

Accepted Manuscript

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PII: S1570-0232(13)00546-1
DOI: <http://dx.doi.org/doi:10.1016/j.jchromb.2013.10.008>
Reference: CHROMB 18575

To appear in: *Journal of Chromatography B*

Received date: 18-6-2013
Revised date: 12-9-2013
Accepted date: 8-10-2013

Please cite this article as: M.K. Kioussi, E.M. Lyris, Y.S. Angelis, M. Tsivou, M.A. Koupparis, C.G. Georgakopoulos, A generic screening methodology for horse doping control by LC-TOF-MS, GC-HRMS and GC-MS, *Journal of Chromatography B* (2013), <http://dx.doi.org/10.1016/j.jchromb.2013.10.008>

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A generic screening methodology for horse doping control by LC-TOF-MS, GC-HRMS and GC-MS

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ABSTRACT

In the present study a general screening protocol was developed to detect prohibited substances and metabolites for doping control purposes in equine sports. It was based on the establishment of a unified sample preparation and on the combined implementation of liquid and gas chromatographic MS analysis. The sample pretreatment began with two parallel procedures: enzymatic hydrolysis of sulfate and glucuronide conjugates, and methanolysis of the 17 β -sulfate steroid conjugates. The extracts were treated for LC-TOF-MS, GC-HRMS and GC-MS assays. The majority of the prohibited substances were identified through a high mass accuracy technique, such as LC-TOF-MS, without prior derivatization. The sample preparation procedure included the formation of methylated and trimethylsilylated derivatives common in toxicological GC-MS libraries. The screening method was enhanced by post-run library searching using Automated Mass spectral Deconvolution and Identification System (AMDIS) combined with Deconvolution Reporting Software (DRS). The current methodology is able to detect the presence of more than 350 target analytes in horse urine and may easily incorporate a lot of new substances without changes in chromatography. The full scan acquisition allows retrospective identification of prohibited substances in stored urine samples after reprocessing of the acquired data. Validation

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