

β-BLOCKERS

β-blockers are a class of frequently prescribed drugs which are used for various indications, but particularly for the treatment of hypertension, angina, cardiac arrhythmia, congestive heart failure and myocardial infarction. They are included in the list of the top 200 prescribed drugs in the USA and Europe. β-blockers are partially eliminated from the human body through excretion, way by which they enter wastewater treatment plants (WWTPs). Their incomplete removal in WWTPs is pointed out as the major source of these compounds in the environment where they had been found at low ng/L levels [1]. At such low levels, pre-concentration is necessary to achieve the desired limits of detection (LOD). The most frequently used β-blockers, selected for investigation in this study, and their structures are shown in Figure 1.

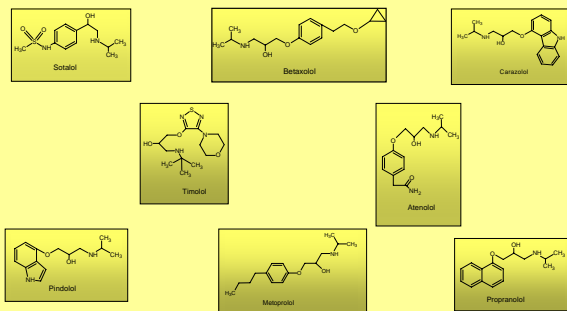


Figure 1. Molecular structure of the β-blockers selected for investigation in this study.

MIPs – MOLECULARLY IMPRINTED POLYMERS

MIPs are artificial antibodies or plastic antibodies prepared by a molecular imprinting technique, in which a functional monomer is polymerized with a cross-linker in the presence of a template molecule. The removal of the template molecule from the resulting polymer leaves molecularly imprinted complementary binding site (s) for the template molecule. MIPs can be used for selective recognition of the template molecule and its structurally related analogues[2].

The main advantages of MIPs, as compared to solid phase extraction (SPE), are:

- higher selectivity for extraction of the target analytes,
- lower co-extraction of matrix interference components, and
- reduction of matrix effects (signal suppression or enhancement) in mass spectrometry coupled to liquid chromatography.

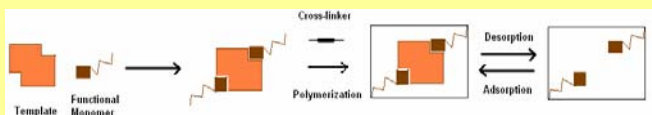


Figure 2 - Concept of Molecular Imprinting

OBJECTIVE

Development of a highly selective and sensitive method, based on MIP extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, for determination of β-blockers (atenolol, carazolol, pindolol, propranolol, sotalol, timolol and metoprolol) in water.

EXPERIMENTAL

Standard Solutions

Individual stock solutions of the analytes were prepared in methanol at a concentration of 1000 mg/mL. A stock standard mixture at a concentration of 20 mg/mL and working standard solutions at concentrations of 2 mg/L, and 1000, 500, 200, 100, 50, 5, 1 and 0.5 µg/L were prepared in methanol-water (75:25, v/v).

MIP extraction protocol

The protocol followed for extraction of the water samples is shown in Fig. 3

LC-MS/MS conditions

The main experimental LC-MS/MS conditions, the equipment and its modes of operation are shown in tables 1 to 3 and in figures 4 and 5.

Table 1 – LC conditions for the separation of the B-Blockers

LC Conditions			
Equipment	Mobile Phase	Column	Flow
Agilent 1100	(Ammonium acetate 5 mmol, pH=5) (Biacetonitrile:methanol, 60:40 gradient)	Purospher®STAR RP-18e (5µm)	0.2 mL/min

Table 2 – MS conditions for the analysis of B-Blockers

MS Conditions					
MS	Ionization Source	Ionization	Mode	ES Ionization Capillary Voltage	Source Temperature
4000 Q-Trap (Applied Biosystems)	TurboIonSpray	ESI positive	SRM	5400 V	700 °C

References

- [1] – M. Gergov, J.N. Robson, E. Duchoslav and I. Ojanpera, Automated liquid chromatography/tandem mass spectrometric method for screening β-blockers drugs in urine. J. Mass Spectrom. 35, 912-918 (2000).
- [2] – J. Haghnaka, Selectivity of affinity media in solid-phase extraction of analytes, Trends in Anal. Chem. 24(5), 2005, 407

Table 3 – Compound parameters for the B-blockers

β-blockers	Mr	[M + H] ⁺	DP - CE	CXP	Transitions	Transitions ratio	LOD (pg)
Atenolol	266.34	267.2	60 – 35	8	267.2 – 145 267.2 – 190	2.45	6
Atenolol d7	273	274	60 - 35	14	274 - 190	-	-
Betaxolol	307.43	308.2	60 – 40	8	308.2 – 116 308.2 – 121	1.49	0.9
Carazolol	298.2	299.2	60 – 35	10	299.2 – 116 299.2 – 222	3.14	0.2
Metoprolol	267.37	268.2	60 – 35	10	268.2 – 121 268.2 – 133	1.14	0.02
Pindolol	248.32	249.2	60 – 30	8	249.2 – 116 249 – 98	8.71	0.3
Propranolol	259.35	260.2	60 – 30	8	260.2 – 116 260.2 – 183	1.48	2.4
Sotalol	272.36	273.2	60 – 25	6	273.2 – 213 273.2 – 255	1.037	0.4
Timolol	316.42	317.2	60 - 30	20	317.2 – 261 317.2 – 244	1.33	0.8

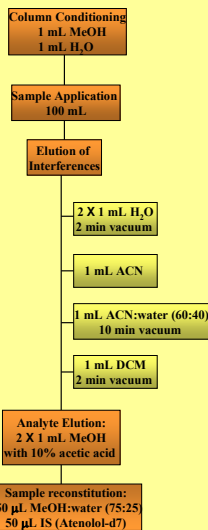


Fig. 3. MIP Extraction procedure



Figure 4. 4000 Q-TRAP (Applied Biosystems/Sciex)

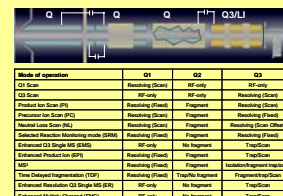


Figure 5. Scheme of the QqLIT instrument (Q TRAP) and description of the various triple quadrupole and trap operation modes

RESULTS

Figure 6 shows the recovery results obtained for each of the target analytes in the study of the breakthrough volume with the MIP used. As can be seen, the highest recoveries were achieved with a sample volume of 100 mL. These are listed in table 4. LODs (see table 3) were determined as the concentration of the compound giving a signal-to-noise (S/N) ratio of 3 for the most intense transition.

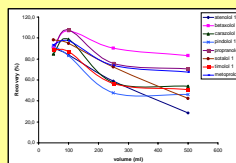


Figure 6 – Determination of the breakthrough volume

Table 4. Recoveries obtained for the analysis of β-blockers in spiked (xxx ng/L) Milli-Q water with the optimized MIP method

β-blocker	Recovery (%)
Atenolol	84
Betaxolol	106
Carazolol	98
Pindolol	83
Propranolol	107
Sotalol	95
Timolol	87
Metoprolol	97

CONCLUSIONS

The extraction protocol developed, based on the use of MIPs specifically designed for the extraction of β-blockers from liquid samples, offers both good efficiency and selectivity in the extraction of the selected β-blockers from environmental water samples. Recoveries achieved for most compounds were higher than 80% and preliminary tests performed with complex, wastewater samples indicate that these materials also efficient at removing matrix interferences. This method is, in addition, simple and fast.

The 4000 Q-Trap system used for analysis adds selectivity and sensitivity to the whole procedure. LODs obtained for the β-blockers investigated were in all instances lower than 6 pg (instrumental LOD), which is equivalent to 3 ng/L in water, considering the method concentration factor of 100.

Acknowledgements

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