Determination of micropollutants in environmental watersamples by gas chromatography-tandem mass spectrometry

PhD thesis

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1. Introduction

From the second half of the 1990's, many studies have drawn the attention to the ever growing threat of water pollution by chemicals coming from both natural and anthropogenic sources, which affect every constituents of the ecosystems- from microorganisms to humans.

This explains how the investigation of the occurrence of pharmaceuticals (antibiotics, anti-inflammatory drugs, analgesics, epileptics, etc), household chemicals and other personal care products (PCPs), such as cosmetics, in the environmental compartments could become one of the most intensively studied areas of Environmental Analytical Chemistry (Fig. 1.).

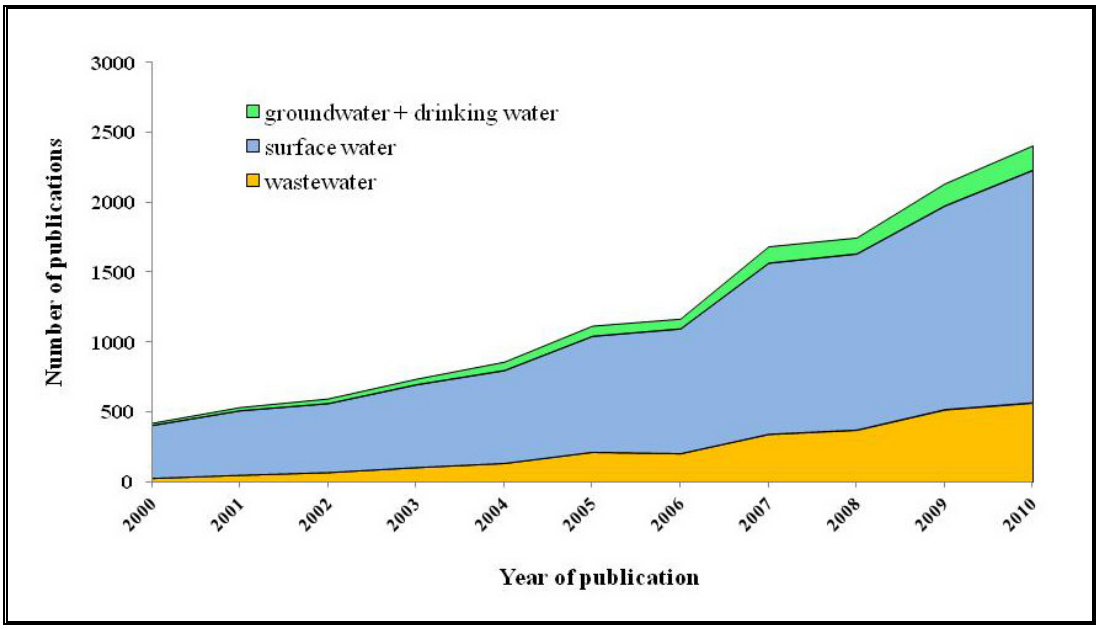

Fig. 1. Number of publications on the determination of pharmaceutical residues in different environmental matrices in the last decade (ground water and drinking water, surface water and waste water)

(Source: Science Direct; keywords: pharmaceuticals/ occurrence/ waste water, surface water, groundwater, drinking water)

2. Aims

The primary aim of my PhD study was to develop a simple and reliable gas chromatography-mass spectrometric method to determine various organic micropollutants present in natural waters. The preliminary work consisted of the analysis of the four most abundant non-steroidal anti-inflammatory analgesics (ibuprofen, naproxen, ketoprofen and diclofenac), since these drugs, taking into consideration their sales figures are estimated to be present not only in wastewater and in the Danube water but potentially even in the drinking water, quantifiable amounts. Later on, the list of target compounds was extended to other household chemicals and pharmaceuticals, such as Endocrine Disrupting Chemicals (EDCs), hormones and bile acids. Although the primarily developed method was proven to be suitable for waste water analysis, it turned out that it was necessary to apply and exploit all the advantages of mass spectrometry for reliable determination of organic contaminants of surface water and drinking water in ng/L concentration.

Therefore, the goals of my PhD thesis were the following:

a) Determination of the four most abundant non-steroidal anti-inflammatory analgesics, namely ibuprofen, naproxen, ketoprofen and diclofenac, in drinking water and Danube water samples by selective ion monitoring (SIM) mass spectrometry;

b) Development of a tandem mass spectrometry (MS/MS) method for the analysis of the above-mentioned compounds through their trimethylsilyl (oxime) ether/ester derivatives;

c) Systematic comparison of the developed MS/MS method with the scanning (FS) and the SIM acquisition techniques;

d) Analysis of the four non-steroidal anti-inflammatory analgesics in water samples from the Danube as well as in drinking water samples by gas chromatography-tandem mass spectrometry;

e) Extension of the MS/MS method for determining further drug residues and other organic micropollutants either in their original form or by their trimethylsilyl (oxime) ether/ester derivatives (simultaneous determination of 42 organic compounds);

f) Qualitative and quantitative determination of the organic micropollutants in environmental water samples by the optimized MS/MS method.

3. Experimental

3.1. Instrumentation

The apparatus consisted of a Varian CP-3800 GC instrument connected to a Saturn 4000 MS ion trap mass spectrometer (Varian, Walnut Creek, CA, USA), equipped with a Varian CP-8400 autosampler and a Varian 1079 Programmable Temperature Vaporizing (PTV) Injector. The system was operated in internal ionization mode. Ionization voltage was 70 eV. The capillary column used was purchased from SGE (Ringwood, Australia): 30m × 0.25 mm; d_f = 0.25 μm. The helium carrier gas flow was 1 mL/min and was kept constant during the temperature gradient program. The temperature of the transfer line, the ion trap and the manifold were 300 °C, 210 °C and 80 °C, respectively. The injector was operated in on-column mode (as a software option). SPE extractions were performed on the Visiprep DL Vacuum Manifold (Catalog No. 57044) from Supelco (Bellefonte, PA, USA). Extracts were dried on Büchi Rotavapor R-200 by means of Büchi Vacuum pump, V-700, both purchased from Büchi (Flawil, Switzerland).

3.2. Samples

Surface water samples were collected with a standard equipment from the River Danube at 0.25 m depth at a distance of 2 m from bank. Tapwater samples were collected from the water system of ELTE and that of Dunaharaszti.

3.3. Materials and reagents

All reagents were of analytical grade. Pyridine and hydroxylamine·HCl were purchased from Reanal (Budapest, Hungary). Hexane, dichloro-methane, methanol, ethyl acetate, hexamethyldisilazane, trifluoroacetic acid and model compounds were purchased from Sigma (St. Louis, MO, USA). Glass microfiber filters (GF/A 125 mm diameter, Cat No. 1820–125) are commercialised by Whatman International (Maidstone, UK). For solid-phase extraction (Oasis, HLB 200 mg Cartridges) (Waters, Milford, MA, USA) were used.

3.4. Solid phase extraction

SPE extractions were performed on the 12- port Visiprep DL Vacuum Manifold (Cat No. 57044) purchased from Supelco (Bellefonte, PA, USA). Prior to extractions, cartridges were conditioned with 5 mL hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL distilled water. Before the SPE enrichment, Danube River and drinking-water samples were filtered through glass microfiber papers. The pH of the water samples (1.5 L or 3.0 L) were adjusted to 4 with 0.1 M hydrochloric acid and with 0.1 M sodium hydroxide. The extractions were carried out with or without spiking with different amounts of standard solutions. The flow rate was 4–5 mL/min. Cartridges were dried under vacuum and elutions were performed with 5 mL hexane, 5 mL ethyl acetate and 10 mL methanol. The pooled fractions were evaporated to dryness by means of a rotary evaporator at 30–40 °C (further on: extract). Blank tests (reagent blanks and SPE blanks) were carried out with each series of samples.

3.5. Preparation of the TMS and TMS (oxime) ester derivatives

Model compounds (10–25 mg/100 mL), weighed with analytical precision were dissolved in water, ethanol, dichloro-methane or in water/ethanol = 1/1(V/V) mixture and further diluted 50 times. Model solutions and extracts were evaporated to dryness at 30–40 °C. The residues were conditioned with 125 μL hydroxylamine·HCl containing pyridine (2.5 g hydroxylamine·HCl/ 100 mL) heated in an oven at 70 °C for 30 min. Thereafter, silylation was made with 225 μL HMDS + 25 μL TFA and heated at 70 °C for 90 min. Samples were taken for the analysis, e.g., after dilutions with HMDS, 1 μL of the diluted solutions was injected into the GC-MS system.

4. Results and discussions

4.1. Summary of the study on non-steroidal anti-inflammatory drug analysis

My results can be summarized as follows:

1. GC-MS-SIM method was developed for the qualitative and quantitative analysis of the four most widely-used non-steroidal anti-inflammatory and analgesic drugs, such as ibuprofen, naproxen, ketoprofen and diclofenac, as their trimethylsilyl (oxim) ether/ester derivatives in water samples from the Danube as well as in tap water.
2. Optimization and validation of the tandem mass spectrometric method was done for the four above-mentioned compounds in order to improve the selectivity of the quantitative analysis.
3. Three acquisition modes, namely FS, SIM and MS/MS ones, were compared, based on the parameters influencing the analytical capabilities, under identical operating conditions and sample preparation.
4. The determination of naproxen and diclofenac by different MS methods gave similar results whereas, for ibuprofen and ketoprofen, only MS/MS gave sufficiently accurate results.
5. Five monthly collected samples of Danube water (namely, in January, September and November 2008 as well as in, April and May 2009) were measured with the validated tandem mass spectrometric method.
6. The concentrations of ibuprofen (3.7-50 ng/L), naproxen (5.7-62 ng/L) and ketoprofen (<LOQ-77 ng/L) were below the maximum limit values recommended by the European Union (200-, 100 and 100 ng/L, respectively).
7. The concentration of diclofenac exceeded the recommended limit value of 100 ng/L, in the case of two samples (224 and 931 ng/L). During the five months of analysis its concentration showed high fluctuations, ranging between 24 and 931 ng/L.
8. None of the investigated NSAIDs could be determined in drinking water by GC-MS/MS.

4.2. Summary of the study on multiresidue analysis

Based on our previous investigations and on literature data, altogether 42 organic components were chosen as target compounds for the extension of the MS/MS method including:

- Different pharmaceuticals and their metabolites
- Derivatives of aromatic carboxylic acid;
- Additives of plastics and cosmetic products;
- Caffeine, as a psychostimulant and ferulic acid, as a natural antioxidant;
- Saturated and unsaturated carboxylic acids and dicarboxylic acids;
- Estrogens;
- Cholesterol and cholic acids.

The above-listed compounds were analysed either in their original form or as trimethylsilyl (oxime) ether/ester derivatives, both by the FS and the MS/MS methods. My primary aim was to develop a GC-MS method capable of determining the above-mentioned 42 compounds, simultaneously.

1. A GC-MS/MS method was optimized and validated to improve the selectivity of the simultaneous qualitative and quantitative determination of 42 compounds.
2. The FS and the MS/MS acquisition techniques were compared based on parameters influencing their analytical capabilities. The LOQ value for the MS/MS for model solutions showed a considerable (on average 3.2-fold) decrease, compared to the LOQ values of the FS technique. In the case of several coelutions, only the MS/MS technique provided sufficiently adequate results in the analysis of water samples.
3. Eight Danube water samples collected in different months (September and November 2008, April and May 2009, March and April 2010 and February and March 2011) were analyzed using the validated MS/MS technique.
4. The concentrations of investigated micropollutants showed large fluctuations, varying between 0.54 ng/L and 3640 ng/L.
5. None of the micropollutants, except diclofenac, was found in any of the samples in a concentration higher than the in force or the recommended maximum limit value allowed for the surface waters.

6. The pollution of the water of the River Danube has decreased significantly during the past two years, as a result mainly of the opening of the Central Sewage Works.
7. By the semi-quantitative analysis of suspended solids, benzoic acid and its hydroxyl derivatives as well as dioctyl-phthalate could be detected in higher concentrations.
8. By the analysis of drinking water taken from Budapest, pharmaceutical residues indicating their contamination could not be detected due to the very modern water treatment techniques used by the Csepel Drinking Water Works.

5. Summary of the new scientific results and achievements included in the thesis

1.1. Novel gas chromatography mass spectrometry with selected ion monitoring (GC-MS-SIM) and gas chromatography tandem mass spectrometry (GC-MS/MS) methods have been developed for the qualitative and quantitative analysis of the four most widely-used non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, naproxen, ketoprofen and diclofenac as their trimethylsilyl (oxim) ether/ester derivatives.

1.2. As a novelty to the field, for the first time, the three acquisition techniques, the full scan (FS) the SIM and the MS/MS ones, have been compared. The three methods have resulted to be equally efficient concerning linearity and recovery, however the lowest LOQ has been achieved by MS/MS (a 2.62–5.67-fold decrease was observed compared to the FS mode).

2. At first, the NSAIDs' content of Danube River and various drinking water samples has been determined with the optimized MS/MS method. The results for naproxen and diclofenac were both reliable and well reproducible for each applied MS technique. On the other hand, in the case of ibuprofen and ketoprofen, MS/MS has been confirmed to have the highest selectivity.

3.1. A multiresidue GC-MS/MS method has been developed. The method was suitable for the simultaneous determination of 42 organic micropollutants without derivatization or as their trimethylsilyl (oxim) ether/ester derivatives. On the basis of our method optimization study, it has been confirmed that, a maximum of four different components per segment could be evaluated by the multiple reaction monitoring (MRM) in order to provide the most adequate signals. Our method could be successfully validated.

3.2. The multiresidue analysis system was utilized for the analysis of the pollutants content of eight samples of the Danube River water collected in different months and two drinking water samples collected at different places. The number of determined and measured compounds in the samples have been on average of 30 by the MS/MS-, and of 21 by FS acquisition method. Comparing the two techniques, it could be stated that in the case of coelution of compounds, the MS/MS provides much more selective and reliable results than the FS. The most abundant pollutants in the examined samples were benzoic acid, phenylacetic acid, and 2-phenylpropionic acid as well as saturated and unsaturated fatty acids. However, our results have clearly shown that, with the exception of diclofenac, the concentration of all the examined micropollutants did not exceed the recommended- and, for dioctyl-phthalate, the maximum concentration limit values currently in force.

3.3. From the results of the measurements performed in 2011, it can be concluded that the decrease in the concentration of organic micropollutants compared to the period between 2008-2010, is due to the opening of the Central Sewage Works of Budapest. As a result of this investment, the former direct inflow of wastewater ceased, therefore in Budapest the efficiency of cleaning of wastewater has increased from 51 to 95%.

6. Publications, lectures, and posters

Publications (basis of thesis):

1. Multiresidue analysis of pollutants present in the aquatic environment
   Á. Sebők, A. Helenkár, A. Vasanits-Zsigrai, K. Sezer, Gy. Záray, I. Molnár-Perl,
   J. Chromatogr. A 1216 (2009) 2288-2301, Impact factor: 4.101

2. The role of the acquisition methods in the analysis of the non-steroidal anti-inflammatory drugs in Danube River by gas chromatography - mass spectrometry
   A. Helenkár, Á. Sebők, Gy. Záray, I. Molnár-Perl, A. Vasanits-Zsigrai,
   Talanta 82 (2010) 600-607, Impact factor: 3.290

3. Determination of Non-steroidal Anti-inflammatory Drugs in Danube River by Gas Chromatography-tandem Mass Spectrometry as Their Trimethylsilyl Derivatives
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   IV. Conference on Environmental Chemistry in the Carpathian Basin, Debrecen, 28-29 March 2008**,** Z. Orosz, V. Szabó, G. Molnár, I. Fazekas, Abstract book (2008) 159-165. ISBN: 978-963-06-4625-3

Publications (published on the subject):

4. Gas chromatography-mass spectrometry of the trimethylsilyl (oxime) ether/ester derivatives of cholic acids: Their presence in the aquatic environment
   Á. Sebők, K. Sezer, A. Vasanits-Zsigrai, A. Helenkár, Gy. Záray, I. Molnár-Perl,
   J. Chromatogr. A 1211 (2008) 104-112, Impact factor: 3.756

5. Investigation of acidic pharmaceuticals in river water and sediment by microwave-assisted extraction and gas chromatography–mass spectrometry
   M. Varga, J. Dobor, A. Helenkár, L. Jurecska, J. Yao, Gy. Záray,
   Microchem. J. 95 (2010) 353-358, Impact factor: 2.579

6. Derivatization and fragmentation pattern analysis of natural and synthetic steroids, as their trimethylsilyl (oxime) ether derivatives by gas chromatography mass spectrometry: analysis of dissolved steroids in waste water samples
   N. Andrási, A. Helenkár, A. Vasanits-Zsigrai, Gy. Záray, I. Molnár-Perl,
   J. Chromatogr. A 1218 (2011) 1878-1890, Impact factor: 4.101

Lectures:

1. Determination of Non-steroidal Anti-inflammatory Drugs in Danube River by Gas Chromatography-tandem Mass Spectrometry as Their Trimethylsilyl Derivatives
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   IV. Conference on Environmental Chemistry in the Carpathian Basin, Debrecen, 28-29 March 2008

2. Determination of micro contamination in Danube River by gas chromatography-tandem mass spectrometry, as trimethylsilyl(oxime) derivatives
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   Meeting of Foundation Lajos Kisfaludy's, 8th March 2010

Posters:

1. Determination of Non-steroidal Anti-inflammatory Drugs by GC-MS/MS in Danube River
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya-Molnár Perl, Gyula Záray
   7. Balaton Symposium, Siófok, 5-7 Sept. 2007
   Researcher's Day, 9 Nov. 2007, L. Eötvös University, Budapest

2. IV. Conference for Waste water Treatment, 29-30 Nov. 2007, Budapest Determination of Nonsteroidal Anti-inflammatory Drugs by Gas-Chromatography-Tandem Mass Spectrometry in Danube River
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   XIII. Italian-Hungarian Symposium on spectrochemistry: environmental contamination and food safety, Bologna, 20-24 April 2008

3. Determination of non-steroidal anti-inflammatory drugs in Danube River by gas chromatography-tandem mass spectrometry as trimethylsilyl derivatives
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   27th International Symposium on Chromatography, Münster, 21-25 Sept. 2008

4. Determination of micro contamination in Danube River by gas chromatography-tandem mass spectrometry
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   Ambulatory assembly of Separation Sciences, Sárvár, 5-7 Nov. 2008

5. The Role of Gas Chromatography-Tandem Mass Spectrometry Acquisition Method in the Analysis of Organic Micropollutants in Danube River
   Helenkár A., Záray Gy., Molnár-Perl I., Vasanits-Zsigrai A.
   8th Balaton Sypmposium on High-Performance Separation Methods, Siófok,
   2-4 Sept. 2009

6. Determination of Micropollutants by Gas Chromatography-Tandem Mass Spectrometry as Their Trimethylsilyl(oxime) Derivatives in Danube River
   András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray
   Colloquium Spectroscopium Internationale XXXVI, Budapest,
   August 30-September 3 2009
   Euroanalysis 2009, Innsbruck, 6-10 September 2009