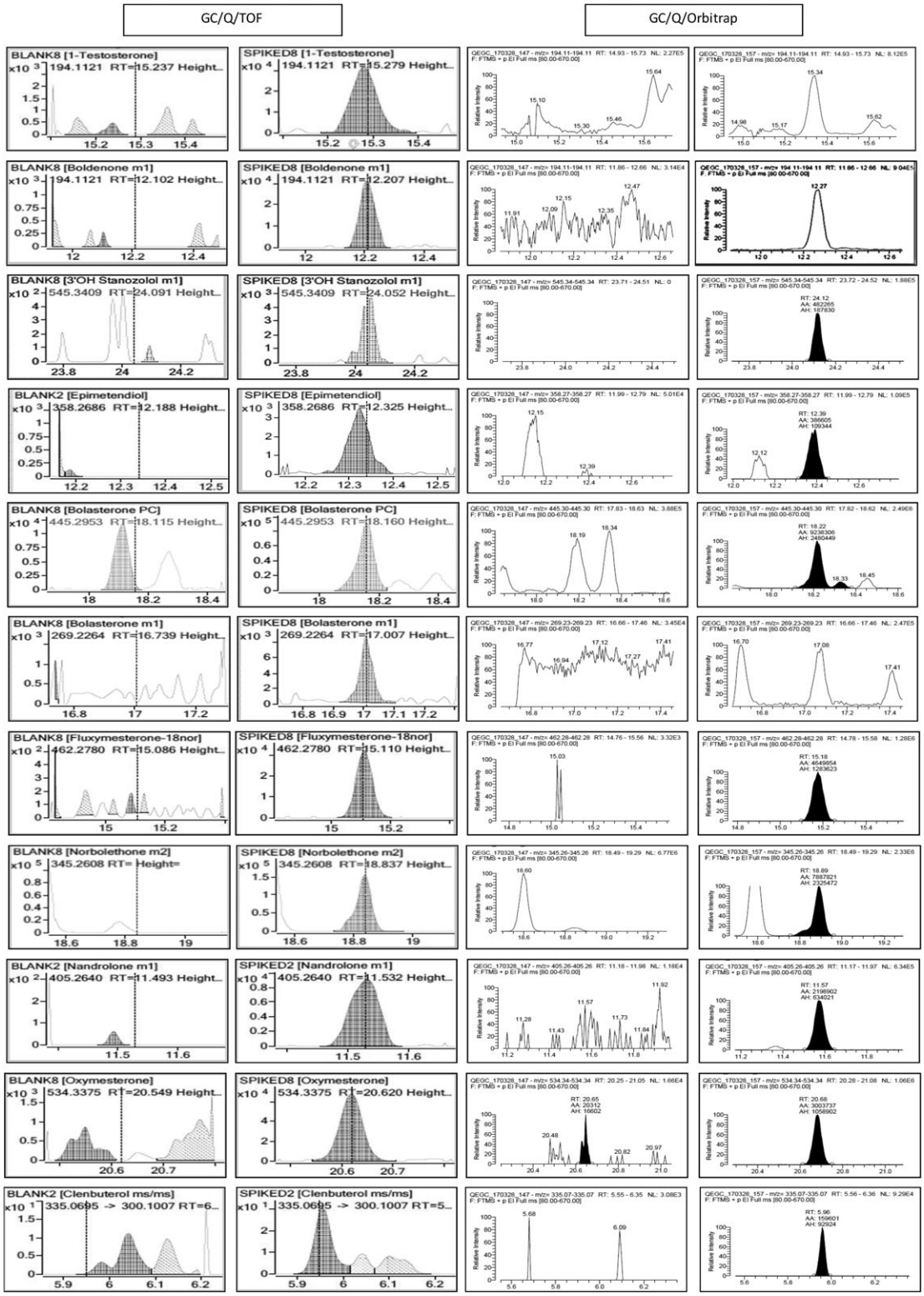

FIGURE 2 Extracted ion chromatograms of main fragment ions of some AAS screened by GC/Q-TOF and GC/QOrbitrap at concentration 50% of MRPL

of the suspicious urine sample. A routine batch of samples analyzed by GC/MS or LC/MS methods may generate tens of thousands of ion chromatograms that have to be reviewed one by one. Consequently, the quality and reproducibility of the ion chromatograms to be reviewed are important in reducing errors during the evaluation of doping tests. In an HR instrument, the mass accuracy of the ion chromatograms influences the quality of their evaluation. If the mass extraction window is too wide, the matrix interference is enhanced; if, however, it is too narrow, there is an increased risk of not detecting a prohibited substance due to mass error. In the current study, the mass extraction windows were optimized and set to 20 ppm for GC/Q-TOF and 5 ppm for GC/Q-Orbitrap, and the same *m/z* values were used for the detection of the analytes. The Orbitrap method can be further optimized via the use of the chromatographic back-flush

device and the inclusion of MS/MS acquisition, both of which were applied only to the GC/Q-TOF analysis in the current study. Both the GC/Q-TOF and the GC/Q-Orbitrap instruments were equipped with a quadrupole mass analyzer that allowed for the acquisition of MS/MS spectra.

3.2 | Quantitative analysis results

The development of a new GC/MS anti-doping screening method that cannot measure endogenous AAS has no practical utility.[10,25] Therefore, the current study included screening of the six endogenous AAS that are listed in Table 3 for both instruments. The data presented in Table 3 demonstrates that the analytical performance of both instruments complies with the WADA specifications.[10] It can be seen that the MU is less than 20% for A and Etio, 25% for 5αadiol and 5βadiol, and less than 20% for T and E. The endogenous concentrations of androsterone and etiocholanolone may be high relative to those of the other AAS (e.g., 8 μg/mL); thus, to avoid detector saturation, the FS MS mode was not used for the Q-TOF, but instead the transition from *m/z* 434.3031 to *m/z* 419.2796 was retraced at lower abundances using the MS/MS data. The GC/Q-Orbitrap method was conducted in FS mode during testing of all the steroids. It should be noted, however, that the Orbitrap platform is less prone to detector saturation than the Q-TOF because the Orbitrap is an ion trapping instrument that can adapt to higher analyte concentrations by reducing the ion acquisition time via automatic gain control and, thereby, allow for the detection of a larger dynamic concentration range than the Q-TOF. Overall, the data acquired from both instruments was in compliance with the specifications given in the technical document TD2016EAAS.[10] In a previous study, a comparison of steroid profile data obtained using GC/Q-TOF and GC/QQQ was carried out.[9]

3.3 | Analysis of a proficiency test sample

The analysis of a proficiency test sample was also successfully conducted using both instruments. The sample was obtained from

TABLE 3 Summary of the results of the quantitative data analysis

Compound	Calibration range (ng/mL)	Bias (%)		Intermediate precision (%)		MU (%)	
		GC/Q-TOF	GC/Q-Orbitrap	GC/Q-TOF	GC/Q-Orbitrap	GC/Q-TOF	GC/Q-Orbitrap
Androsterone (A)	100–8000	15.1	10.9	4.3	4.02	15.7	11.6
Etiocholanolone (ETIO)	100–8000	9.1	5.7	6.2	4.3	11	7.2
5α-Androstandiol (5αdiol)	4–800	5.8	6.5	0.8	4.7	5.9	7.7
5β-Androstandiol (5βdiol)	4–800	5.1	6.1	1.9	5.3	5.5	8.1
Testosterone (T)	2–400	9.1	17.2	1.2	1.1	9.2	17.2
Epitestostrone (E)	2–400	8.4	2.1	9	1.5	12.3	2.6

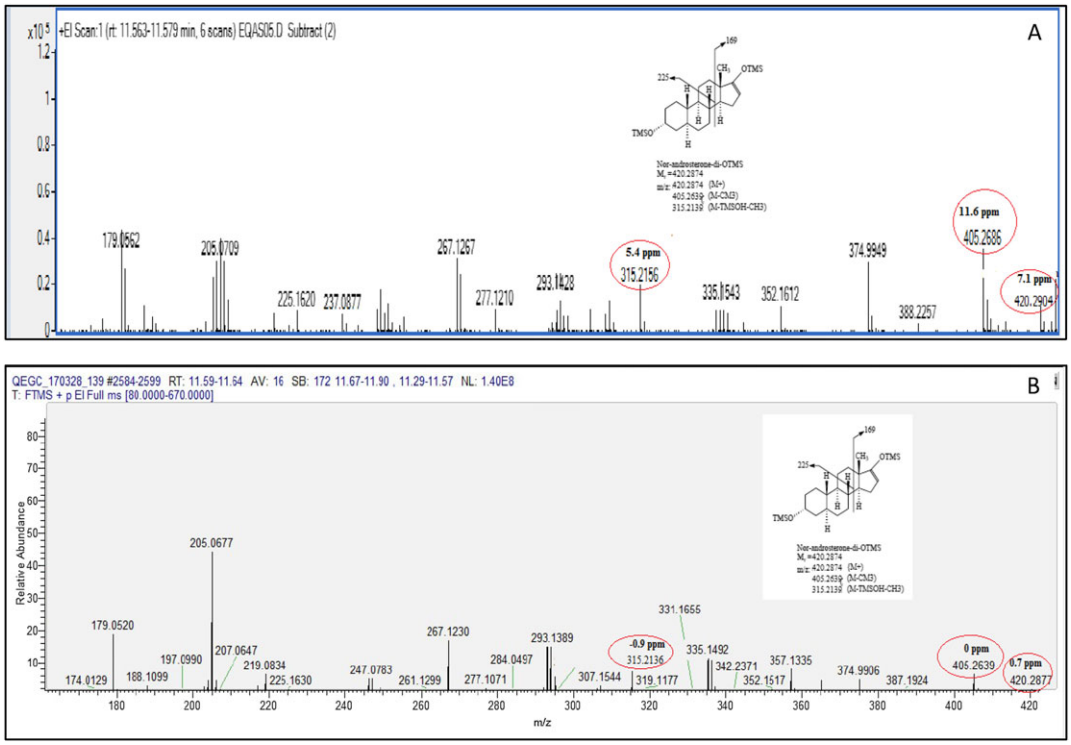

FIGURE 3 Full scan spectrum of proficiency test sample (19-norandrosterone) obtained by (A) GC/Q-TOF and (B) GC/Q-Orbitrap [Color figure can be viewed at wileyonlinelibrary.com]

an individual to whom nandrolone was administered; the two main metabolites of nandrolone, 19-norandrosterone and 19-noretiocholanolone, were detected. Figure 3 presents the FS/HR spectrum of 19-norandrosterone obtained at a concentration of 6.2 ng/mL using both the GC/Q-TOF and the GC/Q-Orbitrap platforms.

4 | CONCLUSIONS

The goal of the current study was to compare the performance of the GC/Q-TOF and GC/Q-Orbitrap molecular profiling technologies during routine anti-doping screening and the assessment of the qualitative and quantitative analyses resulting from the use of both platforms. The data presented herein demonstrate that both molecular profiling platforms can be used for anti-doping screening. It was also shown that it is possible to combine FS/HR acquisition with MS/MS to enhance specificity in order to facilitate screening for AAS. The LODs for both instruments were well below 50% of the MRPL sensitivity level. The identification criteria data shows that a limited number of compounds were not able to reach 100% detectability in the various urine matrices. The mass accuracies were excellent for both instruments; however, the GC/Q-Orbitrap had superior mass accuracy due to its higher resolution. The superiority of the GC/Q-Orbitrap was also demonstrated by the lower matrix effects found in the urine samples in relation to the mass accuracies. Overall, both instruments proved to be sufficiently robust for routine anti-doping testing and analysis. The results of the reprocessing of the existing data files to identify substances that previously escaped detection in FS mode will be presented in a future report.

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