Quantification of pharmaceutical residues in solid samples (Danube sediment and sewage sludge) by coupled gas chromatography-mass spectrometry technique

PhD Thesis

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1. Introduction

In 1974 the population of the Earth was 4 billion and it is estimated to exceed 7 billion by the end of 2011. In every single minute 5 human beings are born and 2 cease. Unbelievably high numbers. A crowd like this uses huge amounts of water day by day, placing enormous burden to our Earth. The ability of our planet to tolerate the environmental risks created by the population growth is more and more limited.

Quantity of medicaments consumed by the world population enters into the surface waters, partly through the sewage disposal system and partly in a direct way. A part of the pharmaceutical compounds and their decomposition products (their metabolites) are highly persistent, i.e. their degradation in the environment is very slow or they are not degradable at all. These molecules react very sluggishly with reagents used in water treatment or even more toxic metabolites are produced during the process. Since part of the surface waters are sources of utility water at the same time, it exists a possibility for these medicaments and their metabolites to return to the consumer via drinking water. Thus, a part of the slowly degrading molecules is recycled to the start of the process. The direct chemical and biochemical effects of these chemicals may have deleterious influence for longer periods and not only for the aquatic creatures, but also to more developed organisms including humans.

Environmental awareness gains increasingly high ground in Europe. Nowadays it happens that a manufacturer itself promotes the possibility of monitoring the fate of their products in the environment. Improvements in coupled chromatographic analytical methods help the present situation, and as a consequence, also detection limits have been decreased significantly.

Research work was initiated at the Department of Analytical Chemistry of the Institute of Chemistry at Eötvös Loránd University (ELTE) about five years ago, aimed at analytical determination of water soluble and persistent drugs and personal care products (substances used in extremely high quantities) in waters and solid samples. I joined this research five years ago. Subject of my PhD Thesis was analytical investigation of four drug molecules having acidic character and selected intentionally, from solid environmental samples by means of the GC-MS technique. I wished to contribute to the solution of this question.

2. Objective

My objectives were the following:

a) To develop a sample preparation method for the analytical determination of the studied compounds (ibuprofen, naproxen, ketoprofen, diclofenac) in sewage sludge and Danube sediment using microwave-assisted extraction, so that the extract obtained after the sample preparation could be utilized for GC-MS and GC-MS-MS measurement procedures developed for the aqueous samples by other members of the research group.

b) Validation of the sample preparation method. Determination of the recovery rate, relative standard deviation (RSD), limit of quantification (LOQ) values.

c) Determination of the level of contamination of the mixed and active sludge (both are products of aerobic degradation) formed as final products of sewage disposal for the decision on their further utilization and treatment.

d) To monitor the level of contamination of samples from the Danube sediment in the long run (one year monitoring), to ensure water quality of the riverbank filtration wells. To explore possible relations between the climatic factors and the changes in contamination level.

e) Modeling of physico-chemical processes (adsorption and desorption) undergoing in natural waters (e.g. Danube) on the selected group of compounds, under near natural conditions, in order to establish major factors influencing the adsorption.

3. Experimental part

Objective of my research was to develop methods that reduce number of multiple phase-changes, and number of sample preparation steps.

Sampling: samples of sewage sludge originate from the North-Pest plant of Budapest Sewage Works Ltd. (Active sludge: sludge formed during biological sewage disposal, it is rich in bacteria performing the microbiological degradation. Mixed sludge: a mixture of sludge formed upon mechanical treatment and the excess of the active sludge; the ratio depends on the parameters of the actual process.)

Following sampling, samples were dried at 40 °C or liophilized. After drying sewage sludge samples were homogenized (these samples have biofilm character and a fibrous-crosslinked structure). Samples were stored in a refrigerator at 4 °C until the preparatory operations. Samples of Danube sediment were obtained from three predetermined sites along the Danube from the Kvassay-zsilip (Kvassay sluice) and Tököl. Samples of the Danube sediment were treated similarly to the sewage sludge samples. Spiked samples from each sample type were also prepared parallel, by the use of standard solutions of the components to be measured. Procedure for the preparation of the spiked samples was the following: to the aqueous suspension of the dried sample were added an aqueous-methanolic solution of the standard solution and stirred vigorously for 30 to 60 minutes.

Specified quantities (0.2g to 1.0g) of the solid samples were subjected to microwave extraction (Milestone START-MOD) at various temperatures, for different time intervals. Solvent was distilled water.

Removal of solid floating substances and apolar organic contaminants of the extracts was accomplished with the so-called DME (dispersive matrix extraction) technique developed by myself. Due to this procedure, the complex matrix containing lots of interfering components interacts with the adsorbent (alumina) for a longer time (by increasing particle size of the sorbent a higher surface area can be attained); role of $KAl(SO_4)_2$: to assist in separating the colloidal phase, to remove phosphate content, to set pH to around 4 which is an optimum for the subsequent purification. Filtrates were purified further by the SPE technique. To this purpose, Oasis-HLB columns were used. The base of the purification in each case is that the sorbent binds the polar compounds containing hydrophobic side chain, and the attached compounds can be removed selectively by the use of appropriate solvents. Following washing by hexane, the bound amphoteric components were dissolved from the adsorbent by

the use of ethyl acetate, then methanol, and evaporated. Conditioning of the sorbent took place also with hexane, ethyl acetate, methanol and water.

Derivatization, measurement, evaluation: the silyl derivatives of the studied compounds and the oxime derivative of Ketoprofen was also prepared. Samples were measured by GC-MS technique (Varian-4000 GC/MS/MS). Total ion chromatograms (TIC), or the Selective Ion Chromatograms (SIM) have been recorded. Evaluation in both cases proceeded based on the selective ions, by integrating the area under the GC curve. Calculation of recovery values were performed in each case by the use of external standards (100% was regarded the standard which was only derivatized and measured without sample preparation).

4. Summary

1.1. A new sample-preparation method have been elaborated for the determination of four selected acidic pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac) from mixed and activated sewage sludge. As a novelty in the field, distilled water as a solvent was applied in order to extract the drug molecules. The essential parts of the method were microwave assisted extraction and a clean-up procedure of the extracts before the SPE technology. The pre-cleaning method was named dispersive matrix extraction (DME). The main features of this clean-up method were the use of a sorbent (alumina) having lower polarity than water, and the application of dispersive forces (shaking) in presence of an electrolyte (alum) in order to decrease the matrix effect considerably.

1.2. The whole sample-preparation procedure was validated. The method resulted in recoveries of 80-105% in the concentration range of 20-2000 ng/g, with relative standard deviation of 10-20%. The LOQ values proved to be 10-20 ng/g.

1.3. The four selected pharmaceuticals in sewage sludge were quantified. The obtained values were as follows for mixed sludge: ibuprofen 28 (±16) ng/g; naproxen 47 (±12) ng/g; ketoprofen 76 (±18) ng/g; diclofenac 73 (±12) ng/g; for activated sludge: ibuprofen 23 (±16) ng/g; naproxen 47 (±15) ng/g; ketoprofen 131 (±21) ng/g; diclofenac 138 (±14) ng/g.

2.1. The new sample-preparation method was adapted for processing of river sediments (Danube sediment). The whole sample-preparation process was validated. The method resulted in recoveries of 95-103% in the concentration range of 2-2000 ng/g, with relative standard deviation of 10-12%. The LOQ values proved to be 2-6 ng/g.

2.2. The concentration of four selected pharmaceuticals in Danube water and sediment was monitored during a one-year-period. The determined values were as follows: ibuprofen: <LOQ, naproxen: LOQ-20 (±9-12%) ng/g, ketoprofen: <LOQ, diclofenac: LOQ-38 (±9-12%) ng/g.

2.3. The measured concentration data of the studied drugs were evaluated in correlation with environmental effects (water temperature, water level, TOC content of sediment). The drug content of Danube sediment increased with the drug concentration of Danube water, as well as with the TOC content of sediment, and it decreased with the temperature of Danube water.

3.1. The rate constants of sorption for the studied pharmaceuticals on sediment samples were determined. The sorption process was characterized by a pseudo-first-order model and the value of the rate constant of sorption was found to be 83 min^{-1} for all the four drug molecules.

3.2. The sorption coefficients (K_d) for the four target pharmaceuticals were determined in a wide range of TOC interval (2-16 mg/g), so that the characteristic parameters of sediment samples (chemical composition, particle size) were hold constant and only in the TOC-content changed in correlation with the TN-content. The highest measured values of K_d were: 0.354±0.013; 0.710±0.008; 1.175±0.009; 1.432±0.016 for ibuprofen, naproxen, ketoprofen and diclofenac, respectively.

3.3. It was proven that the sorption has been developed on a biofilm layer, which – above a certain thickness of layer – inhibits the diffusion to deeper layers.

3.4. It was established that the correlation between K_d and TOC content was linear only to a certain limited value of TOC (7 mg/g). Above this value, the normalization of K_d with the organic carbon content could not provide comparable data. Instead of the normalization process, an empirical function was suggested which described the correlation between K_d and TOC in the whole studied TOC range properly. The parameters of this empirical function were calculated for the four studied pharmaceuticals. The received equations are as follows:

ibuprofen: $K_d*f_{oc} = 0.423*f_{oc} - 0.076$ (R^2: 0.993; SD: 0.03);

naproxen: $K_d*f_{oc} = 0.809*f_{oc} - 0.202$ (R^2: 0.995; SD: 0.05);

ketoprofen: $K_d*f_{oc} = 1.366*f_{oc} - 0.306$ (R^2: 0.997; SD: 0.07);

diclofenac: $K_d*f_{oc} = 1.645*f_{oc} - 0.339$ (R^2: 0.998; SD: 0.07).

5. Conclusions

I. Reproducibility and accuracy of my sample preparatory procedure (90–105±15%) was appropriate on sewage sludge and on Danube sludge as well. Essence of the procedure is that the solid phase is extracted with water as a solvent at 100°C under microwave radiation, then the extracts were purified with Al_2O_3 as an adsorbent and $KAl(SO_4)_2$ as an electrolyte, in order to reduce the matrix effect. Contact of the extract's contaminants and the adsorbent was performed by shaking and their separation by centrifugation. Novelty of the procedure consists in applying water as a solvent, which models the behavior of the water-soluble components in natural environments better than the earlier methods using organic solvents for extraction. On the other hand, the use of an electrolyte causes the colloidal particles (fats and tensides) to precipitate on the adsorbent, thereby forming a readily filterable, clean solution, which is already well treatable by the conventional techniques.

II. On the base of the obtained result it was established that all four pharmaceuticals were present both of sewage sludges. Consequently these compounds could not be eliminated quantitatively during the sewage treatment nor yet more significant enrichment was experienced for ketoprofen and diclofenac in the active sludge, than in the mixed one.

III. The following results were obtained in a one-year monitoring of Danube samples:

- Concentrations of the studied compounds in the Danube water could be correlated with the weather factors. At lower water levels and water temperatures the measured values were higher.
- Concentrations found in Danube sediment depended on the concentrations measured in Danube water and on the total organic carbon (TOC) content of the sediment. Depending on the TOC value, the observed enrichment values were as high as 100 to 2,000. Enrichment as a function of TOC was found linear in the studied range. The obtained values in the sediment were in the range: limit of quantification (LOQ) and 40 ng/g for diclofenac; LOQ and 20 ng/g for naproxen. Ibuprofen and ketoprofen were not detectable.

IV. Model studies performed on the Danube samples gave the following results:

- Adsorption is a relatively fast process and equilibrium is attained within an hour. Pseudo-first-order rate constant found for the process: $k=83min^{-1}$. Measured and calculated saturation value showed good agreement, which fact demonstrates the fitness of the pseudo-first-order model for description of the process.

- Adsorption of the target compounds depends on the pH of the aqueous medium; this dependence is significantly lower in case of diclofenac. Adsorption takes place even if the fully unprotonated form is present, which suggests the importance of ionic interactions during adsorption.
- Adsorption of the target compounds depends on TOC and total nitrogen (TN) content of the sediment. High TOC and TN values promote the enrichment to a certain limit.
- Due to colonization of microorganisms, a biofilm layer is formed on the surface of the sediment, which increases TOC and TN content. Adsorption takes place on the biofilm layer, which was demonstrated by scanning electron microscope (SEM) study.
- The following function was established between the adsorption coefficient (K_d) and the TOC value (for sediments that showed correlation between TOC and TN content):

$$K_d * f_{oc} = A + B * f_{oc}$$

This correlation contradicts to my results obtained for natural samples at the first sight, where a linear relationship was found, however, while studies were performed up to a TOC value of 7 mg/g (f_{oc}=0.7 %) in natural samples, laboratory experiments were extended to the TOC=16 mg/g (f_{oc}=1.6 %) limit. Most likely explanation for the deviation from the linear relationship is, that a free diffusion of the contaminant particles to the deeper layers is limited by the biofilm above certain layer thickness.

Major difference between processes taking place in natural conditions vs. those in laboratory conditions is, that the biofilm layer is permanently growing in natural waters and the contaminated water always contacts with newly formed biofilm layer, while model studies were performed with an inert (dead) biofilm. Hence, always higher enrichment is expectable under natural conditions, than it would be prospected from the results of laboratory adsorption studies.

6. Publications, lectures, and posters

Publications

- J. Dobor, M. Varga, J. Yao, H. Chen, Gy. Palkó, Gy. Záray: A new sample preparation method for determination of acidic drugs in sewage sludge applying microwave assisted solvent extraction followed by gas chromatography–mass spectrometry, Microchemical Journal 94 (2010) 36–41 (Impact factor: 2.505)
- M. Varga, J. Dobor, A. Helenkár, L. Jurecska, J. Yao, Gy. Záray: Investigation of acidic pharmaceuticals in river water and sediment by microwave-assisted extraction and gas chromatography–mass spectrometry, Microchemical Journal 95 (2010) 353–358 (Impact factor: 2.505)

Hungarian publication

- József Dobor, Margit Varga, Gyula Záray: Analytical determination of drug residues from solid matrices using gas chromatography mass spectrometry; Debrecen, IV. Conference on Environmental Chemistry in the Carpathian Basin, Debrecen, 28-29 March 2008, p. 198-204**,** Z. Orosz, V. Szabó, G. Molnár, I. Fazekas, Abstract book (2008), ISBN: 978-963-06-4625-3

Submitted for publication

- J. Dobor, M. Varga, Gy. Záray: Comparative study of sorption of selected acidic drugs on river sediment using microwave assisted extraction and gas chromatography mass spectrometry

Lecture

- M. Varga, J. Dobor, A. Helenkár, Gy. Záray: Determination of acidic drugs in sewage sludge and in Danube sediment by microwave assisted solvent extraction using GC-MS, XXV. Sino-Hungarian symposium, 2009, Budapest

Posters

- József Dobor, Margit Varga, András Helenkár, Gyula Záray: Analytical determination of drug residues from Danube River and sediment after microwave extraction using gas chromatography mass spectrometry, Hungarian Natural History Museum 1083 Budapest, Ludovika place 2-6. GeoExpo, October 2010
- Edit Turcsán, József Dobor, Péter Szőke, Laura Jurecska, Dr. Katalin Barkács: Adsorbed organic halogen compounds, testing for the presence of the Danube water and sediment, L. Eötvös University Faculty Of Science, Cooperative Research Centre for Environmental Studies, IX. Environmental Analysis and Technology Conference Sopron, 7-9 October 2009
- József Dobor, Margit Varga, Gyula Záray: Analytical determination of drug residues from solid matrices using gas chromatography mass spectrometry; Debrecen, IV. Conference on Environmental Chemistry in the Carpathian Basin, Debrecen, 28-29 March 2008