Application Note: 52276

The Determination of Anabolic Steroids in Human Urine using the TSQ Quantum XLS

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Key Words

- TSQ Quantum XLS Triple Quad
- PTV Backflush
- Repeatability
- Selected Reaction Monitoring
- Sports Doping
- Steroids
- Timed-SRM

Introduction

Use of performance-enhancing drugs (PED) in professional sports has sparked widespread media attention across the world. The potential financial and social impact of athletes screening positive for PED's such as anabolic steroids has increased the importance of accuracy in drug screening. Although screening methods exist, the ability to reliably detect and quantitate low concentrations of PED's in complex biological matrices remains a challenge for many laboratories. In recent years, it has become clear that triple quadrupole technology with the high selective hyperbolic quadrupole assembly provides the appropriate tools necessary for low concentration drug screening analysis. The following methodology describes the identification and quanititation of anabolic steroids in Urine matrix using the Thermo Scientific TSQ Quantum XLS mass spectrometer.

Experimental

A Thermo Scientific AS 3000 II autosampler and a Thermo Scientific TRACE GC Ultra gas chromatograph, equipped with a backflush valve B.E.S.T PTV injection port, provided sample introduction into the TSQ Quantum™ XLS mass spectrometer. Chromatographic separation was achieved using a Capillary Guard Gold Column 2 m × 0.53 mm I.D. pre-column (part number 26050-0253) with a Thermo Scientific TraceGOLD TG-5MS 15 m × 0.25 mm I.D. × 0.25 µm column (part number 26098-1300). Additional instrument parameters are displayed in (Table 1). Thermo Scientific QuanLab Forms software provided automated acquisition and processing of all data, including quantitation and ion ratio confirmation calculations.

Thermo Scientific TRACE GC Ultra	
Injection Volume	2 µL injection
Liner	Siltec® baffled liner
Carrier Gas	He, constant flow, 1.4 mL/min
GC Method	Initial 140 °C, Hold 0.1 min, Ramp 40.0 °C/min–180 °C, Ramp 4.0 °C/min–192 °C, Ramp 8.0 °C/min–220 °C, Ramp 40.0 °C/min–300 °C, Hold 2.0 min
Transfer Line	300 °C
TRACE GC Ultra™ PTV	
Injector Temperature	250 °C, splitless injection
PTV Cleaning Step	350 °C, 11 min, clean flow 50 mL/min
Transfer Time (Backflush)	3 min
TSQ Quantum XLS Mass Spectrometer	
Source Temperature	280 °C, closed EI ion volume
Ionization	EI, 70 eV
Emission Current	50 µA
Resolution	0.7 Da Q1, Q3
Collision Gas	Argon, 1.5 mTorr

Table 1: Selected instrument parameters TRACE GC Ultra and TSQ Quantum XLS mass spectrometer

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Method Setup

The TSQ Quantum XLS mass spectrometer was operated in select reaction monitoring mode (SRM). A full scan of a positive control sample at 10 ng/mL was used to determine retention times and parameters for the SRM method. In a SRM method, specific transitions are monitored in a narrow window around the retention time of each individual compound. Optimal monitoring transitions are selected from a group of the most abundant product ions for each selected compound. Figure 1 depicts the selection from five product ion candidates for the precursor 19-norandrosterone.

Data acquisition parameters were established by selecting two ion precursor-product ion transitions for each target compound, and two ions for the deuterated internal standard d3-testosterone (Table 2).

Standards at 10 ng/mL were measured prior to sample analysis to confirm system suitability and provide ion ratio confirmation limits. Five replicates of five unknown specimens in urine matrix were analyzed for clenbuterol, 19-norandrosterone, 17β-methyl-5β-androst-1-ene-3α,17α-diol, 17α-methyl-5β-androstane-3α,17β-diol and 3'-hydroxystanozolol content. Method performance was assessed for repeatability (precision) and conformance to World Anti-Doping Agency (WADA) analysis criteria (Table 3).

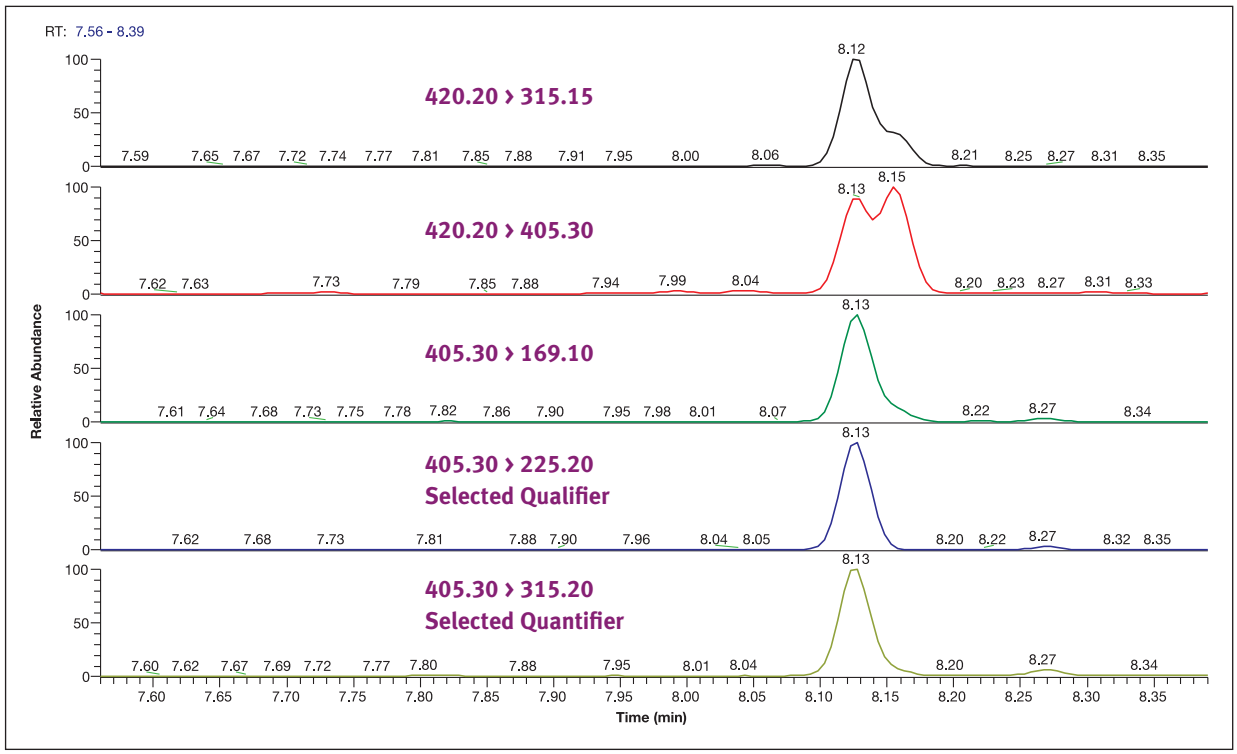

Figure 1: Quantitative and qualitative ions were selected based on greatest sensitivity and minimized interference when analyzed in matrix. Selection of optimal transitions for 19-norandrosterone eluting at RT 8.13 min. *Note: Interference present at RT 8.15 min.*

Compound Name	Quant/Qual	Parent Ion [Da]	Product Ion [Da]	Collision Energy [V]	Retention Time	T-SRM Window
clenbuterol	Qual	335.2	227.2	15	5.08	4.47–5.95
	Quant	335.2	300.2	11	5.08	4.47–5.95
19-norandrosterone	Quant	405.3	315.2	19	8.13	7.60–8.45
	Qual	405.3	225.2	15	8.13	7.60–8.45
17β-methyl-5β-androst-1-ene-3α,17α-diol	Quant	358.3	301.2	10	8.25	7.73–8.77
	Qual	358.3	343.2	15	8.25	7.73–8.77
d3-testosterone internal standard	Quant	435.3	330.3	25	8.53	8.32–9.82
	Qual	435.3	420.3	15	8.53	8.32–9.82
17α-methyl-5β-androstane-3α,17β-diol	Qual	255.2	199.1	15	8.88	8.36–9.15
	Quant	270.2	213.2	14	8.88	8.36–9.15
3'-hydroxystanozolol	Qual	545.3	387.2	34	10.61	10.08–11.58
	Quant	545.3	455.2	36	10.61	10.08–11.58

Table 2: SRM data acquisition parameters

Relative Abundance (% of base peak)	GC-MS/MS
> 50%	±10% (absolute)
25% to 50%	±20% (relative)
5% to < 25%	±5% (absolute)
< 5%	±50% (relative)

Table 3: WADA – maximum tolerance criteria for relative ion intensities

Results and Discussion

SRM method setup allowed monitoring of multiple steroid compounds without dividing chromatograms into segments or the need to select suitable segment breaks.

Chromatograms for quantitative SRM transitions in a single replicate of sample are displayed in Figure 2.

All peaks are clearly defined, and a high response is generated for multiple compounds at low concentrations ensuring sample analysis integrity and the sensitivity needed in drug confirmation analysis.

All compounds were detected in the five urine matrix specimens at various concentrations. Table 4 contains measured sample concentration ranges for each compound and comparison to the established WADA limits of reporting (LOR).

The results in Table 4 demonstrate the ability of the TSQ Quantum XLS to accurately evaluate multiple components at low concentrations within a single sample.

Compound	Calculated Sample Concentration Range (ng/mL)	WADA – LOR (ng/mL)
clenbuterol	0.80–8.09	2.0
19-norandrosterone	1.88–9.41	1.0
17β-methyl-5β-androst-1-ene-3α,17α-diol	0.42–3.98	2.0
17α-methyl-5β-androstane-3α,17β-diol	0.71–6.99	2.0
3'-hydroxystanozolol	4.67–23.56	2.0

Table 4: Calculated sample concentration ranges in comparison to WADA LOR criteria

Quan/qual ion ratio limits were established using results from standard injections in accordance with WADA regulation (Table 3). Average ion ratio values from the each set of five injections for each sample are compared to the established limits in Table 5.

All samples were measured within established ion ratio limits. The maintained ion ratio stability and low RSD values between measurements demonstrate the reproducibility and robustness of the TSQ Quantum XLS and meets the WADA requirements for drug screening analysis.[1]

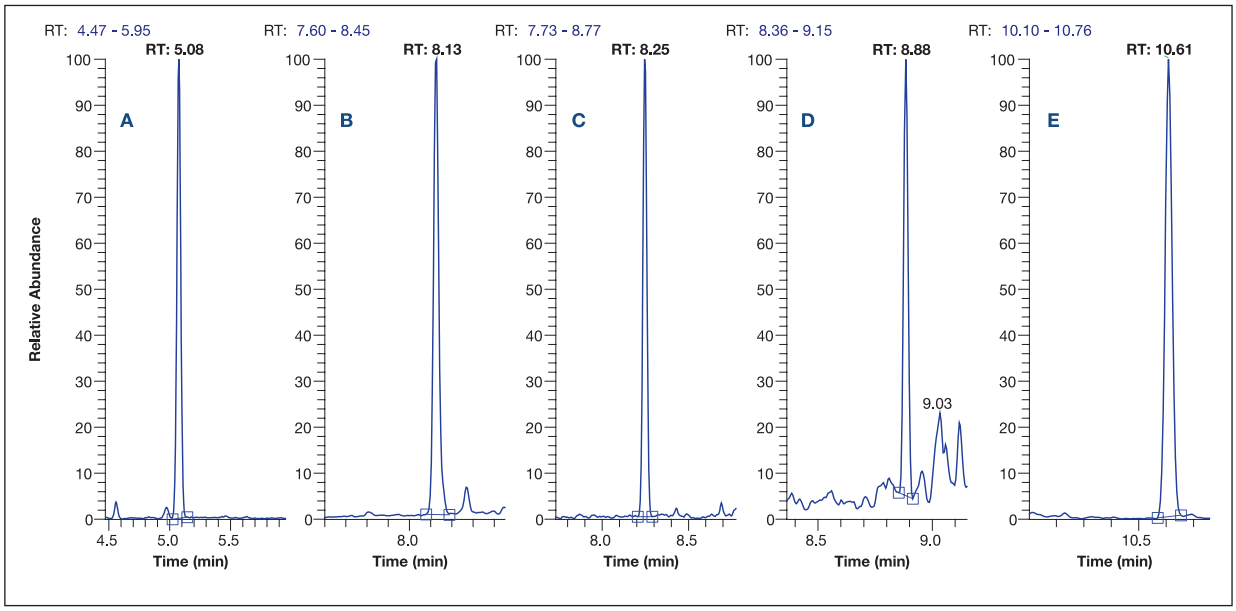

Figure 2: Sample 4: Quantitative SRM transitions for each steroid compound. A) clenbuterol B) 19-norandrosterone C) 17β-methyl-5β-androst-1-ene-3α,17α-diol D) 17α-methyl-5β-androstane-3α,17β-diol E) 3'-hydroxystanozolol

Compound	Limits	Average	RSD
clenbuterol	55.22%–75.22%	64.66%	1.13%
19-norandrosterone	95.08%–115.08%	97.03%	3.17%
17β-methyl-5β-androst-1-ene-3α,17α-diol	8.77%–18.77%	15.74%	7.83%
17α-methyl-5β-androstane-3α,17β-diol	31.14%–51.90%	32.66%	3.20%
3'-hydroxystanozolol	3.92%–13.92%	8.99%	0.27%

Table 5: Resulting quan/qual ion ratios in comparison to calculated assay limits.
Note: percentage values and RSD calculated from five injections for each sample.

A total of thirty 2 µL urine extracts were injected in splitless injection mode using the PTV inlet.[2] The combination of an inert PTV backflush injector, precolumn, column and detection system permitted stable system suitability and optimal chromatography throughout the analysis. Figure 3 shows a comparison of the initial and final injections of the late eluting, low intensity transition (545 > 387) for 3'-hydroxystanozolol.

Despite a late retention time and heavy matrix presence, 3'-hydroxystanozolol peak shape and comfortable detection at the level of interest is well maintained. High performance GC-MS/MS, in combination with stable operation and an inert system setup, ensures accurate analysis results with freedom from interfering matrix compounds.

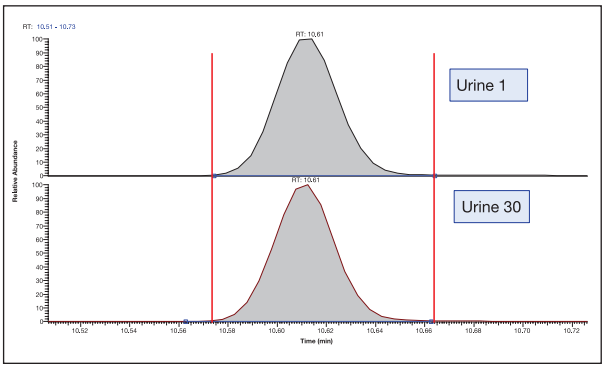

Figure 3: Initial and final sample chromatograms for the 545 > 387 transition of 3'-hydroxystanozolol

Conclusions

The TSQ Quantum XLS was chosen for its ability to detect low level concentrations of steroids in complex biological matrices. When operated in SRM mode, the TSQ Quantum XLS provides excellent sensitivity with the ability to accurately analyze multiple components at various concentrations within a single injection conserving laboratory resources and decreasing sample analysis time.

The PTV backflush system enables optimal chromatography by removing heavy matrix interference with the added benefit of increasing column and source lifetime. These benefits ensure minimal instrument downtime, ultimately saving money and increasing productivity.

QuanLab Forms™ delivered automated sample processing and data review, minimizing the need for extensive data interpretation and ensuring unmatched sample throughput through processing accuracy.

The TSQ Quantum XLS delivers unsurpassed performance and reproducibility when analyzing samples according to WADA guidelines, effectively eliminating heavy matrix effects and providing a stable robust system.

References

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