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Improved screening of anabolic steroids in human urine using a new GC-triple quadrupole mass spectrometer -Preliminary results-

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Introduction

Mass spectrometry, in combination with gas or liquid chromatography (GCMS or LCMS), is the analytical technique of choice for drug testing in sport. To identify the presence of anabolic steroids at trace levels, tandem mass spectrometry (MS/MS) has been applied to gas chromatography based analytical methods. In case of analyzing samples with complex matrices, the application of ion traps or triple quadrupole instruments offers an increased sensitivity and selectivity by an effective elimination of the matrix background. Ion traps are characterized by their high sensitivity in full scan and product ion scan mode and offer the possibility for MS^n. However, due to long cycle times the number of analytes that can be determined simultaneously in MS/MS mode is restricted to 4-5. Furthermore, the quantitation capability and the ion ratio stability are poor. In contrast, triple quadrupole instruments are known for their high sensitivity, selectivity, ion ratio stability and linearity. Thus accurate and reproducible quantification is possible - even in the presence of complex matrices. Additionally, in selected reaction monitoring mode (SRM) these instruments provide the opportunity to test 25–30 analytes simultaneously.

The objective of this work has been to compare the new TSQ Quantum GC triple quadrupole instrument and the PolarisQ ion trap system when analyzing anabolic steroids in human urine. The comparison of these two MS/MS techniques is carried out by considering the parameters linearity, ion ratio stability and reproducibility. The sensitivity of the new GC triple quadrupole instrument is shown by means of selected target analytes.

Sample preparation

The samples were prepared according to the standard operating procedure for anabolic steroids described by Geyer et al [1].

Instrumentation

The GC/EI-MS/MS experiments were performed using a Thermo TraceGC Ultra gas chromatograph interfaced to a PolarisQ ion trap mass spectrometer and a TSQ Quantum GC triple quadrupole mass spectrometer, respectively. The GC system was equipped with a Varian VF-1ms capillary column (length 25 m, i.d. 0,2 mm, film thickness 0.1 µm) and a HP Ultra 1 (length 17 m, i.d. 0,2 mm, film thickness 0.1 µm). A volume of 3 µL of the sample was injected in the GC system which was operated in the split (1:10) mode. The GC oven temperature program started at 185°C, was increased at 5°C/min to 240°C, followed by 20°C/min to 310°C using helium as carrier gas (0.9 mL/min constant pressure). The injector and interface temperatures were set to 300°C and the ion source was operated at 225°C. Ionization was performed using electron ionization (EI) (70 eV).

GC/MSMS analysis

The GC-MS/MS parameters for the analysis of Clenbuterol, 18-norepimetendiol (18-NorEMD), epimetendiol (EMD), 19-norandrosterone (NA), D4-19-norandrosterone (D4-NA), 17α-methyl-5ß-androstane-3α,17ß-diol (3a5b-THMT), 17ß-hydroxymethyl-17α-methyl-18-norandrost-1,4,13-trien-3-one (NW) and 17α-methyl-testosterone (MT, (ISTD)) are listed in Table 1a,b.

Analyte	Precursor ion m/z	Excitation voltage (V)	Product ion range (m/z)	Product ion (m/z)
Clenbuterol	335.1	1.0	220-310	300,227
18-NorEMD	253.1	1.1	180-260	185,197,183
EMD	358.2	1.1	250-350	301, 253
NA	405.3	1.0	220-320	315,225
D4-NA	409.3	1.0	220-325	319,229
3a5b-THMT	270.2	1.0	130-260	213,199,216
NW	339.3	1.0	100-340	243,193,245,159
MT	446.4	1,0	290-360	301,356

Table 1a: MS/MS parameters for the PolarisQ ion trap mass spectrometer.

Analyte	Precursor ion (m/z)	Collision Voltage (V)	Product ion (m/z)	Dwell time (ms)
Clenbuterol	335.1	15	300,227	80
18-NorEMD	253.1	15	185,197	80
EMD	358.2	15	301,253	80
NA	405.3	15	315,225	80
D4-NA	409.3	15	319,229	20
3a5b-THMT	270.2	15	213,199,216	80
NW	442.3	15	339,236,133	80
	339.3	15	243	80
MT	446.3	15	301	20

Table 1b: MS/MS parameters for the TSC Quantum GC triple quadrupole mass spectrometer.

Results

Ion ratio stability

To investigate the ion ratio stability, a reference standard containing the metandienone metabolite NW is prepared and analysed repeatedly. Tabulated data for the ion ratio stability are shown below (Table 2 a, b). Ion transition ratios have been calculated with reference to the most intense (base) transition for NW. Based on these ratios the % RSD values are calculated.

Ion Transition	Ratio to Base Transition				Mean	SD	%RSD
	Injection 1	Injection 2	Injection 3	Injection 4			
339/243	1	1	1	1			
339/193	1.01	0.85	0.65	0.76	0.82	0.13	16.1
339/245	0.42	0.51	0.28	0.39	0.40	0.08	20.5
339/159	0.28	0.49	0.34	0.35	0.37	0.08	21.1
339/145	0.14	0.14	0.19	0.16	0.16	0.02	13.0
339/133	0.26	0.15	0.18	0.18	0.19	0.04	21.2

Table 2a: PolarisQ ion ratio stability of NW over 4 injections.

Ion Transition	Ratio to Base Transition								Mean	SD	% RSD
	Inj.1	Inj.2	Inj.3	Inj.4	Inj.5	Inj.6	Inj.7	Inj.8			
442/339	1	1	1	1	1	1	1	1			
442/236	0.38	0.39	0.41	0.39	0.37	0.39	0.38	0.42	0.39	0.02	4.0
442/133	0.34	0.37	0.35	0.36	0.33	0.34	0.32	0.35	0.34	0.02	4.8
339/193	1	1	1	1	1	1	1	1			
339/243	0.39	0.37	0.38	0.36	0.38	0.37	0.37	0.36	0.37	0.01	2.3

Table 2b: TSQ Quantum GC ion ratio stability of NW over 8 injections.

Reproducibility

Ten different urine samples spiked at the WADA minimum required performance limit (2ng/mL) were analysed to determine the reproducibility of the instrument. For standardisation the internal standard MT was used. Table 3 summarises the PolarisQ and TSQ Quantum GC data as %RSD (n=10).

	Clenbuterol	18-NorEMD	NA	EMD	3a5b-THMT
PolarisQ %RSD	25.1	28.6	25.6	30.4	22.8
TSQ %RSD	8.2	7.9	11	11.9	11.6

Table 3: % RSD values for selected compounds spiked at the WADA MRPL (2ng/mL urine) obtained by PolarisQ and TSQ Quantum GC.

Linearity

To generate the calibration curves, 19-norandrosterone (NA) was analysed in the concentration range from 1 to 10 ng/mL urine. For internal standardisation D4-NA was used (10 ng/mL). Each calibration point was prepared and analysed once. According to the ANOVA test approximation of the linearity was allowed with the equations y = 0,1583x – 0,0284 (R^2 = 0,9416) for the PolarisQ and y = 0,21269x – 0,04 (R^2 = 0,9986) for the TSQ Quantum GC.

Sensitivity

Figure 1 shows examples of extracted ion chromatograms of various anabolic steroids in urine at a concentration level of 0.1 ng/mL. Even at this extremely low concentration (1/20 of the required MRPL) all target compounds can be clearly detected using the TSQ Quantum GC.

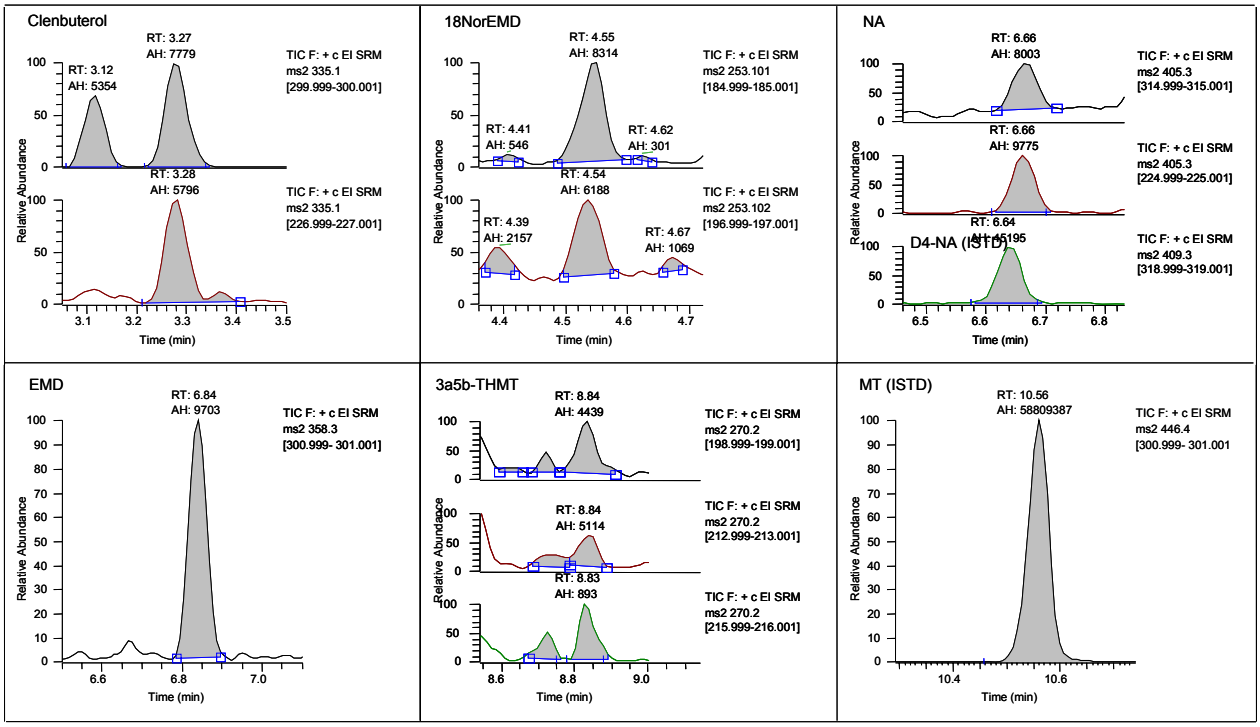

Figure2: Extracted ion chromatograms of various anabolic steroids at 0.1ng/mL in urine.

Conclusions

The TSC Quantum GC triple quadrupole mass spectrometer allows fast screening of anabolic steroids in human urine with highest sensitivity and selectivity. When operated in SRM mode, it can deliver strong confidence in quantitation with highly linear calibration curves, even at trace levels. The system reproducibility and the excellent ion ratio stability guarantee very good identification of compound presence in matrix as well.

References

[1] Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G (1998) Screening procedure for anabolic steroids - the control of the hydrolysis with deuterated androsterone glucuronide and studies with direct hydrolysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke, U (eds.) *Recent advances in doping analysis (5)*, Köln, pp 99-101.