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GC –single and –triple quadrupole mass spectrometry in steroid profiling

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Abstract

The accurate quantification of the six endogenous anabolic steroids androsterone (A), etiocholanolone (Etio), testosterone (T), epitestosterone (E), 5α-androstane-3α,17β-diol (Adiol) and 5β-androstane-3α,17β-diol (Bdiol) for the purpose of the steroid profile model of the Athlete Biological Passport is of utmost importance. Since urinary certified reference material for the six mentioned target analytes is limited, the evaluation of steroid profile results reported by the laboratories in External Quality Assessment Scheme (EQAS) tests is assessed using z-scores.
Currently the community of doping control laboratories utilizes GC quadrupole MS instruments to determine steroid profile values.
Steroid profile results of 18 urine samples, distributed for interlaboratory testing (WADA EQAS exercise), obtained from GC-MS and GC-MS/MS were evaluated based on z-scoring in order to check the GC-MS/MS results for feasibility. Minor differences in concentrations for the six target steroids were detected. An extended variation for T/E is shown between the different technologies, mainly caused by the high specificity of GC-MS/MS.
Most important is the examination of the target relative standard deviation (TSD) for T/E, which is currently fixed at 10%. An extention up to 20% (which is in line with most of the endogenous steroids) would lead to an elimination of questionable / unsatisfactory results of the presented data set.

Introduction

According to requirements of the World Anti-Doping Agency the exact quantification of the six steroid profile variables androsterone (A), etiocholanolone (Etio), testosterone (T), epitestosterone (E), 5α-androstane-3α,17β-diol (Adiol) and 5β-androstane-3α,17β-diol (Bdiol) is crucial [1].
Presently the analysis is performed by means of GC quadrupole MS (GC-MS) instrumentation and the evaluation of the results reported by the laboratories in External Quality Assessment Scheme (EQAS) tests is assessed using z-scores. Recently GC –triple quadrupole mass spectrometry (GC tandem MS = GC-MS/MS), a more specific technology for steroid profiling, became available. Steroid profile results of GC-MS and GC-MS/MS were evaluated based on z-scoring in order to check the GC-MS/MS results for feasibility.

Experimental

Common procedures for the detection of anabolic and endogenous steroids in doping control were applied [2-4]. Quantitative data were achieved utilizing single point calibration. GC-MS/MS T/E ratios were calculated concentration-based, whereas for GC-MS the T/E results were obtained by area value ratios.
Analytical results of 18 urine samples, distributed for interlaboratory testing (WADA EQAS exercise), obtained from Thermo Trace Ultra, connected to TSQ Quantum XLS GC triple quadrupole MS and HP 6890 quadrupole MS coupled to a HP 5973 GC were compared based on z-scores. The calculation of the z-values is based on the consensus value and a target relative standard deviation (TSD) of 20% for each of the steroids, except for Adiol and Bdiol (TSD 25%) and the T/E ratio (TSD 10%). The target relative standard deviations have been set in such a way that an absolute z-score 3 or greater are interpreted as an unsatisfactory performance and a value of between 2 and 3 as questionable.

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Results and Discussion

Marginal differences in concentrations for the six target steroids were detected. No questionable or unsatisfactory result was calculated and most values are situated within z-score area +/-1 (Figure 1).

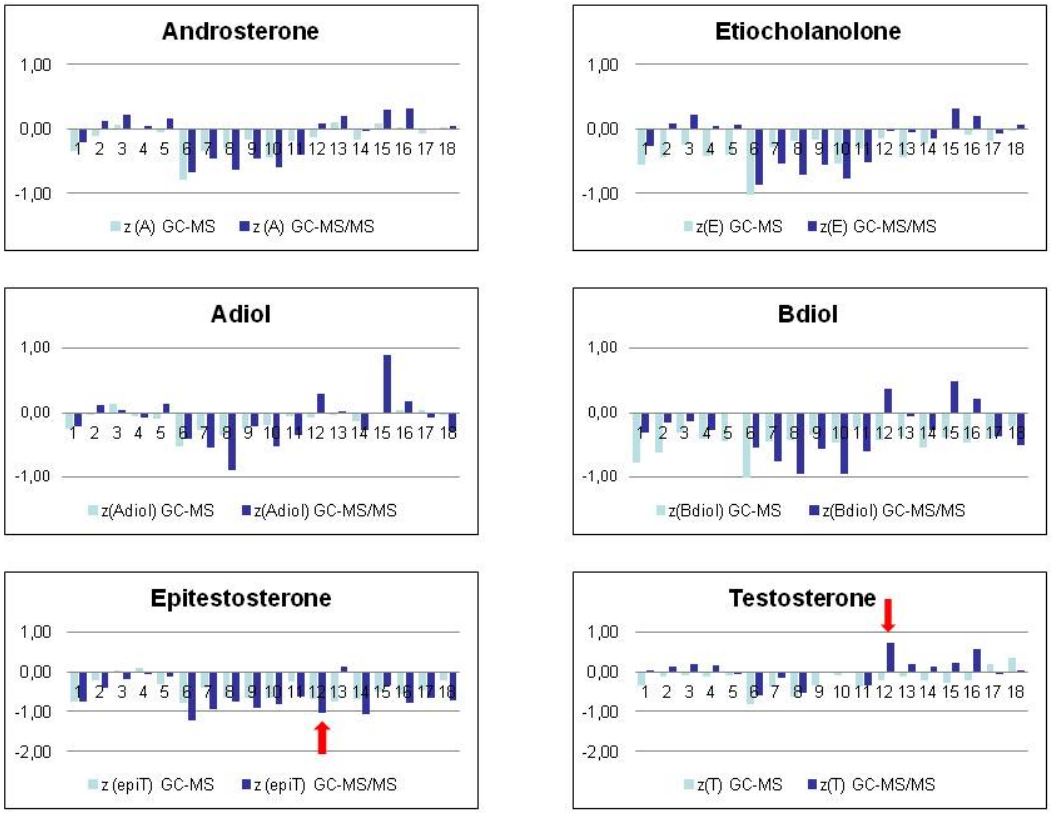

Figure 1: Comparison of z-scores from Athlete Biological Passport steroids, based on GC-MS and GC-MS/MS analysis

The evaluation of the T/E ratios showed three questionable and one unsatisfactory GC-MS/MS results (Figure 2). In specimen 12, the concentrations for T and E are situated within the acceptable range, whereas the insufficient z-score result of the T/E ratio is caused by the combination of a lower concentration for E and an elevated concentration of T.
Most important is the examination of the target relative standard deviation (TSD) for T/E, which is currently fixed at 10%. An extention up to 20% (which is in line with most of the endogenous steroids) would lead to an elimination of questionable/unsatisfactory results of the presented data set.
As depicted in Figure 2, an extended variation for T/E is shown between the different technologies, mainly caused by the high specificity of GC-MS/MS (Figure 3). Further possible reasons for different T/E results are the concentration-based calculation of the T/E ratio and a higher influence of matrix issues on GC-MS/MS.
For an accurate determination of the steroid profile the availability of suitable reference material is essential. The currently available freeze-dried human urine is certified for T, E and T/E. The characterization of the remaining steroids is still pending.

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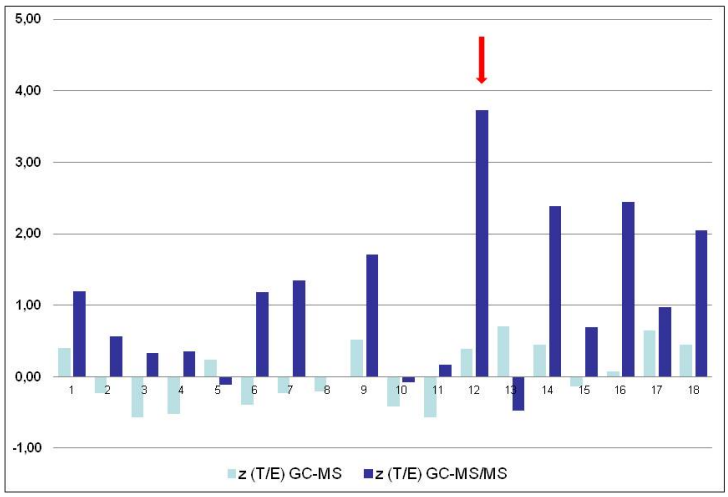

Figure 2: Comparison of z-scores (T/E), based on GC-MS and GC-MS/MS analysis

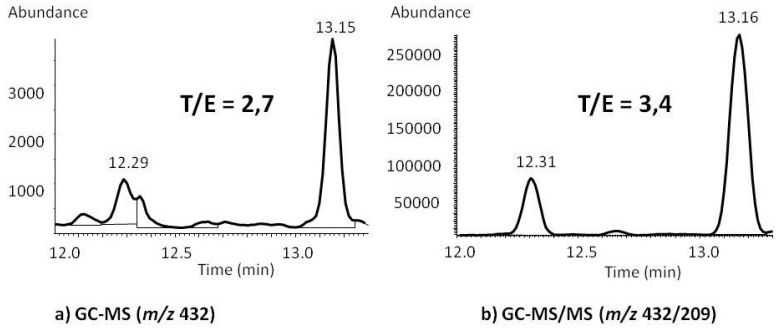

Figure 3: Specificity - Screening chromatograms monitoring T/E

Conclusions

- Due to the high specificity, GC tandem MS technology is an improvement for steroid profiling, especially for urine specimens having low epitestosterone concentrations and coeluting substances.
- The target standard deviation for T/E should be changed to 20%.
- Aspects potentially influencing analytical results of GC-MS and GC-MS/MS measurement necessitate further investigation.

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