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SPE-LC-TOFMS screening method for detection of doping agents in urine

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Screening analysis within doping control is most challenging, because it should be performed in a short period of time, it should cover a vast range of different types of doping agents, the results should be reliable and the report should be legible and unambiguous. An ideal screening method should be able to detect in one run a wide range of acidic, basic and neutral compounds in a urine sample. Especially the detection of hydrophilic compounds is essential, because many of the doping agents are excreted in urine as hydrophilic metabolites. For some compounds, these metabolites are the only way to detect doping.
We developed and validated a general screening method based on solid phase extraction (SPE) and liquid chromatography-time-of-flight mass spectrometry (LC-TOFMS) for 111 different doping agents, including stimulants, ß-blockers, narcotics, ß2-adrenergic agonists, agents with anti-estrogenic activity and diuretics.

Special attention was paid on SPE, because good recoveries were required for hydrophilic compounds. We compared mixed mode cation exchange-reversed phase SPE sorbents, using different non-polar chemistries (C8, C18 and C4) with several washing solvents, and obtained the best results with C8. The final extraction was performed according to IST application note IST 1066 S (International Sorbent Technology). In spite of careful optimization, some of the analytes eluted from the SPE column already in the last methanol wash, and consequently the validation was done from the methanol fraction for these compounds.

Identification by LC-TOFMS was grounded on retention time, accurate mass and isotopic pattern fit. The mass range was set to m/z 50-800. The mass errors were on average below 5 ppm. The mass tolerance window used was 8 ppm. The recoveries were generally between 40-90%. The minimum required performance limits established by World Anti Doping Agency (WADA) were applied to the detection of the analytes.

Being one of the first reports on LC-TOFMS in doping, the present study demonstrates the technique's merits in terms of flexibility and sensitivity in screening. By using accurate mass and isotopic pattern as a basis of identification, preliminary identification is possible even for compounds for which a reference standard (retention time) is not available.

KEYWORDS: *Doping, LC-TOFMS, Screening*

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