Developing the GC-MS method to quantify Testosterone/Epitestosterone ratio in female urine

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Introduction

Quantifying Testosterone/Epitestosterone ratio (T: E) in urine allows drugs to be detected in the sporting world.
T and E are present at low concentrations in female urine. To achieve a good degree of sensitivity and specificity on a GC-MS analysis, urine samples require a multistep procedure. The protocol consists in extracting and concentrating molecules. The main steps are hydrolysis, extraction and derivatisation. Methyltestosterone is added at the beginning of the extraction protocol and used as an internal standard (I.S) in order to correct possible variations or losses.

Finally, the T: E ratio is obtained measuring peak areas of T, E and I.S.

Experimental Conditions

The GC-MS analysis of urine samples was carried out on a Hewlett Packard 6890 gas chromatograph directly coupled to a mass spectrometer: a Hewlett Packard 5973. The selected ion monitoring (SIM) mode was chosen as the acquisition mode. The GC column was an HP1-MS, 100% dimethylpolysiloxane column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25µm.

The oven temperature was : 150°C (1min), 20°C/min → 220°C (18min), 30°C/min → 250°C (5min),10°C/min → 320°C (5min).

The carrier gas was helium at a constant flow rate of 0.8 ml/min. 1µl of the sample was injected in the split less mode. The electron energy was set at 70 eV and the ion source temperature at 280°C.

T and E were identified according to three m/z ratios (mass to charge ratio for the detected ions): 417, 432 and 433. These steroids were quantified thanks to 417 m/z since it was the most intense. For I.S, the ions used for identification were 301, 446 and 447. Ion 301 was also used for quantification.

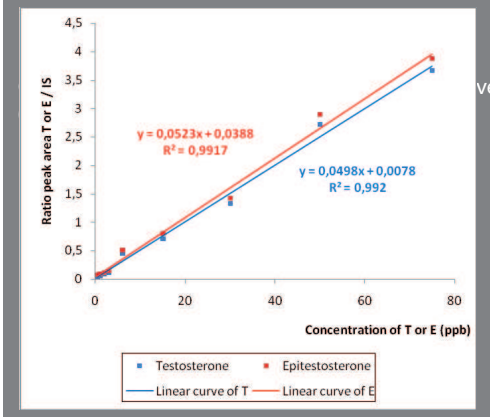
Results

The retention times were 24.83 minutes for E, 26.04 minutes for T and 28.659 for I.S. Two peripheral peaks were present around T at 25.90 and 26.23 minutes. Even if the resolution between each interference and T was equal to 0.5, three interest species were quantified.

Indeed the linearity was verified in the range of concentrations going from 0.5 to 75 ppb since the regression coefficients of internal calibration curves were 0.99 for T and E (Fig1).

The specificity, repeatability and the detection and quantification limits were controlled and validated in the same range of concentrations.

Fig.1: Internal cali



Conclusion

This GC-MS method definitely allowed Testosterone and Epitestosterone to be identified and quantified in the female urine sample thanks to particular retention time, and mass spectrometry.
The detection limit is roughly 3 ppb and quantification limit is around 10 ppb.
All these parameters were developed for the urine sample, therefore, in order to validate this quantitative method, it ought to be tested on white urine.

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