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Improved steroid profiling using GC tandem mass spectrometry - Preliminary results -

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Introduction

The detection of steroidal hormones in human urine is a great challenge in doping control analysis. On the one hand most of these compounds have to be detected at trace levels on the other hand for steroid profiling the quantitation of several endogenous produced steroids is required. The application of GC triple quad instruments allows the fulfillment of these criteria in one analytical run. The objective of this work has been to demonstrate the capability of the TSQ Quantum GC triple quadrupole instrument for steroid profiling. By means of calibration curves the concentrations and ratios of various endogenous steroids including testosterone, epitestosterone, androsterone, etiocholanolone, dehydroepiandrosterone, 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol were determined in routine samples and compared to common GC/MS data. The large advantage of the GC/MSMS technique over the GC quadrupole instruments concerning sensitivity and selectivity at lower concentrations is exemplarily demonstrated by the detection of epitestosterone and testosterone.

Chemicals, reagents and sample Preparation

tert-Butyl methyl ether (TBME) was purchased from KMF Laborchemie (St. Augustin, Germany) and distilled before use. ß-Glucuronidase from Escherichia coli (E. coli) was supplied by Roche Diagnostics (Mannheim, Germany). MSTFA was obtained from Macherey &Nagel (Düren, Germany). T, E, A and Etio were purchased from Sigma-Aldrich (Seelze, Germany). ADiol, BDiol and DHEA were delivered by LGC Promochem (Wesel, Germany). D3T, d3E and d4etio (in-house synthesis, Institute of Biochemistry, German Sport University Cologne, Germany) were used as internal standards. All solutions and buffers were prepared using deionised water (Water Lab System, Millipore, Eschborn, Germany). The samples were prepared according to the standard operating procedure for anabolic steroids described by Geyer et al [1].

Instrumentation

The GC/EI-MSMS experiments were performed using a Thermo TraceGC Ultra gas chromatograph interfaced to a TSQ Quantum GC triple quadrupole mass spectrometer. The GC system was equipped with a HP Ultra1 capillary column (length 17 m, i.d. 0.2 mm, film thickness 0.1 µm). A volume of 1.5 µL of the sample was injected in the GC system which was operated in split (1:15) mode. The GC oven temperature program started at 185°C, was increased at 3°C/min to 240°C, followed by 40°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 250°C. Ionization was performed using electron ionization (EI) at 70 eV. In the MSMS mode argon was used as collision gas.

GC/MSMS analysis

The GC/MSMS parameters for the analysis of testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 5α-androstane-3α,17β-diol (Adiol) and 5β-androstane-3α,17β-diol (Bdiol), d3-testosterone (d3T), d3-epitestosterone (d3E) and d4-etiocholanolone (d4Etio) are listed in table 1.

Analyte	Ion transition (m/z)	Collision Energy (eV)	Dwell time (ms)
testosterone	432 / 209	18	20
epitestosterone	432 / 209	18	20
d3-testosterone (ISTD)	435 / 330	18	20
d3-epitestosterone (ISTD)	435 / 330	18	20
androsterone	434 / 239	18	10
etiocholanolone	434 / 239	18	10
d4-etiocholanolone (ISTD)	423 / 333	13	10
dehydroepiandrosterone	432 / 417	8	20
Adiol	241 / 185	17	20
Bdiol	241 / 185	17	20

Table 1: Mass spectrometric parameters of investigated steroids.

Results and Discussion

Linearity

To generate the calibration curves a mix standard in a DIPA matrix [2] was analysed at different concentration levels. For internal standardisation d4Etio, d3E and d3T were used. Each calibration point was prepared and analysed once. For all analytes, approximation of linearity was allowed according to the Mandel test with the equations listed in table 2.

Analyte	Concentration range (ng/mL)	Equation	Correlation coefficient
T	2 – 100	y = 0.0199x - 0.0140	R^2 = 0.9999
E	2 – 100	y = 0.0409x - 0.0456	R^2= 0.9985
A	250 – 5000	y = 0.0010x - 0.0700	R^2 = 0.9997
Etio	250 – 5000	y = 0.0009x - 0.0302	R^2 = 0.9995
DHEA	10 - 250	y = 0.0017x - 0.0026	R^2 = 0.9993
Adiol	10 – 200	y = 0.0007x - 0.0017	R^2 = 0.9998
Bdiol	20 - 450	y = 0.0008x - 0.0029	R^2 = 0.9999

Table 2: Linearity parameters of investigated steroids.

Precision

For determination of the instrument precision and the batch to batch precision a quality control sample is prepared daily and analyzed several times per sequence. The relative standard deviation (%RSD) values for the different steroids were calculated from their concentrations and are listed in table 3.

Analyte	Instrument precision (%RSD)	Batch to batch precision (%RSD)
A	1,5	6,7
Etio	1,6	7,4
Adiol	6,1	13,1
Bdiol	4,5	12,1
DHEA	3,5	8,9
T	4,9	10,8
E	4,0	11,9

Table 3: %RSD values for instrument reproducibility and batch to batch precision in a QC sample.

Selectivity and Sensitivity

Figure 1 demonstrates the advantage of the GC triple quad instrument over the common used single quad instruments.

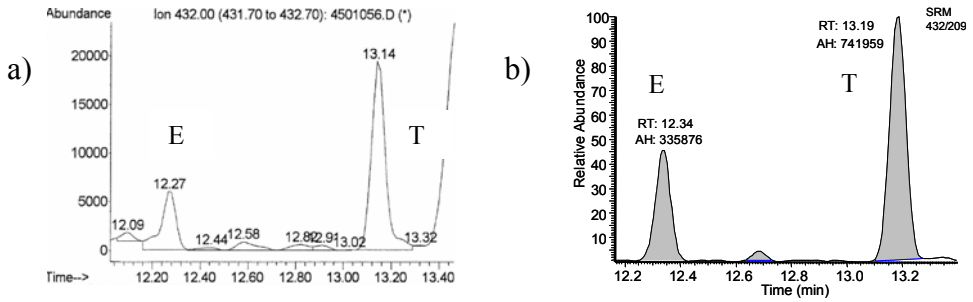

Fig 1: Extracted ion chromatograms for E and T a) GC/MS b) GC/MSMS.

When operating in MSMS mode interfering substances are eliminated and the peak detection and resulting calculation of concentrations is more reliable. The enhanced selectivity and sensitivity is clearly shown using the example of epitestosterone and testosterone.

Comparison of results

To verify the accuracy of this technique the steroid concentrations in doping control samples were determined by calibration curves and compared to the results of the routinely used GC/MS single quadrupole method. The obtained data (table 4) are in good agreement.

	Sample 1		Sample 2		Sample 3		Sample 4	
Analyte	GC/MSMS	GC/MS	GC/MSMS	GC/MS	GC/MSMS	GC/MS	GC/MSMS	GC/MS
A	1242.1	1289.0	571.1	623.0	2508.9	2586.0	1751.9	1792.0
Etio	977.4	992.0	289.6	290.0	2989.9	2917.4	1539.6	1426.3
Adiol	19.4	20.0	16.1	15.0	77.8	82.0	25.6	24.0
Bdiol	53.8	52.0	38.7	37.0	183.7	193.0	63.6	65.0
DHEA	27.4	27.0	12.2	9.5	53.3	58.0	30.5	29.0
E	3.7	3.2	8.5	8.0	91.5	96.8	17.0	16.1
T	2.7	2.8	13.0	13.1	74.5	77.9	19.7	18.7

Table 4: Comparison of steroid concentrations (ng/mL) obtained by GC/MSMS and GC/MS.

Conclusion

The application of the TSQ Quantum GC triple quad instrument allows the detection of a large number of prohibited compounds at trace levels (far below the WADA MRPL) and steroid profiling in one and the same analytical run. When operating in SRM mode, it can deliver strong confidence in quantitation with highly linear calibration curves. The system reproducibility in combination with highest sensitivity and selectivity guarantee very good identification and quantification of compounds 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 U. (eds.) *Recent Advances in Doping Analysis (**5**) Sport und Buch Strauß, Köln*, pp 99-102.
[2] Nolteernsting E, Opfermann G, Donike M (1996) 1-(N,N-Diisopropylamino)-n-alkanes: A new reference system for systematical identification of nitrogen containing substances by gas-chromatography and nitrogen specific or mass spectrometrical detection. In: M. Donike, H.Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) *Recent advances in doping analysis (3). Sport und Buch Strauß, Köln*, pp 369-388.