

*Analysis of pharmaceutical residues and other
organic pollutants in waste waters and in the Danube
River, as their trimethylsilyl (oxime) ether/ester
derivatives by gas chromatography mass spectrometry*

PhD theses

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1. Introduction, Aims

The main aim of my PhD studies was to develop reliable and reproducible methods, in order to introduce also in Hungary the analysis of pollutants present primarily in waste waters and in the Danube River, which will be the basis of the analysis of pollutants in the tap and bottled drinking waters.

My work consists of three main parts relating to the study of the

- (a) non steroidal anti-inflammatory drugs [1],
- (b) to the principle of the presence of cholic acids [2] as well as,
- (c) to the development of a multiresidue analysis system [3]. In the frame of studies (a - c) my points were as follows:

To part (a)

- 1) to compare derivatization conditions for the commonly used reagents (MSTFA, BSTFA, MTBSTFA), including the suggested HMDS+TFA,
- 2) to show the differences in responses - obtained by the internal and external ionization techniques, as well as,
- 3) to perform TIC and SIM elutions, in parallel, in order to select the preferred one,
- 4) to document the reproducibilities of the TMS (oxime) ester derivatives of ibuprofen, naproxen, ketoprofen and diclofenac, in the pg concentration ranges, both from model solutions and from waste-water samples,
- 5) to give an overview on the content of these four NSAIDs measured in the influent and effluent samples of a Hungarian waste-water treatment plant under a 16 months period of time, and,
- 6) to compare the efficiency of their removal under the waste-water treatment process in comparison to those obtained in different other countries.

To part (b)

- 1) to present the fragmentation patterns of the fully derivatized species of cholic-, lithocholic-, chenodeoxycholic-, ursodeoxycholic, 3-hydroxy,7-ketocholanic and dehydrocholic acids (further on: model cholic acids), as their trimethylsilyl (oxime) ether/ester derivatives,
- 2) to optimize the oximation and silylation conditions of model cholic acids in order to get quantitative derivatization,
- 3) to document the linearity of the TMS (oxime) ether/ester derivatives of various amounts of cholic acids from model solution,

- 4) to present the recoveries of all six cholic acids by means of solid phase extraction (SPE), applying the Waters OASIS cartridge, as a function of the pH of the sample to be extracted,
- 5) to demonstrate the reproducibilities in the identification and quantification of the cholic acid contents of four Hungarian influent waste waters and the Danube River,
 - applying different volumes of water samples, and
 - two types of SPE cartridges, varying also the pH of samples (pH 2, pH 4 and pH 7).

To part (c)

- 1) to present a literature overview on recent multiresidue proposals suitable for the analysis of water pollutants,
- 2) to perform a GC-MS derivatization and mass fragmentation pattern study in order to identify and quantify as many pollutants as possible, expected in waste-, in river- and in drinking waters: ready to react with silylating agents, or measurable without derivatization, from a single solution by one injection, simultaneously.

In the frame of these studies further efforts were

- 3) to optimize derivatization conditions for selected pollutants,
- 4) to investigate fragmentation patterns, suitable for quantitation purposes, both of the trimethylsilyl (oxime) ether/ ester derivatives and those of the underivatized species,
- 5) to document SPE recoveries
- 6) to call attention to the primary importance of blank tests,
- 7) to prove reproducibilities based on the quantitation of selective fragment ions of the TMS (oxime) ether/ester derivatives along with underivatized ones, in total of 63 possible pollutants, in different concentration ranges, and
- 8) to show the practical utility of the method by the analysis of the pollutant contents of influent/effluent wastewater and the samples of the Danube River.

2. Experimental

Instrumentation

The apparatus consisted of a Varian 4000 GC-MS/MS system (Varian, Walnut Creek, CA, USA), equipped with a Varian CP-8400 AutoSampler, and with the Septum-equipped Programmable Injector (SPI). The column used was a product of SGE (Victoria, Australia); SGE forte capillary: 30 m x 0.25 mm; $df=0.25\ \mu\text{m}$. The temperature of the transfer line, ion trap and manifold were, in order of listing 280 °C, 210 °C and 80 °C, respectively.

Solid phase extraction

SPE extractions were performed on the Visiprep DL Vacuum Manifold for 12 samples (Cat No: 57044) from Supelco (Bellefonte, PA, USA).

Extracts were dried on a Büchi Rotavapor R-200 by means of Büchi Vacuum pump, V-700, both from Büchi (Flawil, Switzerland).

Materials and reagents

All were of analytical reagent grade. Pyridine, hydroxylamine.HCl from Reanal (Budapest, Hungary). Hexane, methanol, ethyl acetate, hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA) and model compounds were all from Sigma (St. Louis, MO, USA). Glass microfiber filters (GF/A Circles, 125 mm, Cat. No. 1820-125) were from Whatman (Maidstone, UK). Cartridges for solid phase extraction (SPE), in order of listing, Oasis HLB 6cc, and Strata-X 6cc, were from Waters (Milford, MA, USA), and from Phenomenex (Torrance, CA, USA), respectively.

Samples

Influent and effluent waste waters and the samples of the Danube River were obtained with monthly from Budapest Sewage Works Limited.

Sample preparation was consisted of filtering and pH adjusting (pH 2, 4, 7) of 1-3 L waters with diluted hydrochloric acid (36% ~tenfold).

Solid phase extraction

Cartridges, prior to extractions were treated with 5 mL hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL distilled water. Before the SPE enrichment, river and waste-water samples were filtered on glass micro fiber paper. Water samples (0.5, 1.0 L) were adjusted to pH 4 and extractions were followed without or by adding different amounts of standard solutions, with a rate of 4-5 mL/min. Cartridges have been dried by vacuum, and elutions were performed, in order of listing with 5 mL hexane, 5 mL ethyl acetate, and with 14 mL methanol. The unified eluents were reduced in volume, evaporated to dryness by means of a rotary evaporator at 30-40 °C (further on: extract).

Preparation of the TMS (oxime) derivatives

Model compounds (20-25 mg/100 mL, weighed with analytical precision were dissolved in ethanol or in water/ethanol=1/1 (v/v) solution and further diluted for 10 x, 100 x, or further on. Model solutions (10-500 µL) and the extracts were rotary evaporated to dryness at 30-40 °C. The residues were treated with 125 µL hydroxylamine.HCl containing pyridine (2.5 g hydroxylamine.HCl/100 mL). Oximation was followed at 70 °C for 30 and 60 min, as well as

at 100 °C for 30, 90 and 120 min. Thereafter silylation was performed with 225 µL HMDS and with 25 µL TFA. Vials were further heated in the oven, at 70 °C for 90 min at 100 °C for 60 and for 120 min. Finally as optimum derivatization condition for oximation 70 °C and 30 min and for silylation 70 °C and 90 min were selected. At each condition samples were taken for analysis, eg. after dilutions with hexamethyldisilazane, 1 µL of the diluted solutions were injected into the GC-MS system.

Separation of the TMS(oxime) derivatives

Under gradient conditions, the optimized temperature programs different for both the column and the SPI were as follows:

- (a) injections were made at 100 °C, and held at 100 °C for 0.5 min, then heated to 270 °C (200 °C/min), with a 3 min hold at 270 °C,
- (b) column temperature starts at 100 °C, held for 1 min, then heated up to 300 °C (20 °C /min for basic studies, total elution time 21 min, or 10 °C /min for the waste water analysis, total elution time 31 min), with a 10 min hold at 300 °C in both cases.

3. Results, Conclusions

3.1 Reliable, reproducible fast and cost-effective gas chromatographic mass spectrometric method has been developed for the identification and quantification of four non-steroidal anti-inflammatory drugs as their trimethylsilyl (oxime) ester derivatives.

3.2 Derivatization, ionization and data evaluation methods have been studied and optimized: it was shown that

(a) trimethylsilylation with HMDS+TFA ensures the same efficiency as MSTFA and BSTFA, (strictly under the same conditions), and,

(b) it is also the most cost-effective reagent.

3.3 It was demonstrated that internal ionization technique furnished 15-25 times higher responses in comparison to the external one.

3.4 On the basis of parallel TIC and SIM elutions, performing data evaluations on the basis of the same selective fragment ions, in the case of waste-water analysis, TIC elutions were preferred. Since, TIC elutions

(a) provide higher responses, and

(b) are available in archived form for future interest.

3.5 Reproducibilities in the quantitation of ibuprofen, naproxen ketoprofen and diclofenac, both from model- and from waste-water solutions, in the 7-250 pg ranges proved to be between 0.93 and 4.11 RSD%.

3.6 The four NSAIDs' content determined in the influent and effluent waste-waters of a Hungarian waste-water treatment plant was followed under a 17 months period of time. Our results, in accordance with those measured in Canada, in the United States, in Spain and in Croatia revealed uniformly, that both (i) the level of these four NSAIDs and (ii) the efficiency of their removal do not differ significantly: due the worldwide popularity of these NSAIDs, and to the very similar elimination processes applied for their removal.

3.7 The gas chromatographic mass spectrometric identification and quantification conditions of six cholic acids, such as cholic-, lithocholic-, chenodeoxycholic-, ursodeoxycholic-, 3-hydroxy,7-ketocholanic- and dehydrocholic acids have been optimized.

3.8 Fragmentation patterns of all six model acids provided the expected masses of molecular ions, the molecular ions less by one methyl radical, less by one TMSO, TMSOH, Si(CH₃)₄ and further characteristic fragment ions.

3.9 As the practical utility of the method, in the frame of this study a new group of waste water pollutants have been introduced.

3.10 In the influent waste water five different cholic acids, in the Danube river three species have been identified and measured. The total cholic acid contents of waste water samples were extremely high, close to 300 µg/L, while in the Danube river 4 µg/L cholic acids were found.

3.11 Reliable and reproducible gas chromatographic mass spectrometric, multiresidue method has been developed for the analysis of pollutants, as their trimethylsilyl (oxime) ether/ester derivatives, (caffeine and phthalates in their initial forms), from one solution by a single injection.

3.12 Due to detailed derivatization and mass fragmentation studies 63 compounds were identified and quantified, on the basis of their selective fragment ions, within 31 min.

3.13 Detailed studies were performed to clear up the reality and importance of blank tests.

(i) Blank values were characterized for the first time by numerical data. It was shown that

(ii) impurity products originated partly from the reagents, partly, however primarily from the SPE cartridges.

(iii) Based on the use of seven different SPE cartridges it has been confirmed that these blank values are independent of the size and producer of cartridges.

(iv) Due to the considerable blank values, to carry out blank test with each series of analyses is inevitable necessary.

3.14 The practical utility of the proposal was shown by the identification and quantification of the pollutants, present in the influent and effluent wastewaters of a Hungarian wastewater treatment plant (WWTP), (under six months period of time and in the Danube River for two months.

3.15 As to the efficiency of the Hungarian WWTP, characterized with the average removal of the total of pollutants, it proved to be 96% (3.40%, RSD), under 6 months period of time.

4. Novel scientific results; Summary

4.1 The identification and quantification of the four non steroidal anti-inflammatory drugs, such as ibuprofen, naproxen, ketoprofen and diclofenac, as their trimethylsilyl (oxime) ether/ester derivatives, were suggested for the first time. The silylation of these four drugs has been optimized as a function of the derivatization reagent and those of the time and temperature of reactions. The differences of the two electron ionization techniques (internal and external) have been examined: the sensitivity of the internal ionization technique proved to be 15-25 folds greater compared it to the sensitivity of the external one.

4.2 The non steroidal, anti-inflammatory drug contents of Hungarian influent and effluent waste waters and the Danube River have been determined, at first.

4.3 The analysis of six cholic acids, including cholic, lithocholic, chenodeoxycholic-, ursodeoxy-cholic, 7-ketolithocholic and dehydrocholic acids, as their trimethylsilyl (oxime) ether/ester derivatives, by GC-MS has been optimized for the first time.

4.4 The cholic acids have been determined in environmental matrices at first. In the Hungarian influent waste water samples five, in the Danube River samples three cholic acids were found.

4.5 The multiresidue analysis system, suitable for simultaneous identification and quantification of 63 compounds, expected as environmental pollutants, has been described at first:

(i) as their trimethylsilyl (oxime) ether/ ester derivatives,

(ii) on the basis of their selective fragment ions, within 31 min, by means of GC-MS.

(iii) It has been confirmed, that in favor of the keto group containing pollutants, the oximation step is advantageous.

4.6 The multiresidue analysis system has been utilized for the analysis of the pollutant content of Hungarian waste-waters and the samples of the Danube River, at first: in the waste water samples 51, in the Danube River 25 pollutants have been identified and quantified.

4.7 On the basis of our detailed study, the importance of the all-time preparation and quantitative consideration of blank tests (reagent and SPE blank test samples) has been confirmed at first:

(i) the amounts of the same pollutants originated from the derivatization and from the SPE processes have been quantitatively differentiated.

(ii) Comparing 7 various SPE fillings, it could be stated that the overwhelming part of impurities are originated from the SPE process,

(iii) the quality and quantity of pollutants are independent on the cartridges' sizes and producers, as well as,

(iv) to take them into consideration, from the point of view of the reliability and reproducibility in pollutants' analysis, proved to be unavoidable necessary.

5. Publications, lecture, posters (basis of theses)

Publications:

[1] Á. Sebők, A. Vasánits-Zsigrai, Gy. Palkó, Gy. Záray and I. Molnár-Perl: Identification and quantification of ibuprofen, naproxen, ketoprofen and diclofenac present in waste-waters, as their trimethylsilyl derivatives, by gas chromatography mass spectrometry.

Talanta 76 (2008) 642-650

[2] Á. Sebők, K. Sezer, A. Vasánits-Zsigrai, A. Helenkár, Gy. Záray, I. Molnár-Perl: Gas chromatography-mass spectrometry of the trimethylsilyl (oxime) ether/ester derivatives of cholic acids: their presence in the aquatic environment.

J. Chromatogr. A 1211 (2008) 104-112

[3] Á. Sebők, A. Vasánits-Zsigrai, A. Helenkár, Gy. Záray, I. Molnár-Perl: Multiresidue analysis of pollutants as their trimethylsilyl derivatives, by gas chromatography mass spectrometry.

J. Chromatogr. A 1216 (2009) 2288-2301

Lecture:

Ágnes Sebők: Analysis of pharmaceutical residues and other pollutants in waste waters as their trimethylsilyl (oxime) ether/ester derivatives by GC-MS

Hungarian Association of Chemists, Session for young scientist (November 20, 2007, Budapest)

Posters:

2. Á. Sebők, A. Vasánits-Zsigrai, Gy. Záray and I. Molnár-Perl: Analysis of non-steroidal, anti-inflammatory drugs present in waste waters, as trimethylsilyl derivatives, by GC-MS.

Rámcová smernice o vodách, Český Krumlov, October 10-12, 2006).

3. Sebők Ágnes, Zsigrai-Vasánits Anikó, Helenkár András, Perl-Molnár Ibolya, Záray Gyula: Analysis of the non steroidal anti-inflammatory drugs in waste waters as trimethylsilyl derivatives, by GC-MS.

III. Conference for waste water pollutants, (November 30, 2006, Budapest).

4. Á. Sebők, A. Vasánits-Zsigrai, Gy. Palkó, Gy. Záray, I. Molnár-Perl: Advances in the Gas Chromatographic Mass Spectrometric Analysis of Pharmaceuticals, Present in the Aquatic Environment.

7th Balaton Symposium, Siófok, Hungary (September 5-7, 2007).

5. Ágnes Sebők, András Helenkár, Anikó Vasánits-Zsigrai, Gyula Záray, Ibolya-Molnár Perl: Advances in the Gas Chromatographic Mass Spectrometric Analysis of Pharmaceuticals, Present in Waste Water.

XIII. Italian - Hungarian symposium on Spectrochemistry Environmental Contamination and Food Safety, (April 20-24, 2008, Bologna, Italy) .