UHPSFC-MS/MS AS A VIABLE OPTION IN DOPING CONTROL ANALYSIS

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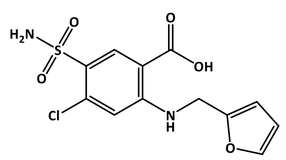
INTRODUCTION AND THE AIM OF THE WORK

A two-steps approach is generally implemented in **doping control analysis**, including generic screening and selective confirmation steps, if applicable. High throughput methods as well as sufficient sensitivity and specificity are needed to avoid false positive/negative results and to enable the control of a large range of structurally diverse substances. During both screening and/or confirmation analyses, chromatographic approaches (LC and GC) coupled to mass spectrometry (MS) are the methods of choice as they can meet all the analytical requirements in terms of speed, selectivity and sensitivity. Supercritical fluid chromatography (SFC) has recently become very popular due to the properties of the supercritical fluids and the possibility to use high flow-rates leading to decrease in analysis time and high separation efficiency, especially when using sub-2-µm particles, known as ultra-high performance supercritical fluid chromatography (UHPSFC). The information concerning the use of SFC in doping control is still very limited.

The **aim of this work** was to examine the potential and applicability of UHPSFC-MS/MS for doping screening in urine samples. Two different multi-analyte approaches were developed for two groups of diverse compounds, including **relatively polar substances** (such as diuretics, stimulants and narcotics) in a first instance and secondly, **more challenging categories** including **anabolic agents**, hormones, metabolic modulators, synthetic cannabinoids and glucocorticoids.

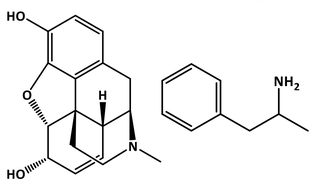
EXPERIMENTAL DESIGN

METHOD 1:



110 compounds

STATIONARY PHASES:
Acquity UPC^2 BEH, BEH 2-EP
100 x 3.0 mm, 1.7 µm
column temperature: 40 ^oC

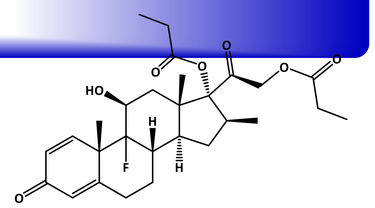


MOBILE PHASE:
CO_2 + methanol + additive
gradient elution: 2—40 % OM, 4 min + 3 min
flow-rate: 1.5 ml/min

BACK PRESSURE REGULATOR: 120 bar
MAKE-UP: EtOH, 0.3 ml/min

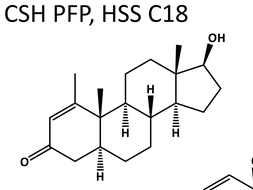
SAMPLE PREPARATION:
dilute and shoot with 75 % ACN
10 x dilution

METHOD 2:



100 compounds

STATIONARY PHASES:
Acquity UPC^2 BEH, BEH 2-EP, CSH PFP, HSS C18
Torus 2-PIC, 1-AA, diol, DEA
100 x 3.0 mm, 1.7 or 1.8 µm
column temperature: 40 ^oC



MOBILE PHASE:
CO_2 + methanol + additive
gradient elution: 2—40 % OM, 4 min + 4 min
flow-rate: 1.3 ml/min

BACK PRESSURE REGULATOR: 150 bar
MAKE-UP: MeOH, 0.3 ml/min
SAMPLE PREPARATION:
SLE extraction with MTBE
5 x preconcentration

UHPSFC-MS/MS METHOD 1

Fig. 1: Distribution of individual 110 analytes under UHPSFC-MS/MS and UHPLC-MS/MS conditions.
Both UHPLC-MS/MS and UHPSFC-MS/MS methods were optimized in terms of mobile phase composition, ESI source conditions and make-up solvent for UHPSFC-MS/MS. As a mobile phase, 0.1 % formic acid in water/ACN and CO_2/MeOH based mobile phase with the addition of 10 mM ammonium formate + 2 % water were found to be the most generic conditions for UHPLC-MS/MS, and UHPSFC-MS/MS, respectively.

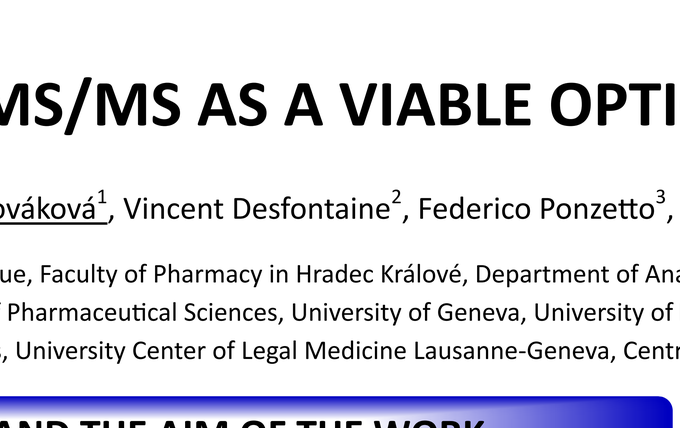
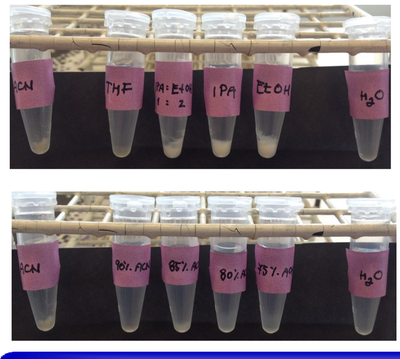


Fig. 2: A choice of dilution solvent for urine.



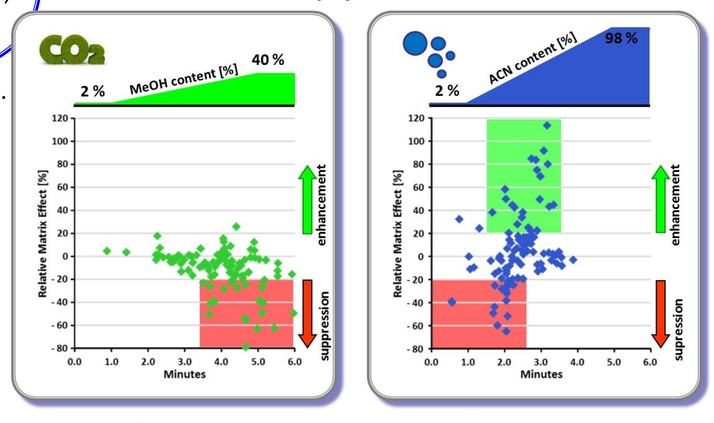


Fig. 3: Matrix effects evaluation, comparison of UHPSFC and UHPLC.

Fig. 4: A choice of SFC stationary phase for the analysis of anabolic steroids.
8 different stationary phases were tested using CO_2/MeOH + 20 mM ammonium formate. The best results in terms of retention, selectivity and peak shape were obtained on Torus diol column.

SLE protocol:
loading 1 ml urine
elution 3 ml MTBE
evaporation
reconstitution in 50 % ACN
agitation 5 min

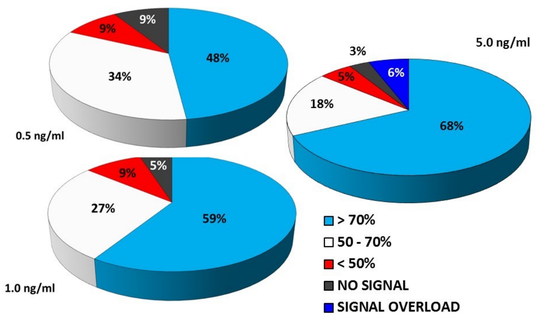


Fig. 5: Recovery of SLE procedure.

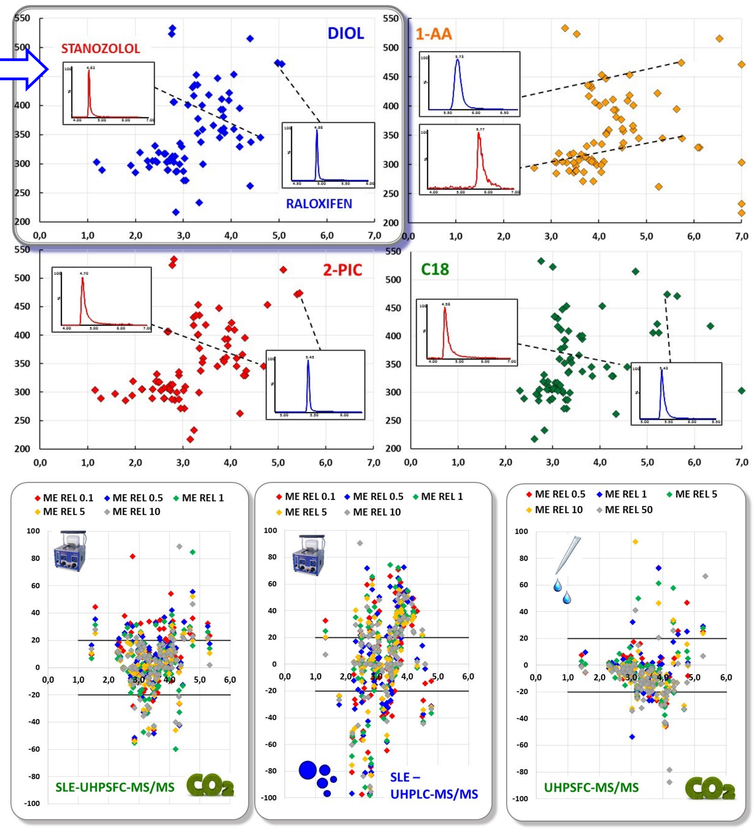


Fig. 6: Matrix effects —comparison of UHPLC and UHPSFC, SLE and DS.

CONCLUSIONS

Both UHPSFC-MS/MS methods were finely optimized and subsequently they were applied for the analysis of urine samples. Furthermore, they were compared to the current state-of-the art methods, especially UHPLC-MS/MS. 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 very short analysis time (up to 8 minutes), enabling the application of these two methods for screening purposes as an alternative technique to LC-MS/MS or GC-MS/MS.

Requested MRPLs (minimum required performance limits) were reached for 207 compounds out of 210 using UHPSFC-MS/MS. The method sensitivity was dependent on experimental conditions (mobile phase, make-up solvent) and mass analyser. Nevertheless, it was adequate for screening purposes using simple dilute and shoot approach (method 1) or SLE in case of more challenging analytes (method 2). The difference in sensitivity between UHPLC-MS/MS and UHPSFC-MS/MS was analyte dependent and also mass analyser dependent. UHPSFC-MS/MS was influenced by matrix effects to a lesser extent compared to UHPLC-MS/MS.

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