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Validation of rapid UPLC/MS/MS qualitative screening method for detection of β-blockers in urine samples

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Introduction

Due to the unsatisfactory results of a GC-MS screening method for β-blockers, a rapid, sensitive screening procedure for the analysis of 20 β-blockers was developed with use of Ultra Performance Liquide Chromatography tandem mass spectrometry. The method allows to detect β-receptor blocking agents enroled to the WADA 2009 Prohibited List [1].

Chemicals and reagents

Standards of β-blockers were acquired from: Sigma (Acebutolol, Alprenolol, Atenolol, Labetalol, Metoprolol, Nadolol, Oxprenolol, Pindolol, Propranolol, Timolol), Synthelab (Betaxolol), Merck (Bisoprolol), Oberval (Carteolol), Boehringer Man&SmithKline Bee (Carvedilol, Metipranolol), Chemie Lizn AG (Celiprolol), Gensia Europe Ltd. (Esmolol), Allergan (Levobunolol), Schering (Mepindolol) and Hexal (Sotalol).

Sample preparation

The urine sample preparation was carried out as described by Deventer *et al.* [2]. Most of β-blockers have secondary or tertiary amine function, so they can be extracted at basic pH [3]. For 1 mL of urine 5 µL of Mefrusid (internal standard) solution was added (10 µg/mL), followed by addition of 125 mg of K_2CO_3. A Liquid/liquid extraction was performed by rolling for 20 min with 3 mL ethyl acetate. After centrifugation the organic layer was transferred into a new tube and evaporated until dry under nitrogen at 40°C. The remaining residue was dissolved in 100 µL of 10% MeOH, the initial mobile phase.

Instrumentation

The screening was carried out on a Waters UPLC Acquity coupled with a MicroMass Quattro Premier XE tandem MS. The screening method was performed with a Waters UPLC BEH C18 column (2.1 x 100 mm; 1.7 µm). The mobile phase consists of 2 mM CH_3COONH_4 in H_2O and in MeOH. A 5 min. long gradient elution at flow rate 0.6 mL/min was applied. The screening was performed in positive ion mode only. For each β-blockers two MRM transitions were defined. The data acquisition parameters were compiled in Table 1.

Tabel 1. Data acquisition parameters.

Compound	Ret. time [min]	Cone voltage [V]	1st MRM transition	Coll. energy [V]	2nd MRM transition	Coll. energy [V]
Acebutolol	2.27	25.00	337.26>98.00	25.00	337.26>116.12	20.00
Alprenolol	2.95	25.00	250.14>116.05	20.00	250.14>173.18	20.00
Atenolol	1.37	30.00	267.20>145.00	25.00	267.20>190.14	20.00
Betaxolol	2.97	30.00	308.20>98.17	25.00	308.20>116.08	25.00
Bisoprolol	2.74	25.00	326.18>116.05	20.00	326.18>133.12	35.00
Carteolol	1.86	25.00	293.30>202.20	25.00	293.30>237.20	15.00
Carvedilol	3.17	30.00	407.20>224.29	25.00	407.20>283.30	25.00
Celiprolol	2.50	25.00	380.28>251.30	25.00	380.28>324.30	20.00
Esmolol	2.45	35.00	296.15>145.02	25.00	296.15>219.20	20.00
Labetalol	2.74	20.00	329.20>90.94	35.00	329.20>162.04	25.00
Levobunolol	2.35	25.00	292.20>201.10	25.00	292.20>236.27	20.00
Mepindolol	2.02	25.00	263.09>116.08	20.00	263.09>147.98	30.00
Metipranolol	2.82	30.00	310.20>165.18	30.00	310.20>191.20	25.00
Metoprolol	2.35	25.00	268.30>133.06	30.00	268.30>159.10	25.00
Nadolol	1.97	25.00	310.26>201.20	25.00	310.26>254.30	20.00
Oxprenolol	2.61	25.00	266.12>98.07	25.00	266.12>116.03	20.00
Pindolol	1.77	30.00	249.20>116.14	20.00	249.20>133.99	25.00
Propranolol	2.91	25.00	260.14>116.19	20.00	260.14>183.19	20.00
Sotalol	1.20	20.00	273.16>213.22	20.00	273.16>255.26	15.00
Timolol	2.37	35.00	317.16>244.26	20.00	317.16>261.27	15.00
Mefrusid (IS)	2.73	25.00	382.97>129.05	20.00		

Results and Discussion

The developed method exhibits very good recovery levels for most of the studied β-blockers (detailed recoveries were collected in Table 2) and therefore they can be easily detected by UPLC/MS/MS as shown in figure 1.

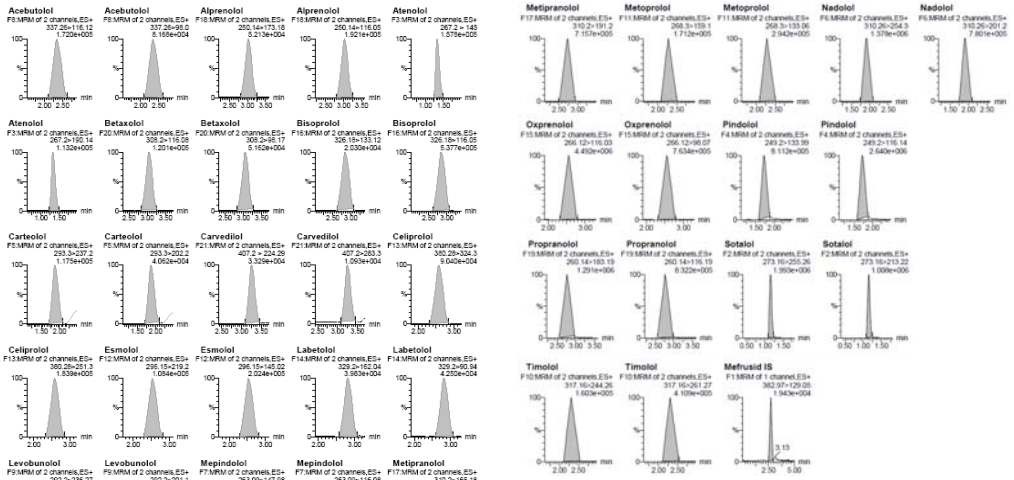

Fig. 1. Chromatograms of QC sample containing prohibited β-blockers listed in Table 1. All standards were at concentration of 50 ng/mL.

Table 2. Extraction recoveries of β-blockers.

Compound	LOD [ng/mL]	RSD [%]	Extr. Recov. [%]; n=4	Compound	LOD [ng/mL]	RSD [%]	Extr. Recov. [%]; n=4
Acebutolol	50	6.7	94.7	Levobunolol	50	1.7	94.6
Alprenolol	50	8.0	93.6	Mepindolol	50	11.2	92.0
Atenolol	50	5.5	50.1	Metipranolol	50	2.9	88.0
Betaxolol	50	2.0	87.9	Metoprolol	50	11.9	97.7
Bisoprolol	50	2.4	92.6	Nadolol	50	7.5	69.4
Carteolol	50	5.9	91.1	Oxprenolol	50	5.5	92.2
Carvedilol	50	6.1	95.8	Pindolol	50	8.2	96.3
Celiprolol	50	5.9	92.0	Propranolol	50	11.3	97.2
Esmolol	50	6.5	95.3	Sotalol	50	5.4	32.4
Labetalol	50	6.6	95.0	Timolol	50	7.6	94.9

Limits of detection (LODs) for all examined β-blockers were determined. A negative urine sample was spiked with all β-blockers standards at concentrations of 500 ng/mL (WADA MRPL) or 50 ng/mL (10% of WADA MRPL) [4]. LODs for all β-blockers in the method were evaluated in urine at the level of 50 ng/mL. According to the WADA Technical Document TD2009MRPL it is not recommended to report presence of β-blockers below 10% of the MRPL. In order to simplify sample preparation, the hydrolysis step in the extraction procedure was omitted as described in [2,5].

Administration study urines

A retrospective analysis of urine samples deemed as positive with previously applied procedure (with hydrolysis step) as well as samples collected from volunteers that had taken one dose of selected β-blockers were performed. The urine from the volunteers was collected from 0.5h to 14h after administration. Analysis of those samples confirmed the presence of prohibited β-receptor blocking agents.

Variations in the composition of aromatic ring of β-blockers cause differences in their metabolism. Excretion of β-blockers in unchanged form in urine vary from 1% for alprenolol, carvedilol and propranolol, 5% for labetalol, 9-12% for acebutolol, up to 76% for carteolol [6]. Due to the high sensitivity of LC/MS/MS technique the described method allows to confirm a presence of prohibited β-blockers in real urine samples with screening by the parent drug. The same conclusion was drawn by Deventer [3]. A retrospective analysis of urine

samples after administration of acebutolol, alprenolol, labetalol, oxprenolol, nadolol, sotalol, betaxolol, and carvedilol with use of the extraction procedure described in this paper confirmed beyond all doubts the presence of prohibited β-receptor blocking agents in all samples. The developed method was also successfully applied during two WADA EQAS tests. Moreover, the described method might be developed by addition of others β-receptor blocking agents such as befunolol, bunitrolol, bupranolol, butofilolol, carazolol, cloranolol, indenolol, moprolol, nebivolol, nifenalol, penbutolol, talinolol, toliprolol, etc.

[1] World Anti-Doping Agency. The 2009 Prohibited List. International Standard, Montreal (2009) http://www.wada-ama.org/rtecontent/document/2009_Prohibited_List_ENG_Final_20_Sept_08.pdf (access date 12.08.2009)

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[3] Deventer K. (2006) *Liquid Chromatography-Mass Spectrometry, an evolution in doping analysis*, PhD Thesis, The University of Gent

[4] World Anti-Doping Agency. Technical Document TD2009MRPL Minimum Required Performance Levels For Detection of Prohibited Substances, Montreal (2009) http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-IS-Laboratories/WADA_TD2009MRPL_EN.pdf (access date 12.08.2009)

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[6] Moffat AC, Osselton MD, Widdop B. (2003) *Clarke's Analysis of Drugs and Poisons in pharmaceuticals, body fluids and postmortem material*, The Pharmaceutical Press, London, Chicago