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Combined solid phase extraction procedure for extensive doping screening

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Introduction

The list of prohibited substances by World Anti-Doping Agency (WADA) includes hundreds of compounds with variable chemical properties [1]. Doping control laboratories have to be able to screen and identify these substances from a limited volume of biological sample at a limited time-frame. Therefore, the selection of an appropriate sample preparation method is important and mainly specifies the quantity and amount of target compounds. Extensive sample preparation procedures have been applied in fields of toxicology [2], environmental and food [3], as well as horse [4,5] and human doping control analysis [6]. The aim of this study was to develop a single sample preparation method for doping agents in a 1-mL aliquot of urine sample regardless of their chemical properties. The compounds were extracted with two mixed-mode solid phase extraction (SPE) columns with C8 and either strong cation or anion exchange mechanisms.

Experimental

Isolute® HCX and HAX (130 mg) columns by Biotage (Uppsala, Sweden) were chosen and optimization of the method was based on previous study (HCX) [7] and Technical note 127 (HAX) [8]. Combined SPE procedure is presented in Fig.1. An aliquot (1 mL) of spiked human urine sample was first hydrolyzed with β-glucuronidase. At the first phase (SPE I), basic and neutral compounds were collected while acidic compounds were eluted in washes to

the second phase (SPE II). Elution fractions from both columns were combined as one sample for analysis. The extraction recoveries were determined in urine as four replicates spiking analytes before and after extraction. Samples were analyzed with liquid chromatographic/time-of-flight mass spectrometric (LC/TOFMS) technique using electrospray ionization (ESI) in positive and negative mode. The applicability of the procedure was demonstrated with eleven different doping agents with different chemical properties covering seven prohibited substance classes as listed in Table 1. Selected compounds were spiked at minimum required performance limit (MRPL), with exception of phenylephrine and clenbuterol which were spiked at ten times MRPL level.

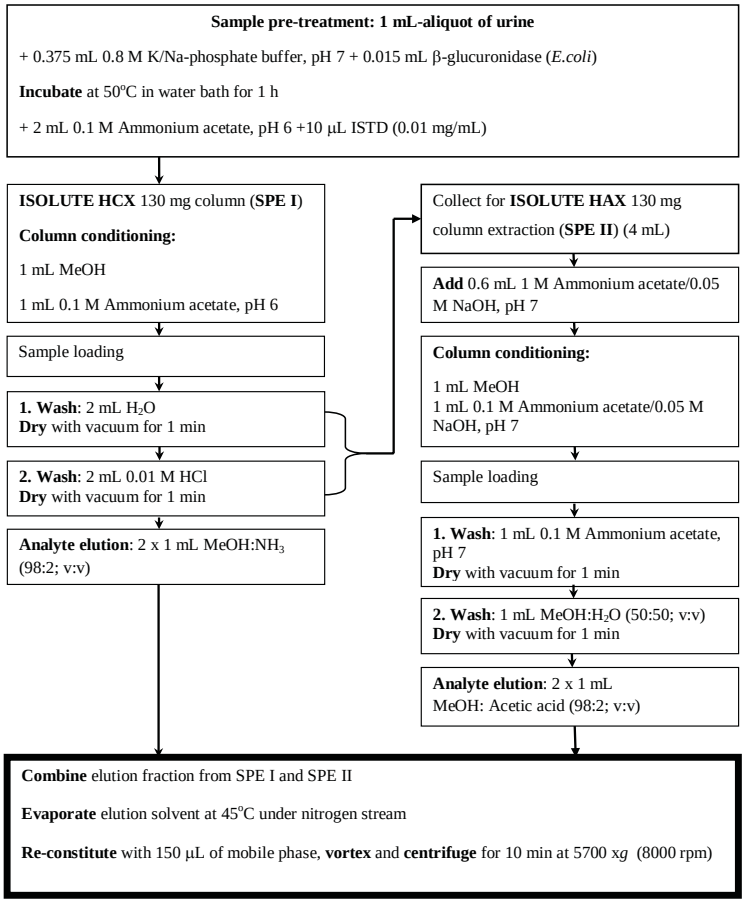
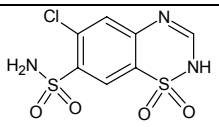
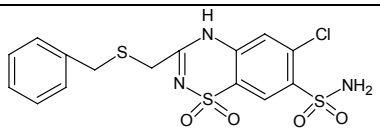
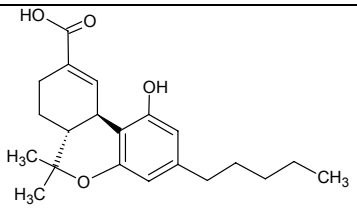
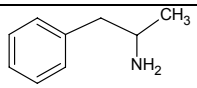
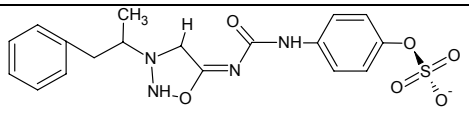
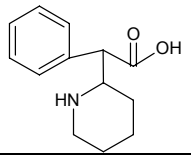
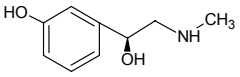
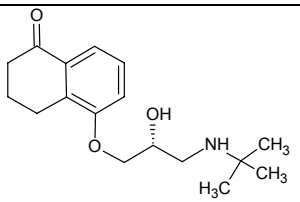
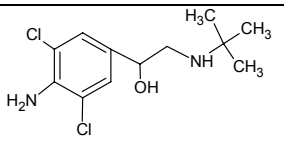
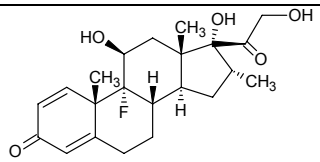
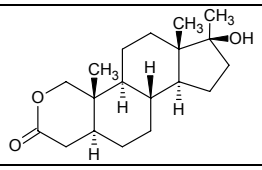


Figure 1. Combined solid phase extraction (SPE) diagram for screening of doping agents.

Results and Discussion

The results are summarized in Table 1. Combined extraction procedure was applicable to different types of doping agents with relative (to ISTD, dibenzepin) extraction recoveries over 50% for the majority of the compounds.

Table 1. Results of the combined extraction method for the studied compounds in urine.

Compound	**Structure**	**ESI Polarity**	**Concentration µg/mL**	**S/N**	**Extraction recovery%**
Chlorthiazide		-	0.25	142	10
Benzthiazide		-	0.25	664	104
THC-COOH		-	0.015	422	37
Amphetamine		+	0.50	27	95
p-OH-mesocarb-sulphate		+	0.50	134	90
Ritalinic acid		+	0.50	194	86
Phenylephrine		+	5.0	34	52
Bunolol		+	0.50	466	75
Clenbuterol		+	0.020	196	69
Dexamethasone		+	0.030	92	77
Oxandrolone		+	0.010	192	100

All of the studied compounds could be detected specifically and no interfering compounds were detected in blank urine samples at the corresponding retention times of the analytes (Figure 2). Signal to noise ratios (S/N) were >27 for all studied compounds.

The presented sample pretreatment method combining two separate SPE steps of HCX and HAX enables extensive doping screening covering most of the compound classes listed in WADA´s code. All the materials required for the procedure were general laboratory regents and the total sample consumption was minimized to 1 mL. Simultaneous extraction of compounds with different chemical properties allowed the preparation of one single sample for LC-TOFMS analysis.

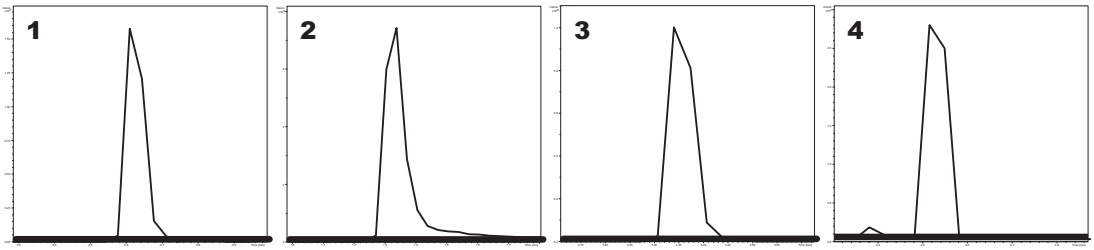


Figure 2. Examples of the extracted ion chromatograms (EIC) of the studied compounds and blank urine (bold line). Chromatographic separation was based on a gradient elution on a C18 rapid resolution column. 1= Benzthiazide $[M-H]^-$ m/z 429.97622, RT= 4.60 min, 2= Amphetamine $[M+H]^+$ m/z 136.11208, RT=1.34 min, 3= Bunolol $[M+H]^+$ m/z 292.19072, RT= 3.34 min, 4= Dexamethasone $[M+NH_4]^+$ m/z 409.22590 RT= 4.51 min.

Acknowledgement
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References

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