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Improved screening method for doping control using a GC-QQQ tandem mass spectrometer

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Introduction

To improve the screening of anabolic steroids and other prohibited substances, detection of which using GC-MS mass spectrometry does not allow in some cases to obtain the results satisfying MRPL requirements, a more sensitive method using the Agilent 7000 Series Triple Quad GC/MS in MS/MS mode was developed. The selected reaction monitoring mode (SRM) allows the detection of target compounds at trace levels in complex matrixes. Additionally, this method provides an opportunity to conduct the screening process, confirmation and quantification in one analysis. In the SRM mode, this method allows to test up to 30 analytes simultaneously.

The developed method allows the identification of 27 anabolic steroids (Table 1). Besides those anabolic steroids, this method was also used for the detection of other classes of substances such as aromatase inhibitors-formestane, letrozole and diuretics – canrenone. To perform the process, we first selected the precursor ion, obtained by electron ionization (EI), in the full scan mode (50-700 Da). Then, we generated the fragmentation of the selected ion in the product ion scan mode using different values of collision energy (from 20 to 60 CE) and selected its product ions for their further optimization. We carried out this process in the SRM mode for the selected pairs of precusor/product ions using CE values varying from 5 to 35 CE in the increments of 5. For each pair, the CE value with the maximal abundance was selected. Next, a general method for all pairs in the SRM mode was created and 10 different blank samples of urine were analyzed. Those ion pairs that showed a significant interference with the matrix were excluded from the method. The final verification of the method was carried out on urine samples spiked with the listed substances in concentrations corresponding to their MRPL levels and below.

The obtained results confirmed that the new GC-QQQ instrument provides a higher level of confidence in the detection of banned substances.

Sample preparation

The samples were prepared according to the standard operating procedure for anabolic steroids described by Geyer et al.; in addition to the standard procedure, the solid-phase extraction of samples is performed before the main procedure. For this purpose, 2 ml of urine was eluted through an Amberlite XAD-2 column. The column (Pasteur pipette closed with a glass bead of 2 mm in diameter) is washed two times with 2 ml of distilled water and absorbed fraction is eluted with 2 ml of methanol.

Instrumentation

The GC/EI-MS/MS was performed using Agilent 7000 Series Triple Quadrupole GC/MS. The GC system was equipped with the GC HP Ultra 1 column (21 m, i.d. 0, 2 m, film thickness 0.1 µm). A volume of 2 µl of the sample was injected in the GC system wich was operated in the split (15.8:1) mode. The GC oven temperature program started at 186^{o}C, was increased at 2 ^{o}C /min to 240 ^{o}C, followed by 22 ^{o}C/min to 320 ^{o}C using helium as carrier gas (0.8 ml/min constant pressure). The injector and interface temperatures were set to 300 ^{o}C and the ion source was operated at 230 ^{o}C, electron ionization (EI) was used (70 eV).

GC/MS/MS analysis

The GC/MS/MS parameters for the analysis of the following substances are listed in Table 1. The limit of detection is shown in Table 1.

Figure 1 shows examples of extracted ion chromatogramms of the substances listed above in urine at the WADA minimum required performance levels.

Conclusion

The GC/MS/MS allows fast screeing of anabolic steroids and other substances in human urine with highest sensitivity and selectivity. When operated in SRM mode, it can deliver strong confidence in detection of compounds of interest at MRPL level. The system reproducibility and the excellent ion ratio stability guarantee very good identification of compound presence in complex matrices.

Table 1. GC/MS/MS parameters and LOD's

Analyte	Precursor ion (m/z)	Collision energy for each product ion respectively (V)	Product ion (m/z)	Dwell time (ms)	LOD (ng/ml)
1-androstenediol	434.6	5, 10, 5, 5, 25	405, 195, 182, 143, 127	30	5 ng/ml
1-androstenedion	430.6	5	194, 147	40	10 ng/ml
	415.6	15	221		
Bolasterone M1	284.5	5, 10, 15, 15, 15	227, 213, 185, 159, 145	40	5 ng/ml
Danazol M	558.9	25, 10, 15, 20	193, 418, 220, 207	40	10 ng/ml
Desoxymethyltestosterone	345.6	10, 15	201, 189	40	5 ng/ml
Fluoxymesterone M	462.7	10, 10, 10, 20	357, 337, 208, 193	30	5 ng/ml
Epimetendiol	358.5	10	301	40	2 ng/ml
	447.2	15	143		
Methandienone new M	339.0	5, 10, 10, 30	243, 193, 145, 119	30	5 ng/ml
Metenolone M1	446.8	5	431	40	10 ng/ml
	431.7	5, 20	341, 119		
19-norandrosterone	405.6	5,10	315,225	40	1 ng/ml
	420.6	20	225		
(3α5β-THMT)	435.7	5	345	40	2 ngml
	270.4	5, 15	199, 213		
Methasterone P	462.8	10, 20, 10, 15, 15	216, 201, 156, 143, 301	40	5 ng/ml
Oxymesterone	534.3	20, 30, 20	389, 301, 239	40	5 ng/ml
3′-hydroxystanozolol	560.9	10, 5, 20	254, 545, 143	40	2 ng/ml
Clenbuterol	335.4	5,10	300, 227	40	1 ng/ml
	337.4	5	300	40	
α- zeranol	433.6	5, 10, 20	389, 309, 295	30	5 ng/ml
β-zeranol	433.6	10, 5, 10, 20	415, 389, 309, 295	30	5 ng/ml
Formestane	518.9	25, 25, 25, 30	233, 195, 167, 131	30	25 ng/ml
Canrenone	412.3	5, 15, 10, 15	397, 299, 246, 180	30	0.125 mkg/ml
Mesterolone M	448.1	5, 15	433, 253	20	10 ng/ml
	433.4	5	253		
Metenolone P	446.2	30, 35, 15	208, 195,179	40	10 ng/ml
17-Epioxandrolone	363.4	20	161	40	10 ng/ml
	308.3	10, 15, 15	176, 161, 117		
Norbolethone 5α	435.6	20, 20	255, 159	40	10 ng/ml
Norbolethone P	301.3	15, 20,15	272, 169, 143	20	10 ng/ml
4-Chlorodehydro-methyltestosterone	478.4	5, 5, 20, 10	373, 240,225, 142	20	10 ng/ml
Calusterone M	374.2	20, 20	170, 156	40	10 ng/ml
Letrozole M	290.9	30, 35	190, 160	40	25 ng/ml
Oxandrolone P	363	15	161	40	10 ng/ml
	308.3	10	117		

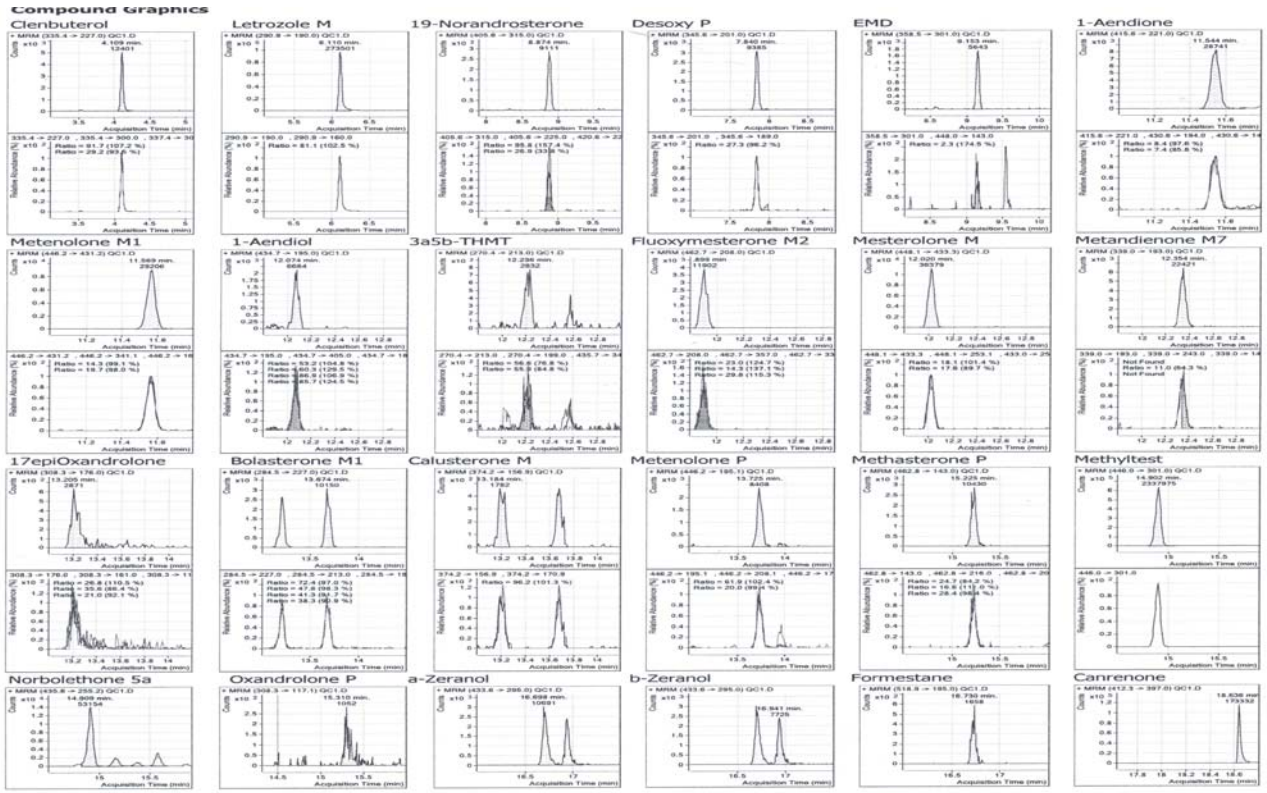

Figure1: Extracted ion chromatograms of some substances listed above in the urine at the MRPL level.

References

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