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A rapid method for the analysis of doping substances that are excreted mainly in non-conjugated form in urine.

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Abstract

A method was developed to reduce the analysis time of diuretics and other doping agents that are excreted mainly as parent compound and/or as their metabolites in non-conjugated form. The main principle is the use of the Thermo Scientific TurboFlow technology to perform online sample preparation. The use of the mass-spectrometer to conduct the analysis simultaneously in two modes, positive and negative, gives additional time savings and the gain in number of analyzed substances.

Introduction

The increasing number of substances banned by WADA requires the development of rapid detection methods. Usually, sample preparation consumes the significant part of analysis time. It can be shortened using appropriate equipment. The direct urine injection used in some methods [1-3] requires sensitive mass spectrometer instruments to reach the MRPL. The online sample preparation allows us to concentrate substances during the analysis [4-6]. In our laboratory, polar substances such as diuretics that are excreted mainly without polar tail (glucuronide, sulphate) were prepared by solid phase extraction (SPE). We then used the direct injection of urine and replaced the SPE step by the online SPE. The Thermo Scientific Turboflow technology uses two columns. One column is used for the separation of salts, very polar substances, and substances with big molecular weight. The second column is used for the chromatographic separation. The mass spectrometer that collects data simultaneously in two modes, positive and negative, allows us to significantly shorten the chromatographic time.

Experimental

Sample preparation:
The preparation consists of only the addition of the internal standard at MRPL concentration to the autosampler vial with 1 mL urine and centrifugation.

Instrumentation:
MS: Thermo Scientific*TSQ Vantage*
Pumping system: Transcend, two 600 Pumps, Valve Interface Module (VIM)
Precolumn: TurboFlow HTLC C8 XL 1.0x50 mm, Thermo Scientific
Column: Hypersil GOLD, 2.1×50 mm, 1.9 µm, Thermo Scientific
Injector: HTS PAL System. Injection volume: 100 µL.
MS parameters: Ion source: HESI; Vaporizer temperature: 400°C; Nebulizer gas: 50 au; Capillary temperature: 300°C; Ion sweep gas pressure: 1.0 au; Aux gas pressure: 15 au; Spray Voltage: 3000 V; Collision gas pressure: 1.5 mtorr; SRM mode with cycle time of 1.6 s (au: arbitrary unit)

 ISBN 978-3-86884-040-7

LC parameters:

- Loading step (Fig.1) during 45 sec
  Loading pump: 1 mL/min (water)
  Eluting pump: 0.3 mL/min (10% acetonitrile, 90% 5mM ammonium acetate)

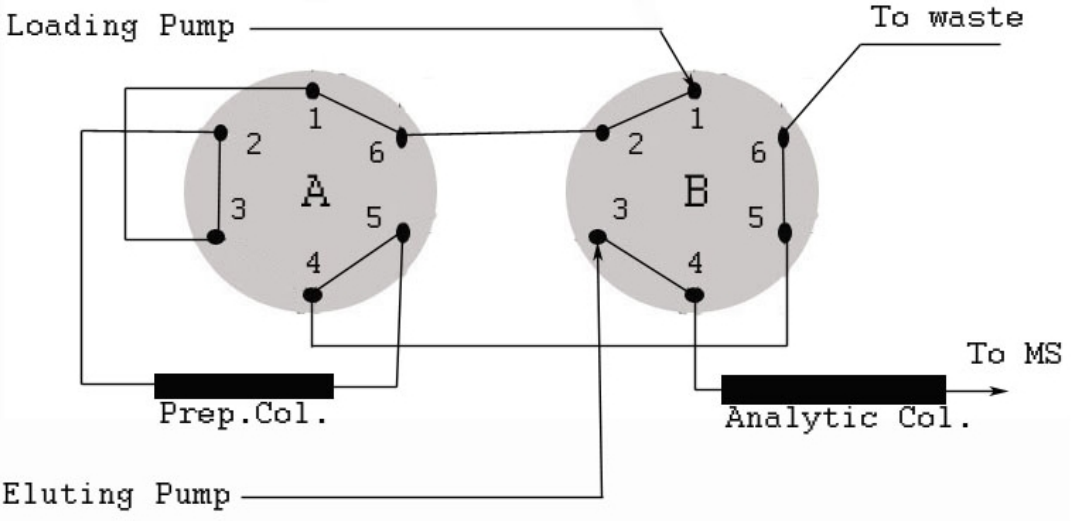

Fig.1: Loading (Initial) step

- Eluting step (Fig.2)
  Loading pump: 0.1 mL/min (water)
  Eluting pump: linear gradient to 90% acetonitrile, 10% 5mM ammonium acetate at 0.3 mL/min during 4 minutes. Wash with 100% acetonitrile 1 minute at 0.4 mL/min, and 2 minutes for initial state
  Loading pump: 1 mL/min (water)
  Eluting pump: 0.3 mL/min (10% acetonitrile, 90% 5mM ammonium acetate)

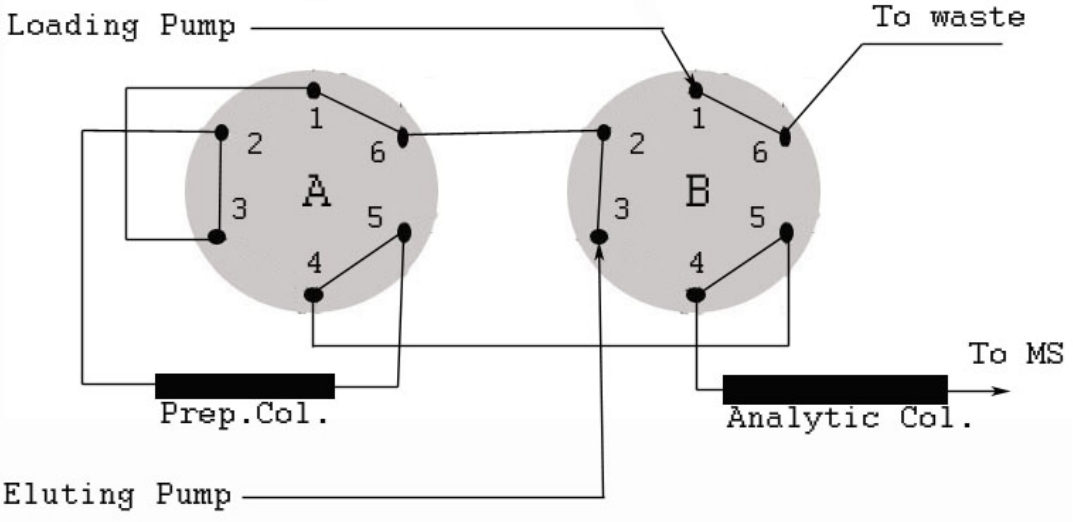

Fig.2: Eluting step

ISBN 978-3-86884-040-7

SRM parameters: see Table 1

Substance	Parent	Product (CE, eV)	S-lens	Polarity	LOD (ng/mL)
Acetazolamide	223	181(15), 164(20), 73(29)	67	Positive	50
Althiazide	382	341(18), 269(22), 205(31)	85	Negative	5
Amiloride	230	171(17), 143(26), 116(31)	77	Positive	5
Andarine desacetylhydroxy glucuronide	590	261(35), 205(35)	120	Negative	n/a
Andarine desacetylhydroxy sulfate	494	261(30), 205(35)	100	Negative	n/a
Bendroflumethiazide	420	289(25), 328(29), 197(48)	91	Negative	5
Benzthiazide	430	308(23), 228(43), 193(57)	92	Negative	5
Bumetanide	365	240(14), 156(35), 184(23)	104	Positive	10
Buthiazide	352	205(26), 269(25), 78(38)	115	Negative	5
Chlorothiazide	294	214(31), 179(47), 215(28)	100	Negative	10
Chlorthalidone	322	150(48), 185(31), 243(22)	108	Positive	50
Clopamide	346	250(21), 169(29), 138(44)	107	Positive	5
Cyclopenthiazide	378	205(25), 269(26), 78(52)	121	Negative	5
Cyclothiazide	388	269(20), 205(35), 322(29)	140	Negative	5
Desmopressin	533	508(23), 516(14), 395(17)	115	Negative	50
Epitizide	424	300(17), 310(20), 269(30)	92	Negative	25
Eplerenone	415	163(21), 121(33), 337(18)	91	Positive	50
Eplerenone hydroxyl Met.	431	355(25), 337(25), 255(25)	85	Negative	n/a
Etacrynic acid	301	243(16), 192(30), 207(26)	45	Negative	5
Ethyl glucuronide	221	75(15), 85(15), 113(15)	40	Negative	n/a
Finasteride carboxy Met.	401	102(30), 357(28)	90	Negative	n/a
Fluticasone Propionate carboxy Met.	451	395(22), 329(23), 73(25)	97	Negative	n/a
Furosemide	329	285(17), 205(23), 126(38)	71	Negative	50
GW1516 sulfoxide	468	154(20), 212(20)	100	Negative	n/a
GW1516 sulfone	484	426(25), 170(25)	100	Negative	n/a
Hydrochlorothiazide	296	269(21), 205(23), 78(31)	60	Negative	10
Hydroflumethiazide	330	239(25), 303(22), 160(43)	77	Negative	5
Indapamide	366	132(14), 130(23), 91(38)	83	Positive	5
Mefruside(IntStd)	381	189(31)	86	Negative	5
Mersalyl Acid	236	121(25), 151(16), 179(11)	65	Positive	n/a
Meticrane	274	118(33), 182(25), 210(21)	70	Negative	50
Metolazone	366	259(19), 179(38), 151(37)	99	Positive	5
Methyclothiazide	358	322(16), 258(19), 194(24)	82	Negative	5
Ostarine glucuronide	564	185(40), 445(20)	120	Negative	n/a
Ostarine hydroxyl glucuronide	580	134(30), 404(20)	120	Negative	n/a
Piretanide	363	236(29), 282(22), 238(19)	115	Positive	5
Polythiazide	438	398(17), 324(22), 418(15)	105	Negative	5
Probenecid	284	240(18), 140(26), 164(25)	73	Negative	25
RSR13	342	256(19), 107(44), 122(31)	100	Positive	5
Spironolactone	341	107(30), 91(52), 187(22)	110	Positive	100
4-amino-6-(trifluoromethyl)benzene-1,3-disulfonamide	318	214(25), 200(29), 239(25)	95	Negative	10
3-chloroaniline-4,6-disulfonamide	284	78(39), 205(25), 169(25)	65	Negative	25
Triamterene	254	237(25), 104(36), 141(42)	100	Positive	5
Trichlormethiazide	380	242(23), 244(22), 215(34)	73	Negative	10
Xipamide	353	274(28), 127(36), 78(37)	110	Negative	5

n/a: not available

Table 1: Method characteristics

ISBN 978-3-86884-040-7

Results and Discussion

The sensitivity (spiked blank urines at MRPL and low concentrations) and selectivity (blank urines) were assessed during the validation process. The obtained results show no significant differences between the new and the old method. For some substances, the sensitivity was significantly less than MRPL. The selectivity was good since one ion transition is enough for the detection of most substances. The substances that elute early require more than one ion transition. Three ion transitions allow us to evaluate a suspicious peak. The use of precolumn makes the analytical column last longer, although the precolumn needs to be changed frequently (after 300-400 injections) even when the back-flushing is used (switching valve "A" at initial step, Fig.1).

The online sample preparation can be implemented on the Agilent 1100 LC system column compartment with one valve, using the built-in "Chemstation" feature or such as on Fig.3. The guard columns such as Zorbax C-8 (or C-18) 2.1x12.5, 5µm can be used as the precolumn for sample preparation. This configuration is being tested in our laboratory.

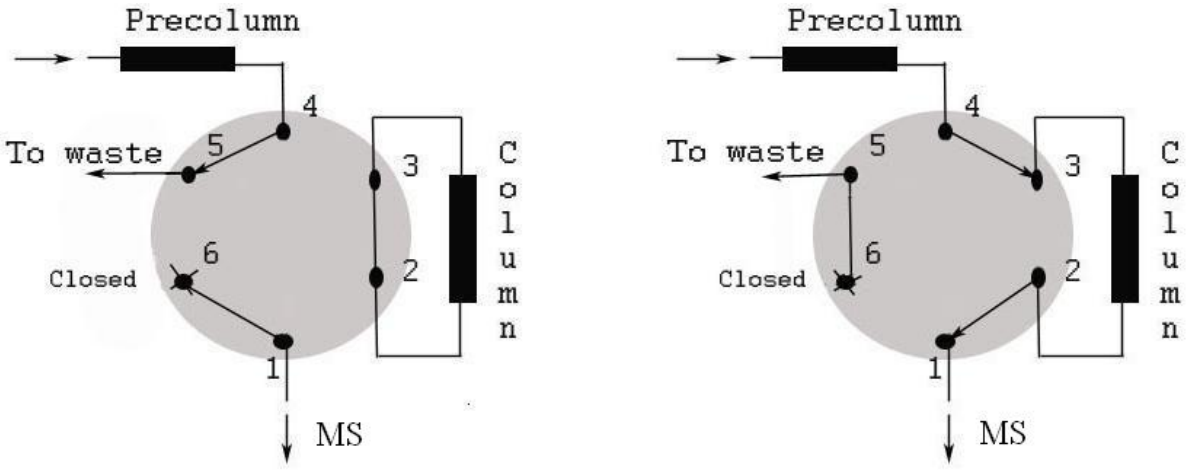

Fig. 3: Loading and elution

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 ISBN 978-3-86884-040-7