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Ultra high performance supercritical fluid chromatography coupled with tandem mass spectrometry for screening of doping agents. II: Analysis of biological samples

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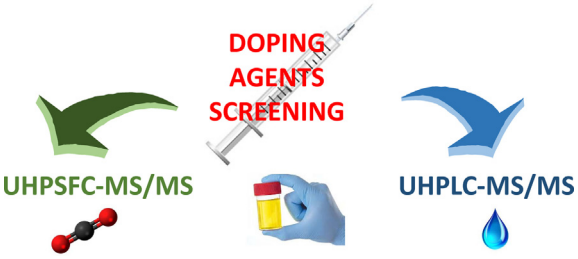
HIGHLIGHTS

- 110 doping agents were tested in UHPLC–MS/MS and UHPSFC–MS/MS.
- Urine matrix was successfully analyzed in UHPSFC–MS/MS.
- Higher sensitivity was achieved in UHPSFC–MS/MS for 32% of the compounds.
- UHPSFC–MS/MS was less susceptible to matrix effects than UHPLC–MS/MS.
- UHPSFC–MS/MS can be considered for the screening of doping agents, as an alternative to UHPLC–MS/MS.

GRAPHICAL ABSTRACT



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ABSTRACT

The potential and applicability of UHPSFC–MS/MS for anti-doping screening in urine samples were tested for the first time. For this purpose, a group of 110 doping agents with diverse physicochemical properties was analyzed using two separation techniques, namely UHPLC–MS/MS and UHPSFC–MS/MS in both ESI+ and ESI− modes. The two approaches were compared in terms of selectivity, sensitivity, linearity and matrix effects. As expected, very diverse retentions and selectivities were obtained in UHPLC and UHPSFC, proving a good complementarity of these analytical strategies. In both conditions, acceptable peak shapes and MS detection capabilities were obtained within 7 min analysis time, enabling the application of these two methods for screening purposes. Method sensitivity was found comparable for 46% of tested compounds, while higher sensitivity was observed for 21% of tested compounds in UHPLC–MS/MS and for 32% in UHPSFC–MS/MS. The latter demonstrated a lower susceptibility to matrix effects, which were mostly observed as signal suppression. In the case of UHPLC–MS/MS, more serious matrix effects were observed, leading typically to signal enhancement and the matrix effect was also concentration dependent, i.e., more significant matrix effects occurred at the lowest concentrations.

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1. Introduction

The global fight against doping in sport is governed since 1999 by the World Anti-Doping Agency (WADA). Among its prerogatives, WADA was responsible for the implementation and acceptance of the World Anti-Doping Code, a set of harmonized rules and regulations within sport and anti-doping organizations. In conjunction to the Code, five international standards, describe in detail all the aspects and procedures related to anti-doping analysis including the List of Prohibited Substances and Methods [1]. This list is annually updated and currently contains over 250 compounds that are forbidden in sport and must be monitored by anti-doping laboratories. The great structural diversity and wide range of physicochemical properties of these substances represent a challenging task from an analytical point of view. In this context, the analytical approach for anti-doping analyses must exhibit both high selectivity and sensitivity in complex matrices such as blood or urine and consists in two steps. A screening is initially performed to determine the presence or absence of a doping agent in the biological sample. This step must be fast, selective and sensitive, to limit as much as possible the risk of false-negative and false-positive results. In the case of a suspicious result, the latter must be confirmed in a second instance through a targeted analysis on the potentially incriminated substance, including possible metabolite(s) and/or biomarker(s). For qualitative assays, this second step should provide sufficient identification capabilities accuracy and precision to confirm the presence of the prohibited substance in the suspicious sample. Detection sensitivity performance of these two steps is strictly defined by the WADA, which imposes minimal required performance levels (MRPL) that all laboratories should be able to attain [2].

During screening and/or confirmation analyses, chromatographic methods (LC and GC) coupled to mass spectrometry (MS) are the methods of choice [3–7] as they can meet all the analytical requirements in terms of speed, selectivity and sensitivity. For the initial screening of doping agents, urine samples have to be analysed [3–7]. To ensure high throughput approach, a simple “dilute and shoot” procedure is commonly employed as it also permits to decrease the analysis cost and minimize possible errors occurring during sample preparation step. However, this simple approach is prone to matrix effects due to the presence of high salt concentrations in urine samples. Supercritical fluid chromatography (SFC) have not yet been employed in anti-doping screening, despite its convenient properties, such as speed of analysis, high separation efficiency and environmental friendliness [8–11]. Successful coupling of SFC with MS has already been described in the scientific literature [12–14] and also more recently with modern SFC–MS platforms [15]. However, the use of SFC–MS for the analysis of biological materials has been scarcely reported [16–19] even though the compatibility of SFC–MS with sample preparation techniques using organic solvents for the extraction is evident. The reason was probably the insufficient quantitative performance of the old SFC–MS platforms, which did not meet demanding criteria of method validation for biological samples [20]. Moreover, considering the dilution of urine with water, such a strategy is well compatible with RPLC mode, but the injection of highly aqueous sample could be critical in SFC, as reported elsewhere [20–22].

The aim of this study was to examine the applicability of UHPSFC–MS/MS for the screening of doping agents in urine samples and to compare its performance with current state-of-the-art UHPLC–MS/MS approach. Both methods were applied for the analysis of 110 doping agents to assess method sensitivity, linearity and matrix effects.

2. Experimental

2.1. Chemicals and reagents

Reference standards of doping agents including 6-acetylmorphine, 6-hydroxybromantan, alfentanil hydrochloride, amfepramone hydrochloride, amiloride hydrochloride monohydrate, aminoglutethimide, amiphenazole, amphetamine, androstatriendione (ATD), bambuterol hydrochloride, benzoylecgonine, benzphetamine, benzthiazide, benzylpiperazine, buprenorphine hydrochloride, canrenone, carphedon, cathine hydrochloride, cathinone hydrochloride, chlorphentermine hydrochloride, clobenzorex, cocaine, codeine, cropropamide, dextromoramide tartrate, ephedrine, eplerone, etilefrine, ethamivan, famprofazone, fenbutrazate hydrochloride, fencamfamine, fencamine, fenetyline hydrochloride, fenfluramine, fenproporex, fenoterol, fentanyl, formoterol fumarate dihydrate, furfenorex cyclohexyl sulfam, heptaminol, hydrocodone, hydromorphone, hydroxymesocarb, isometheptene mucate, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-methamphetamine (MDMA), mefenorex, mefentanyl hydrochloride, mephedrone, mephentermine hemisulfate, mesocarb, methamphetamine hydrochloride, methcathinone, methedrone hydrochloride, methoxyphenamine hydrochloride, methylecgonine, methylephedrine, methylphenidate, morphine monohydrate, 4-methylthioamphetamine (MTA), *N*-ethylnicotinamide, nikethamide, norfenfluramine hydrochloride, norfentanyl hydrochloride, octopamine, ortetamine, oxilorfine hydrochloride, oxycodone, oxymorphone, *p*-hydroxyamphetamine, pemoline, pentetrazole, pethidine hydrochloride, phendimetrazine ditartrate, phenmetrazine hydrochloride, phenpromethamine, phentermine hydrochloride, phenylephrine hydrochloride, pipradrol, PMA (*p*-methoxyamphetamine) hydrochloride, piretanide, prenylamine, prolintane, propylhexedrine hydrochloride, pseudoephedrine, salmeterol, selegiline, sibutramine, strychnine, sufentanyl, synephrine, terbutaline hemisulfate, torasemide, triamterene, tuaminoheptane were kindly provided by the Swiss Anti-Doping Laboratory (Epalinges, Switzerland). Acetazolamide, bendroflumethiazide, bumetanide, chlortalidone, chlorthiazide, clopamide, hydrochlorthiazide, ethacrynic acid, furosemide, indapamide, methylclothiazide, metolazone, probenecid and xipamide were purchased from Sigma–Aldrich (Buchs, Switzerland).

Methanol (MeOH), ethanol (EtOH), isopropanol (IpOH), acetonitrile (ACN) and formic acid (FA) of ULC/MS grade were provided by Biosolve (Chemie Brunschwig, Basel, Switzerland). Ammonium formate and tetrahydrofuran (THF) were provided by Sigma–Fluka (Buchs, Switzerland). Pressurized gas CO_2 4.8 (>99.998%) was purchased from Carbagas (Gümlingen, Switzerland). Ultra-pure water was provided by a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Standard solutions

The stock solutions of doping agents were prepared in methanol at a concentration of 1 mg mL^{-1}. These solutions were further diluted with pure water and a mixture of water–ACN 25:75 (v/v) for UHPLC–MS/MS and UHPSFC–MS/MS, respectively, to reach a concentration of 1 μg mL^{-1}. For sensitivity and linearity tests, these standard solutions were further serially diluted until achieving response with S/N ~10 to obtain limit of quantitation (LOQ) and S/N ~3 to obtain limit of detection (LOD). Method linearity was evaluated with standard solutions in the range 0.001 ng mL^{-1} to 1 μg mL^{-1} on the Waters Xevo TQ-S detector.