Preliminary investigations of diuretic screening method converted from HPLC/MS to UPLC/MS

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AIM: The conversion of a qualitative diuretic screening method used in sports doping analysis from HPLC/MS (total run time of 10 min) to Ultra Performance Liquid Chromatography (UPLC)/MS, where the analysis time is reduced without any loss of sensitivity and selectivity.

METHODS: The investigations were performed on nine diuretics plus one internal standard, these compounds were taken from the WADA prohibited list for 2005. Human urine samples (500µL) were prepared by a simple and rapid solid-phase extraction (SPE) procedure using Oasis HLB cartridge (1cc, 30mg); this preparation also incorporated a three-fold concentration step. Chromatography was performed using a Waters Acquity UPLC system. Analytes were separated on an Acquity UPLC BEH C18 (2.1 × 100mm, 1.7mm) using an gradient elution with 0.1% ammonium hydroxide in water (A) and 0.1% ammonium hydroxide in acetonitrile (B). The gradient started from 95% A to 10% A over 2 min before returning to initial conditions for 1 min, which resulted in a total run time of 3 min. An injection volume of 10 µL was used. A Quattro micro tandem mass spectrometer with a Z-spray ion source was used for analysis; electrospray was performed in negative ionisation mode (ESI-). The MRM conditions were optimised for each compound to achieve maximum sensitivity, while the collision gas was maintained constant at 2.0 × 10-3 mbar for all MRM analyses.

RESULTS: A limited validation was undertaken. For all compounds, responses were linear over the investigated range (50–500ng/mL). Intra-assay precision was satisfactory with CV's for spiked QC samples < 20%. The use of the SPE procedure was demonstrated to be very efficient and gave reproducible extraction recoveries i.e. > 78% for all analytes. Limits of quantitation were estimated to be 50 ng/mL with limits of detection ranging from 0.1 to 25 ng/mL, which meets the minimum required performance limits, as specified by WADA, of 250 ng/mL for the detection of diuretics compounds within athlete urine specimens. The method was applied to the analysis of blind spiked samples (n = 6) provided by the WADA accredited laboratory, HFL.

CONCLUSION: Preliminary investigations into the developed UPLC/MS for the screening of diuretics (masking agents) have demonstrated the significant time benefit that can be achieved with UPLC, with at least a three fold decrease in the total run time when compared to more traditional HPLC methods. This method provides a rapid, robust and sensitive solution for high-throughput sports doping analysis, particularly for the screening for prohibited diuretics. The successful application of this method to the analysis of blind samples demonstrates the feasibility of UPLC/MS for use in a WADA accredited laboratory. Following the introduction of tandem mass spectrometers which utilise fast scanning T-wave technology, there is potential to reduce the analysis time further and screen for more diuretic compounds.

KEYWORDS: *Diuretics, UPLC/MS; Qualitative, Sports doping, Screening*

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