Molecularly imprinted polymers (MIPs) as an innovative solid-phase extraction sorbent for the analysis of antidepressants in environmental waters

Kristof Demeestere[1], Mira Petrović[2,3], Meritxell Gros[2,4], Jo Dewulf[1], Herman Van Langenhove[1] and Damiá Barceló[2,4]

[1]Research Group EnVOC, Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[2]Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluis Companys 23, 80010 Barcelona, Spain
[4]Institut Català de Recerca de l'Aigua (ICRA), Parc Científici Tecnológic de la Universitat de Girona, c/Pic de Peguera, 15, 17003 Girona, Spain
E-mail contact: Kristof.Demeestere@UGent.be

1. Introduction

As a result of their growing use and limited biodegradability, pharmaceuticals are found in aquatic systems, such as sewage treatment plant (STP) effluents, surface waters, and even drinking waters. Pollution by pharmaceuticals occurs at parts per billion (ppb, µg/L), but more often at parts per trillion (ppt, ng/L) concentrations. Among the more than 3000 registered pharmaceutical ingredients, psychiatric drugs deserve particular interest, because of at least two reasons [1]. First, psychoactive drugs like antidepressants and benzodiazepines belong to the most widely prescribed pharmaceuticals, with e.g. fluoxetine (Prozac) having been prescribed to over 34 million people worldwide since 2001. Second, psychoactive drugs and their metabolites may enter the environment through various pathways (e.g. via STP effluent or by land application of biosolids) and may affect the metabolics of aquatic and/or terrestrial organisms. Toxicological studies recently indicated that at ambient concentrations antidepressants induce biological effects in fish, mollusks and aquatic invertebrates.

Despite the widespread use and potential environmental effects of psychiatric drugs and antidepressants in particular, few analytical methods exist to detect these pharmaceutical compounds in environmental matrices. However, accurate and precise trace quantification of these emerging contaminants is indispensable as the first step to better understand their environmental occurrence, behavior, exposure and effects. Given the complexity of environmental matrices and the trace concentrations of the pollutants of interest, sample preparation – typically performed with solid-phase extraction (SPE) and leading to improved selectivity and sensitivity – is the most challenging and time consuming step in the entire analytical sequence. In this context, molecularly imprinted polymers (MIPs), i.e. synthetic polymers showing a highly specific recognition ability towards analytes structurally related to the template molecule used during their preparation, offer promising potential as SPE sorbents [2]. However, the use of MIPs for SPE is a rather new and limited explored research domain, particularly for the analysis of pharmaceuticals. Therefore, we present innovative research results showing for the first time the potential of a novel MIP (developed by MIPTechnologies, Lund, Sweden) as a SPE sorbent for the trace analysis of seven psychiatric drugs in environmental waters.

2. Materials and methods

Extraction of the target compounds (fluoxetine, paroxetine, citalopram, trazodone, venlafaxine, lorazepam and diazepam) was performed from both HPLC grade water and real environmental waters. For the latter, influent and effluent was sampled at the STP del Baix Llobregat (Barcelona, Spain) receiving both domestic and industrial waste waters. Llobregat river water was sampled at the Sant Joan Despi drinking water plant nearby Barcelona. All samples were filtered through 1.0 and 0.7 µm glass fiber filters and 0.45 µm nylon membrane filters (Whatman, UK).

The development, optimization and validation of the novel MIP-based SPE (MISPE) method was done in three steps [3]. First, the sample loading (pH and volume), washing and elution (selection of appropriate solvents) steps were intensively investigated with spiked HPLC grade water (spiking concentration of 0.5 µg/L) to gain insight into the kind of sorptive interactions, and to bring forward a protocol yielding the highest extraction recoveries for the target analytes. Second, in order to assess the applicability of the novel MIPs for the analysis of real environmental waters, the MISPE method has been validated in river water, STP effluent

and STP influent. Finally, the performance of the new MISPE method has been compared with two recently reported HLB-based methods in terms of matrix effects and method detection limits (MDL).

Chromatographic separation of the target compounds and internal standards was performed on an Acquity UPLC^{TM} system (Waters, Milford, MA, USA) equipped with a C_{18} column (d_p: 1.7 µm; 2.1 x 50 mm) and making use of a binary mobile phase gradient consisting of acetonitrile and water (0.1% formic acid). Electrospray Ionisation (ESI, positive mode) triple quadrupole mass spectrometry (Micromass, Manchester, UK) operated in MRM mode was used for quantification purposes.

3. Results and discussion

Our detailed optimization study indicated differences in interaction mechanisms among the different compounds, with the selective serotonin reuptake inhibitors (SSRIs) paroxetine, fluoxetine and citalopram showing the highest tendency to sorb at the imprinted sites in the polymer cavities via selective hydrophilic interactions. The other compounds, however, were much more susceptible to washing losses by moderate or weakly polar solvents like acetonitrile and dichloromethane, indicating that non-selective (weak) interactions in the polymer matrix are a more prominent type of binding mechanism. At optimized conditions, i.e. sample loading at natural pH, followed by washing with water and acetonitrile, and elution with acidified methanol, SSRIs do not show breakthrough up to 200 mL of sample volume, even in dirty samples like STP influent. Their extraction recoveries exceed 70% in real environmental waters and are, as well as the precision (RSD < 15%), similar as those obtained when using HLB polymers as the SPE sorbent. On the contrary, compared to HLB, significantly lower MISPE recoveries are obtained for venlafaxine (up to a factor of 7), trazodone (up to a factor of 5), and diazepam (up to a factor of 20) illustrating the selective character of the MIP sorbent, even within one subgroup of pharmaceuticals.

Second, with the novel MISPE-UPLC-MS-MS method, MDL down to 0.5 ng/L in river water and STP effluent and influent can be obtained for the three most selectively retained compounds. Compared to a recently reported HLB method developed for multi-residue pharmaceutical analysis, these MDL are up to a factor of 7 lower. The higher selectivity of the new MIP protocol resulting into cleaner extracts and hereby minimizing matrix effects induced ion suppression and baseline noise indicated to be the most probably explanation. The effect of selectivity is confirmed by considering MDL obtained with a second HLB method specifically optimized towards antidepressant extraction. Even in relative dirty samples like STP influent, this HLB-2 method including a rather strong washing step approximates the MISPE method in terms of sensitivity, exemplified by MDL not more than a factor of 2 lower with the latter method.

4. Conclusions and outlook

Making use of an innovative MIP-based SPE method for sample pre-concentration and clean-up, next to advanced UPLC-MS-MS analysis, this work brings forward a novel selective and sensitive method for the trace quantification of selected antidepressants in environmental waters.

Future research should focus on selective SSRIs extraction from even more complex matrices like sludge or fluid extracts from solid environmental samples. At the end, implementation of this new type of selective chemical trace analytical techniques together with biological assays in integrated monitoring studies might be very helpful to increase our knowledge on the fate, behavior and ecological effects of these emerging micro-pollutants in the environment.

5. References

[1] Schultz MM, Furlong ET. 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Anal Chem 80: 1756-1762.

[2] Pichon V, Chapuis-Hugon F. 2008. Role of molecularly imprinted polymers for selective determination of environmental pollutants: a review. Anal Chim Acta 622: 48-61.

[3] Demeestere K, Petrović M, Gros M, Dewulf J, Van Langenhove H, Barceló D. Trace analysis of antidepressants in environmental waters by molecularly imprinted polymer-based solid-phase extraction followed by ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry. Anal Bioanal Chem, in press: *doi:10.1007/s00216-009-3270-2*.

Acknowledgement - The authors acknowledge the financial support for a research stay abroad provided by the Research Foundation - Flanders. Brian Boyd from MIPTechnologies (Lund, Sweden) is greatly thanked for supplying the MISPE cartridges; whereas Waters (Milford, USA) is gratefully acknowledged for providing the Oasis HLB SPE cartridges and the UPLC analytical column.