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Screening and confirmation analysis of stimulants, narcotics and beta-adrenergic agents in human urine by hydrophilic interaction liquid chromatography coupled to mass spectrometry

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ABSTRACT

The chromatographic behaviour of 44 polar compounds (23 beta-adrenergic agents, 11 stimulants, 4 narcotics and 6 phenolalkylamines) included in the list of prohibited substances and methods of the World Anti-Doping Agency, has been investigated under hydrophilic interaction liquid chromatography conditions by application of different mobile phase compositions (percentage of the organic solvent, type and amount of mobile phase additive and ionic strength) and column temperatures. Detection of analytes was performed by a triple quadrupole mass spectrometer in positive ionization mode and selected reaction monitoring acquisition mode after liquid/liquid extraction. Data collected using as stationary phase type-B silica materials from different producers, showed that the best chromatographic conditions in terms of peak shape, selectivity and chromatographic retention were obtained using an initial percentage of acetonitrile of 90%, a column temperature of 35 °C, a mobile phase pH of 4.5 and ammonium acetate (5 mM) and acetic acid (0.1%) as mobile phase additives. The selected chromatographic conditions were used to develop screening and confirmation analytical procedures to detect polar compounds in human urine for antidoping purpose. The developed methods were validated in terms of specificity, matrix effect, linearity, precision, accuracy, sensitivity, robustness and repeatability of retention times and relative ion abundances. Such methods offer attractive alternatives and considerable advantages over traditional approaches especially for the analysis of the phenolalkylamines.

1. Introduction

In the last years forensic and pharmaceutical laboratories have been particularly interested in the development of rapid procedures to cope with a large number of samples and to reduce the time required for results delivery. In the specific field of drug testing in sport, the main goal of the antidoping laboratories is to develop screening methods able to check for a number as wide as possible of xenobiotics included in the World Anti-Doping Agency (WADA) list of prohibited substances and methods [1]. For this purpose, in the last years reversed-phase liquid chromatography (RPLC) coupled to mass-spectrometry has been the most widely used technique in the development of almost "all-in-one" screening procedures [2–12] because of its versatility and ability to retain and resolve a large number of heterogeneous compounds. However the analysis of highly hydrophilic, ionic and polar compounds often requires a highly aqueous mobile phase to achieve column retention, which can cause (i) a decrease in sensitivity in electrospray ionization (ESI) mass spectrometry (MS), because high-aqueous content mobile phase is not conducive enough to allow sufficiently adequate ionization, and (ii) a decrease in stationary phase performance with a parallel reduction of its lifetime of operation. Normal phase liquid chromatography (NPLC) could be used as alternative to RPLC for the analysis of polar compounds, but its use is limited because: (i) hydrophilic compounds may not be well soluble in non polar solvent systems, (ii) the non-polar solvents employed are quite expensive, often toxic and environmentally unfriendly, and (iii) the ionization efficiency from non-polar solvents and consequently the sensitivity is diminished. In 1990, Alpert [13] proposed the hydrophilic interaction liquid chromatography (HILIC) technique. HILIC is characterized by an hydrophilic stationary phase and an aqueous–organic solvent mobile phase with a high organic-solvent content that has an increased solubility for the hydrophilic compounds and shows excellent compatibility with the mass spectrometry. Four mechanisms might control analyte retention in HILIC: (a) partitioning between a water-rich layer

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Table 1
Compounds pK_a and LC–MS/MS parameters.

Compounds	pK_a	SRM (m/z)	Collision energy (eV)
Beta-blockers			
Acebutolol	9.4	338/56; 338/72; 338/98; 338/145	40; 40; 35; 30
Alprenolol	9.5	250/56; 250/74; 250/98; 250/116	40; 35; 35; 30
Atenolol	9.6	267/56; 267/74; 267/98; 267/116	45; 45; 40; 35
Betaxolol	9.4	308/56; 308/74; 308/98; 308/116	40; 40; 35; 35
Bisoprolol	9.6	326/56; 326/74; 326/98; 326/116	40; 35; 30; 30
Carvedilol	9.6	407/100; 407/224; 407/283	40; 35; 30
Celiprolol	9.7	380/56; 380/74; 380/233; 380/251	40; 35; 30; 30
Esmolol	9.5	296/56; 296/74; 296/98; 296/116	40; 40; 35; 35
Metoprolol	9.6	268/56; 268/74; 268/98; 268/116	40; 40; 30; 30
Nadolol	9.4	310/57; 310/74; 310/123; 310/151	40; 35; 30; 30
Nebivolol	8.2	406/44; 406/123; 406/151	40; 35; 30
Oxprenolol	9.5	266/56; 266/72; 266/116; 266/255	35;35; 30; 20
Penbutolol	9.4	292/57; 292/74; 292/133; 292/236	40; 40; 35; 30
Pindolol	9.7	249/56; 249/74; 249/98; 249/116	40; 40; 35; 35
Propranolol	9.5	260/56; 260/74; 260/116; 260/155	40; 40; 35; 30
Sotalol	9.4	289/106; 289/133; 289/213	40; 35;30
Timolol	8.2, 9.4	317/57; 317/74; 317/188; 317/261	40; 35; 30; 20
Beta2-agonists			
Bambuterol	N.F.	368/72; 368/249; 368/294	35; 30; 30
Fenoterol	8.5, 10.0	304/107; 304/197	40; 35
Procaterol	N.F.	291/162; 291/231; 291/273	35; 30; 25
Salbutamol	9.3, 10.3	240/148; 240/166; 240/222	35; 30; 30
Salbutamol deuterated (ISTD)	9.3, 10.3	243/151	35
Salmeterol	N.F.	416/232; 416/380; 416/398	30; 25; 20
Terbutaline	8,7, 10.0, 11.0	226/107; 226/125; 226/152	35; 35; 30
Stimulants			
Amphetamine	10.1	136/65; 136/91; 136/119	50; 30; 25
Benzamphetamine	6.6	239/65; 239/91; 239/119	50; 30; 25
Cathine	9.4	152/65; 152/91; 152/119	50; 30; 25
Dimethylamphetamine	9.8	164/46; 164/65; 164/91; 164/119	50; 40; 30; 25
Ephedrine	9.6	166/65; 166/91; 166/119	50; 30; 25
Ephedrine deuterated (ISTD)	9.6	169/91	30
Fenethylline	9.2	342/91; 342/119; 342/181; 342/207	35; 30; 30; 25
MBDB	9.1	208/51; 208/77; 208/105; 208/135	65; 50; 30; 25
MDA	9.6	180/105; 180/133; 180/163	40; 40; 30; 25
MDEA	9.4	208/72; 208/105; 208/135; 208/163	65; 40; 30; 25
MDMA	9.0	194/58; 194/105; 194/135; 194/163	65; 35; 25; 20
Niketamide	3.5	179/72; 179/80; 179/108	55; 50; 25
Phenolalkylamines			
Etilefrine	9.0, 10.2	182/56; 182/65; 182/77; 182/91; 182/107; 182/134	35; 35; 35; 30; 30; 25
Nylidrin	N.F.	300/91; 300/150; 300/282	35; 30; 25
Norfenefrine	8.7	154/91; 154/107; 154/119	35; 30; 25
Octapamine	N.F.	154/91; 154/107; 154/119	35; 30; 25
Oxilofrine	N.F.	182/91; 182/107; 182/135	35; 30; 25
Pholedrine	9.4	166/79; 166/107; 166/151	40; 30; 25
Alfentanyl	6.5	417/197; 417/268; 417/385	35; 30; 25
Fentanyl	6.5	337/105; 337/188; 337/216	35; 30; 25
Methadone	8.3, 8.9	310/105; 310/159; 310/223; 310/265	45; 40; 30; 25
Pentazocine	8.5, 10.0	286/69; 286/159; 286/185; 286/218	45; 40; 30; 25

N.F., not found.
Underscored ion transitions were used for the recovery and quantitative validation.

on the surface of the stationary phase and the more hydrophobic mobile phase, (b) hydrogen bonding with the thin layer of water on the stationary phase, which depends on the acidity or basicity of the analytes and stationary phase, (c) electrostatic interaction with the negatively charged residual silanol groups in the stationary phase, which depends on the acidity or basicity of the analyte and on the mobile phase pH and (d) dipole–dipole interactions, which rely on the dipole moments and polarizabilities of molecules. The predominant retention mechanism can depend not only on the characteristic of the analytes under investigation but also on the selection of column temperature, mobile phase composition (pH, salt concentration and organic solvent selected) and stationary phase (bare-silica particle or chemically bonded silica particle) [14–20]. HILIC is primarily used for separating polar compounds or compounds that are not sufficiently retained under RP-HPLC, such as peptides [21,22], amino acids [13], oligonucleotides [23], polar compounds in natural products and drug substances [14,23–29]. Recent publications illustrated the influence of different factors such as stationary phase type [30,31], column temperature [32], type and amount of mobile phase modifier and ionic strength [16,20,23] on the chromatographic retention, selectivity and sensitivity of different classes of polar compounds. Several guidelines and experimental designs were suggested in order to develop and to optimize a HILIC method [16,18,33,34]. Starting from these suggestions, here we evaluated the potentiality of unmodified silica stationary phase from four different manufacturers as an