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Fast screening by UHPLC-HRMS in high-throughput doping control

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Abstract

Background

Anti-doping Laboratories invest a significant effort trying to maximise productivity by reducing the number of screening methods required to cover the WADA Prohibited List [1]. For this purpose, ultra-high pressure liquid chromatography (UHPLC) coupled to high resolution mass spectrometry (HRMS) is becoming more and more popular, since full data capture allows for retrospective analysis and represents a very flexible approach when new substances must be incorporated in the screening method. Nowadays benchtop HRMS instruments offering polarity switching capability are available and researchers are investigating the benefits of this technique [2,3].

Methods

An Exactive (Thermo Fisher) high-resolution mass spectrometer coupled to an Acquity UHPLC pump (Waters) was used. Fast gradient chromatography (10 min cycle time) was performed on a Waters Acquity BEH C18 column (5.0×2.1 mm, 1.7 µm) to analyse samples after direct β-glucuronidase hydrolysis of urine followed by solid-phase extraction with mixed mode polymeric-cation exchange cartridges (Agilent Plexa PCX). Data were acquired in full scan in positive and negative ionisation modes, in combination with CID fragmentation.

Results and Discussion

We developed a comprehensive screening method based on UHPLC-HRMS for the detection of around 200 analytes across the WADA Prohibited List classes of substances. Full data capture at 25,000 resolution (FWHM) was performed and extracted ion chromatograms were automatically generated using ±5 ppm m/z and ±0.5 min retention time windows to obtain data outputs that are easy to review. CID fragmentation was used to increase selectivity for specific analytes, reducing the number of false suspects to a minimum. Fast polarity switching permitted the analysis of a wide range of analytes with very different chemistries within a single chromatographic run. The solid-phase extraction procedure developed allowed extraction of all analytes and sample pre-concentration to achieve the required sensitivity to comply with the Minimum Required Performance Levels (MRPL) set by WADA (TD2010MRPL)[4].

Conclusions

The UHPLC-HRMS screening method presented enables detection of a large number of analytes and was used successfully at the London 2012 Olympic and Paralympic Games for the analysis of over 5,000 samples. More recently, the method has been transferred to a Q-Exactive HRMS instrument that shows better sensitivity and allows for the detection of the analytes at the recently enforced 2013 MRPL (TD2013MRPL)[5].

This work has now been published [6].

References

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