Analysis of doping and forensic drugs in urine using high-resolution GC/Q-TOF

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Introduction

In doping control and forensic toxicology applications, there is a high requirement for sensitivity and quantitative capability as well as an increasing demand for screening approaches, opening the possibility for retrospective analysis. GC triple quadrupole systems are highly valued for their sensitivity and wide dynamic range, making it a perfect instrument for targeted analysis and quantitation [1]. However, the disadvantages of using GC Triple Quadrupole instruments include a limited scope of compounds able to be analyzed in one run and the lack of capability to perform retrospective analysis. These limitations motivate analysts to keep looking for alternative solutions, such as high resolution accurate mass instruments capable of both high sensitivity and wide dynamic range in complex matrices.

In this study, we are examining the potential for high-resolution accurate mass 7250 GC/Q-TOF equipped with low energy EI source, for both quantitative and screening aspects of doping control and forensic drugs applications.

Experimental

The study was performed in two steps. First, two urine samples were spiked with a number of most challenging compounds (mostly steroids) at World Antidoping Agency (WADA) specified Minimal Required Performance Levels (MRPL) [2] and lower, to evaluate the resolving power, sensitivity and mass accuracy in matrix of the 7250 GC/Q-TOF. In addition, feasibility of using low electron energy for the analysis of these compounds was evaluated.

As a second step, six urine samples (four men and two women) were spiked with a new set of compounds (28 total) that were selected based on not only their notorious analytical difficulty but also compound class diversity, and included anabolic steroids, stimulants, sedative and anesthetic agents used in horse doping, beta-blockers and diuretics among others. Concentration levels varied from 1/10 to twice the MRPL as defined by the World Anti-Doping Agency (WADA) [2].

One milliliter of each urine sample was extracted, dried and derivatized using a mixture of MSTFA/NH4I/Ethanethiol at 80°C for 30 min.

GC/MS analysis was performed using an Agilent 7890B GC system coupled to a high resolution 7250 GC/Q-TOF, equipped with EI source allowing low-energy ionization (Figure 1). Instrument parameters are shown in Table 1.

Experimental

An Accurate mass Personal Compound Database and Library (PCDL) was created to enable automated building of quantitation methods as well as for potential use in the a downstream screening approach. The data were processed using MassHunter Quantitative Analysis software version B.09 and Qualitative Analysis software version B.08 SP1. Unknowns Analysis (a part of MassHunter Quantitative Analysis) was used for SureMass feature detection and NIST14.L library search to identify additional potential compounds of interest in urine.

Figure 1. Agilent 7250 GC/Q-TOF

GC and MS Conditions:	
Column	HP-1MS, 12 m, 0.25 mm, 0.25 µm
Injection volume	1 µL
Split mode	Splitless
Split/Splitless inlet temperature	280 °C
Oven temperature program	110 °C for 0.1 min 70 °C/min to 125 °C for 0.15 min 35 °C/min to 186 for 0.15 min, 2.2 °C/min to 204 °C, 20 °C/min to 245 °C, 50 °C/min to 270 °C, 75 °C/min to 320 °C, 1.1 min hold
Carrier gas	Helium at 1 mL/min constant flow
Transfer line temperature	310 °C
Ionization mode	Standard EI at 70 eV; low electron energy EI at 17 eV, 15 eV
Source temperature	230°C
Quadrupole temperature	150°C
Mass range	50 to 650 m/z
Spectral acquisition rate	3-5 Hz

Table 1. GC/Q-TOF conditions

Results and Discussion

Evaluation of mass accuracy, sensitivity as well as feasibility of using low electron energy

An example of spectra (as PCDL accurate mass library entries) are shown in Figure 2. For compounds with relatively small molecular ions at 70 eV, a potential benefit of using lower electron energy to increase the relative abundance of the molecular ion can be suggested.

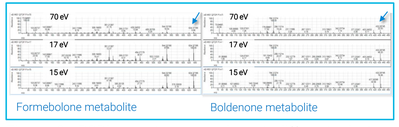

Figure 2. PCDL spectra examples at different electron energies. Arrows point to the molecular ion.

Examples of EICs obtained for MRPL levels at 70 eV as well as lower electron energy are shown in Figure 3.

Mass error for the characteristic ions of the target compounds in solvent as well as spiked into urine at MRPL levels was around or below 1 ppm in most cases (Figure 4).

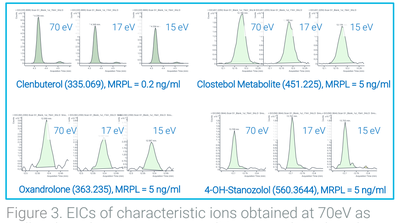

Figure 3. EICs of characteristic ions obtained at 70eV as well as low electron energy conditions are plotted on the same absolute abundance scale for each compound.

Quantitative Analysis

To evaluate a quantitative approach using 7250 GC/Q-TOF, extracted, spiked and derivatized urine samples from 6 different persons were analyzed. Compound selection included the most analytically challenging anabolic steroids (due to their lowest MRPL and significant endogenous interferences). Success in this approach indicates that the method would likely detect virtually every component from a typical screening (about 300 compounds). The results are summarized in Figure 5 (A,B) and Table 2. An example of a calibration curve across a broader concentration range from an independent experiment is shown in Figure 5C.

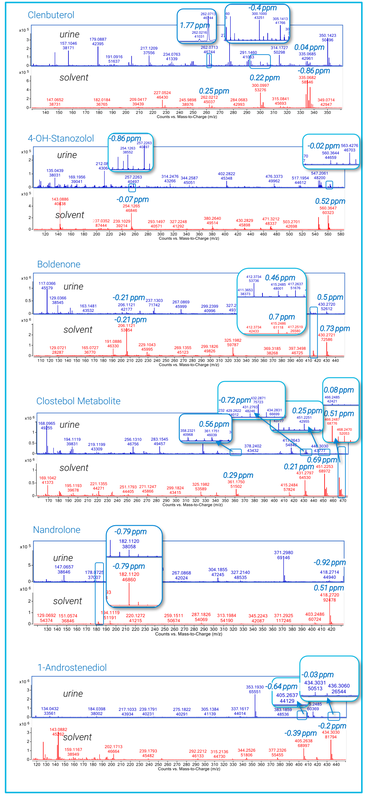

Figure 4. Mass accuracy for characteristic ions and resolving power (shown below m/z) in solvent and urine (MRPL). All spectra are background-subtracted.

Results and Discussion

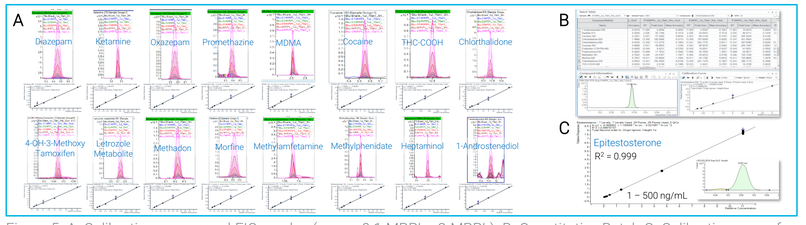

Figure 5. A. Calibration curves and EIC overlay (range: 0.1 MRPL - 2 MRPL). B. Quantitative Batch C. Calibration curve for epitestosterone from 1 to 500 ng/mL.

Untargeted Analysis

An untargeted approach using Unknowns Analysis was able to identify a number of additional compounds of potential interest such as lidocaine, bambuterol, famprofazone, miconazole, umbelliferone and clobenzorex among others (Figure 6).

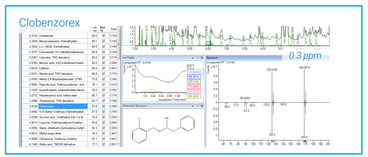

Figure 6. Unknowns Analysis of urine extract for detection of untargeted compounds of interest using NIST library.

Name	Application	MRPL, ng/ml	RT	Target m/z	%RSD
Amfetamine	Stimulant	100	1.4	192.1203	5.7-16.5
Methylamfetamine	Stimulant	100	1.6	130.1047	3.3-11.4
Barbital	Horse doping	50	2.2	313.1398	4.3-6.7
Heptaminol	Stimulant	100	2.5	188.1285	0.9-7
MDMA	Stimulant	100	2.5	130.1048	1-5.6
Methylphenidate	Stimulant	100	3.2	156.1203	1.1-3.4
Ketamine	Horse doping	50	3.3	278.0888	1.1-5.2
Mepivacaine	Horse doping	50	4.1	261.1543	1.5-27.5
Oxprenolol	Beta blocker	100	5.3	222.1071	2.8-6
Cocaine	Stimulant	100	5.6	182.1176	0.9-2.4
Methadon	Narcotic	50	6.2	381.2482	0.9-2.7
Promethazine	Horse doping	50	6.4	284.1342	2.3-26.5
Letrozole metabolite	Aromatase Inhibitor	20	6.8	291.0948	1.2-2.3
Atenolol	Beta blocker	100	7.8	144.1203	2.4-15.5
Diazepam	Horse doping	50	8.5	284.0711	0.7-2.7
Oxazepam	Horse doping	50	8.6	429.1216	1.2-2.3
Morfine	Narcotic	50	10.5	429.2150	0.7-3
1-Androstenediol	Anabolic agent	5	11.9	405.2640	4.6-28.6
5a-Methyltestosteron metabolite	Anabolic agent	5	11.9	143.0887	4.7-22.8
17a trenbolone	Anabolic agent	5	12.1	307.1513	2-16
Endoxifen (-CSH7N)	Hormone Antagonist	20	12.5	460.2248	2.7-11.1
THC-COOH	Cannabinoid	15	12.8	488.2773	1.8-3.6
Norbolethone metabolite	Anabolic agent	5	12.9	157.1043	1.3-15
Fluoxymesterone tetrol metabolite	Anabolic agent	5	13.1	552.3281	8.4-24.7
Chlorthalidone	Diuretic	200	13.3	539.1074	2.4-12.2
4-OH-3-MetoxyTamoxifen	Aromatase Inhibitor	20	13.5	72.0808	2.4-6.1
16 Hydroxy Furazabol	Anabolic agent	5	13.8	231.1231	3.6-18.2
Prednisolone	Corticosteroid	30	13.9	630.3407	3.2-12.4

Table 2. Quantitation results summary showing %RSD range for MRPL levels.

Conclusions

- Quantitation and untargeted screening approaches for doping and forensic drugs in urine were explored using high-resolution GC/Q-TOF.
- For most compounds spiked to urine at MRPL levels % RSD was below 20%. 22 compounds demonstrated linearity of R2>0.99 in most matrices.

References

[1] Van Gansbeke W, Polet M, Hooghe F, Devos C, Van Eenoo P. Improved sensitivity by use of gas chromatography-positive chemical ionization triple quadrupole mass spectrometry for the analysis of drug related substances. JOURNAL OF CHROMATOGRAPHY B-ANALYTICAL TECHNOLOGIES IN THE BIOMEDICAL AND LIFE SCIENCES. 2015;1001:221–40.

[2] WADA, TDMRPL2018: minimum required performance levels for detection and identification of non-threshold substances.