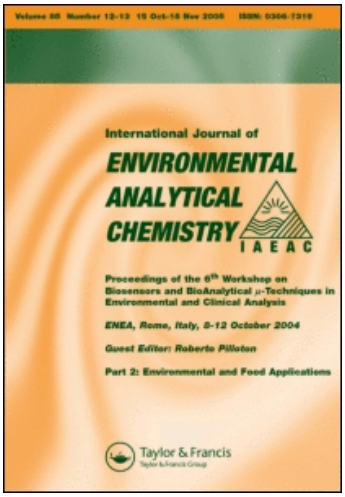

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Pharmaceutical Drug Residues on Suspended Particulate Material in Surface Water

Markus Himmelsbach[a]; Wolfgang Buchberger[a]; Hermann Miesbauer[b]
[a] Department of Analytical Chemistry, Johannes-Kepler-University, Linz, Austria [b] Office of the State Government of Upper Austria Department of Water Supply and Distribution, Laboratory for Water Protection, Linz, Austria

Online publication date: 17 September 2010

To cite this Article Himmelsbach, Markus , Buchberger, Wolfgang and Miesbauer, Hermann(2003) 'Determination of Pharmaceutical Drug Residues on Suspended Particulate Material in Surface Water', International Journal of Environmental Analytical Chemistry, 83: 6, 481 — 486
To link to this Article: DOI: 10.1080/0306731031000101392
URL: http://dx.doi.org/10.1080/0306731031000101392

Intern. J. Environ. Anal. Chem.
Vol. 83, No. 6, pp. 481–486, June 2003

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF PHARMACEUTICAL DRUG RESIDUES ON SUSPENDED PARTICULATE MATERIAL IN SURFACE WATER

MARKUS HIMMELSBACH[a], WOLFGANG BUCHBERGER[a,*] and HERMANN MIESBAUER[b]

[a]*Department of Analytical Chemistry, Johannes-Kepler-University, Altenbergerstrasse 69, A-4040 Linz, Austria;* [b]*Office of the State Government of Upper Austria, Department of Water Supply and Distribution – Laboratory for Water Protection, Goethestrasse 86, A-4020 Linz, Austria*

(Received 16 October 2002; in final form 27 January 2003)

Recent studies have demonstrated the presence of traces of various pharmaceutical drugs in the aquatic environment. Comprehensive data about the distribution of such compounds between the aqueous phase and suspended particulate material are still missing. In the present article a gas chromatographic method with mass spectrometric detection is presented which allows the determination of the particle-bound fraction of some pharmaceuticals commonly found in surface water. Determination limits are between 2 and 12 ng/g particles. Results from surface water samples indicate the possibility that less hydrophilic pharmaceuticals like mefenamic acid are present as suspended particulate material, although the amounts are small in comparison with the concentrations found in aqueous phase. Additional work will be necessary to evaluate the full importance of particle-bound pharmaceuticals with respect to transportation in the environment.

Keywords: Drug residue analysis; Water analysis; Suspended particulate material

INTRODUCTION

In several cases administered pharmaceutical drugs are excreted without major metabolism and enter municipal sewage systems. Even in sewage treatment plants the elimination of drug residues may be incomplete, so that effluents of these plants are the major source for introduction of pharmaceutical drugs into the aquatic environment. Recently, several analytical methods based on GC and HPLC have been developed for these analytes (see for example [1–6]). Published results indicate that a range of different pharmaceuticals of quite diverse chemical structures can be found in surface water samples at low to mid ng/L levels. These concentrations are probably not yet a major problem for drinking water supply. Nevertheless, ecotoxicity of drug residues is still a matter of debate so that further analytical measurements are necessary.

*Corresponding author. Fax: +43 732 2468 8679. E-mail: wolfgang.buchberger@jku.at

ISSN 0306-7319 print: ISSN 1029-0397 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0306731031000101392

Analytical methods available for pharmaceutical drug residues in water most often include a pre-concentration step based on solid-phase extraction. In this case, filtered water samples are generally used. According to our knowledge, detailed investigations on the amounts of pharmaceuticals absorbed to suspended particulate material (SPM) are still missing. Data on distribution of pharmaceutical drugs between the aqueous phase and SPM may be interesting to get a better understanding of the transport in the aquatic environment. Therefore, the present work aims at the development and application of analytical methods for determination of SPM-bound pharmaceuticals in surface water, whereby carbamazepine, clofibric acid, diclofenac, ibuprofen, mefenamic acid and naproxen have been selected as analytes. These compounds are commonly found in surface waters so that they may be considered as environmentally relevant.

The determination of acidic pharmaceuticals like clofibric acid, diclofenac, ibuprofen, mefenamic acid and naproxen by GC-MS after derivation with pentafluorobenzyl bromide and is a relatively straightforward approach [7,8]. Such a method has been used routinely for water samples [4] but not yet for analysis of SPM. Carbamazepine can be analyzed by GC without derivatization, although it is well known that this analyte undergoes some decomposition into the corresponding iminostilbene during injection. Nevertheless, monitoring both the peak of the carbamazepine and its decomposition product allows a good quantitative determination of this analyte. The detection can be done at the same m/z ratio for both peaks, since m/z of the major fragment ion of carbamazepine is identical with m/z of the decomposition product.

With respect to multi-compound methods, HPLC would be the preferred tool for trace analysis of pharmaceutical residues. Unfortunately, the performance of the combination of HPLC with MS by means of atmospheric pressure ionization may be strongly affected by the matrix, especially when more complex samples like extracts from solids are analyzed. Therefore, in the present case GC was chosen in combination with electron impact MS, which is much less prone to interference by the matrix.

EXPERIMENTAL

Chemicals

Standards of the pharmaceutical drugs were obtained from Sigma (St. Louis, MO, USA), desmetryn from Riedl-de-Haen (Seelze, Germany), toluene and ethyl acetate of ultra resi-analyzed quality from Baker (Deventer, The Netherlands). Florisil and pentafluorobenzylbromide were obtained from Supelco (Bellefonte, PA, USA). All other chemicals were of p.A. quality from Merck (Darmstadt, Germany).

Sampling and Sample Pretreatment

Samples of SPM were collected by means of a sampler developed by Reinemann and Schlemmer [6]. After collection, the SPM was separated from the remaining aqueous phase by filtration and was freeze-dried. An amount of 5 g dry material was suspended in 50 mL water. 2 g calcium carbonate were added and the pH was adjusted to 2 by concentrated hydrochloric acid. After 30 min 100 mL acetone were added and the suspension treated in an ultrasonic bath for 30 min. The particulate material was separated

from the liquid phase by centrifugation. The liquid phase was mixed with 200 mL 3% aqueous sodium chloride and extracted three times with 70 mL ethyl acetate each. The combined organic phase was dried by sodium sulfate, concentrated to 3 mL at a rotavapor, and passed through a mini-column filled with 1 g of florisil. The column was washed with 1 mL ethyl acetate. The combined eluates were brought to dryness in a stream of nitrogen and redissolved in 300 μL of toluene containing 6 μL pentafluorobenzyl bromide. After addition of 4 μL triethylamine, the mixture was treated at 90°C for 60 min. Finally, 20 μL of a solution of 10 mg desmetryn dissolved in 10 mL toluene were added as internal standard and the volume adjusted to 1 mL with toluene. This solution was used for analysis by GC-MS.

Analysis by GC-MS

The instrumentation for GC-MS analysis consisted of a HP 5890 Series II gas chromatograph (Agilent, Palo Alto, CA, U.S.A.) equipped with a split–splitless autoinjector and coupled to a HP 5989 A quadrupole mass spectrometer. Separations were carried out by means of a HP 1701 column, 30 m × 0.25 mm i.d., film thickness 0.25 μm. Helium was used as mobile phase at 70 kPa. The injection volume of 2 μL was injected in the splitless mode at 250°C. After 1 min at 90°C the temperature of the oven was raised to 150°C at a rate of 30°C/min, to 210°C at a rate of 3°C/min, and to 280°C at a rate of 15°C/min. The interface temperature was 300°C, the temperature of the ion source (electron impact) 100°C, and the quadrupole temperature 174°C. The mass spectrometer was operated in the SIM-mode at the following m/z ratios (the bold numbers indicate the m/z ratios used for quantitative analysis, whereas the other m/z ratios were used for qualitative confirmation of peak identity): **213**, 198, 171 (16.00 to 18.80 min), **394**, **386**, 236, **193**, 161, 118, 128 (18.80 to 24.00 min), **475**, **421**, **410**, 242, 236, 223, 214,194, **193**, 185 (24.00 min to end of run).

RESULTS AND DISCUSSION

The procedure used for sample pretreatment was partly based on previous experience with pesticide residues on SPM. It turned out to be essential to soak the dry sample in acidified water before extraction of the analytes by addition of an organic solvent miscible with water like acetone. Clean-up of the extract by liquid–liquid extraction with ethyl acetate and passage of the organic phase through a small column filled with florisil was sufficient for the subsequent derivatization step and GC-MS analysis.

For preliminary experiments on the recovery of the analytes from SPM, 2 g sample were spiked with 200 μL of a methanolic solution containing 200 ng of each analyte. After drying, the sample was treated as described above. It turned out that the recoveries strongly depend on the chemical properties of the water from where the SPM sample had been obtained. Samples collected from water of high hardness (more than 3 mmol/L) yielded satisfactory recoveries, whereas the recoveries from samples collected from water of low hardness were zero except for carbamazepine (see Table I). Particles from water of high hardness contained a considerable amount of calcium carbonate, which dissolved upon adjustment of the pH. Therefore, the ionic strength of the solution of such samples was considerably higher than for samples from water of low hardness. An effect of the ionic strength on the recovery cannot be excluded.

TABLE I Recoveries of pharmaceutical drug residues from spiked suspended particulate material originating from water of high hardness (sample 1) and from water of low hardness (sample 2)

Analyte	*Recovery* (%)		
	Sample 1	*Sample 2*	*Sample 2 mixed with $CaCO_3$*
Ibuprofen	91.0	0	96.9
Clofibric acid	95.1	0	0
Carbamazepine	91.6	102.0	99.0
Naproxen	82.5	0	87.5
Mefenamic acid	101.0	0	87.2
Diclofenac	94.0	0	86.5

In another series of experiments solid calcium carbonate was added to those samples that had been collected from water of low hardness. This addition increased the recoveries to a reasonable level (see Table I) except for clofibric acid which showed still recoveries close to zero. The results clearly indicate the necessity to determine the recoveries separately for each sampling site. At the moment the reasons for the loss of clofibric acid are not yet known and this analyte was excluded from the list of analytes for the present study.

In case of 5 g sample, the determination limits (signal-to-noise ratio of 5) were 11 ng/g for carbamazepine, 12 ng/g for clofibric acid, 11 ng/g for diclofenac, 9 ng/g for ibuprofen, 2 ng/g for mefenamic acid and 4 ng/g for naproxen, provided that no major interfering peaks from the matrix were present in the chromatogram.

The limited amount of SPM available did not allow a thorough evaluation of the repeatability of the analytical method. Nevertheless, a rough estimate of repeatability could be achieved by using solid calcium carbonate as sample which was spiked with 100 ng of each analyte per 1 g sample. These spiked model samples were subjected to the whole analytical procedure. The results of six replicates yielded recoveries between 90 and 100% for all analytes with relative standard derivations ranging from 2.5 to 4.3%. Among others, a series of measurements was done at the river Feldaist in Upper Austria, where the population density was around average. At a sample site located approximately 1 km downstream a sewage treatment plant, previous measurements of pharmaceutical drug residues in water (after filtration) had indicated the presence of diclofenac, carbamazepine and mefenamic acid at concentrations between 10 and 150 ng/L. At this site, several samples of SPM were taken. The length of the sampling was approximately three days for each sample in order to get about 5 g of SPM. During one month, nine samples could be obtained. In two thirds of the SPM samples, mefenamic acids could be detected at concentrations between 4 and 24 ng/g. All other analytes were below the detection limits. A typical chromatogram of a SPM sample is given in Fig. 1.

A more detailed study of the adsorption behaviour of pharmaceuticals was carried out in a series of lab experiments involving 4 L of water spiked with 100 ng/L of each analyte. After addition of 4 g SPM (free of residues), the suspension was stirred for 24 h. The SPM was separated from the aqueous phase by filtration and analyzed as described above. No pharmaceuticals could be found at the SPM except mefenamic acid; its concentration was 12 ng/g. It should be noted that mefenamic acid exhibits the highest octanol–water partition coefficients (P_{ow}) among all compounds investi-

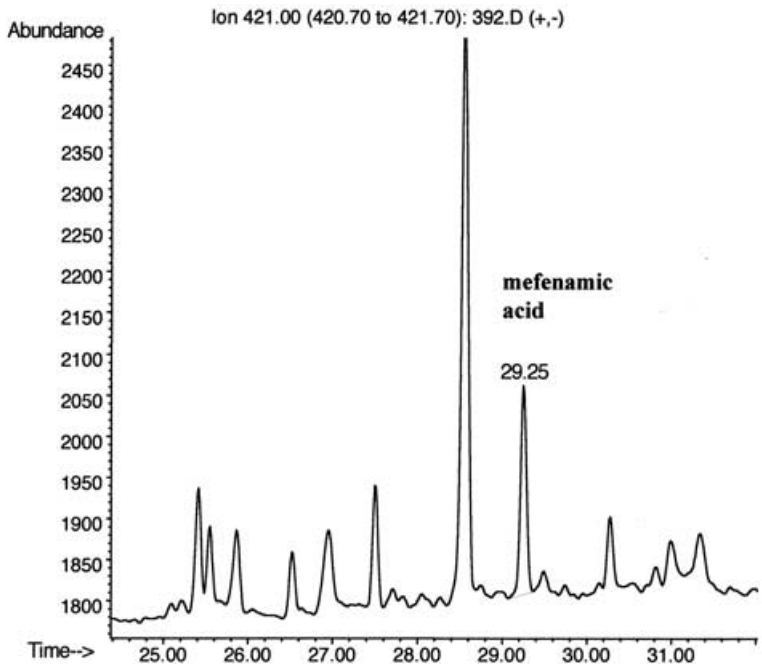

FIGURE 1 Chromatogram of the extract from a typical sample of suspended particulate material containing mefenamic acid (peak at 29.25 min).

gated within this study. Further work is in progress to investigate the adsorption of other compounds with high P_{ow} values.

In this context it may be interesting to compare the results from the present study with results for other anthropogenic contaminants that are continuously introduced into the environment via sewage treatment plant effluents. During recent years, synthetic musk fragrances have been identified in the aquatic environment [9]. They are essential ingredients in fragrances for numerous consumer products and are persistent enough to survive sewage treatment plants and to enter surface waters. Determinations of synthetic musk fragrances in river water and on SPM [10] demonstrated that the total concentrations were in the order of 100 ng/L and therefore in the same range as drug residues. SPM-bound residues were in the order of 500 ng/g and considerably higher than for drugs. One should keep in mind that synthetic musk fragrances are quite hydrophobic, which explains the higher concentrations on SPM and confirms the idea that hydrophobicity is one of the main driving forces for adsorption on SPM in water. On the other hand, investigations published in the literature [10] also point out that the presence of humic acids in water decreases the adsorption to SPM. Therefore, it seems necessary to carry out additional work with water samples containing varying concentrations of humic acids in order to get a more accurate picture about the presence of drug residues bound to SPM.

Another group of contaminants measured in water and on SPM were polycyclic aromatic hydrocarbons (PAHs) [10]. Concentrations in the order of 10 ng/L in water were associated with concentrations in the order of 500 ng/g SPM. Not surprisingly, PAHs show a considerably stronger tendency to adsorption than pharmaceutical drugs of less hydrophobic character included in our study.

CONCLUSIONS

In the present work first efforts have been made to differentiate between particle-bound and dissolved residues of pharmaceutical drugs frequently found in the ng/L range in the aquatic environment. One might argue that pharmaceuticals which show a strong tendency to adsorption at SPM might be less relevant from the point of environmental chemistry, because in the sewage treatment plants they would be eliminated from the aqueous phase via the sludge. Nevertheless, in the present study it could be demonstrated that in surface waters pharmaceutical drug residues can be found in particle-bound form. The amount of pharmaceuticals at SPM seems to be much lower than the amounts dissolved in the aqueous phase. At the moment all data necessary to calculate an exact balance for the fate of pharmaceuticals in the aquatic environment are not yet available. A final judgement of the relevance of particle-bound pharmaceuticals seems to be possible only after comprehensive analytical work including a much broader range of analytes.

Acknowledgement

Financial support from the Austrian Science Foundation, grant P13906CHE, is gratefully acknowledged.

References

[1] T.A. Ternes, *Trends Anal. Chem.*, **20**, 419–434 (2001).
[2] S. Öllers, H.P. Singer, P. Fässler and S.R. Müller, *J. Chromatogr. A*, **911**, 225–234 (2001).
[3] T.A. Ternes, M. Bonerz and T. Schmidt, *J. Chromatogr. A*, **938**, 175–185 (2001).
[4] F. Sacher, F.T. Lange, H. Brauch and I. Blankenhorn, *J. Chromatogr. A*, **938**, 199–210 (2001).
[5] A. Putschew, S. Schittko and M. Jekel, *J. Chromatogr. A*, **930**, 127–134 (2001).
[6] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton, *Environ. Sci. Technol.*, **36**, 1202–1211 (2002).
[7] T. Heberer and H.-J. Stan, *Intern. J. Environ. Anal. Chem.*, **67**, 113–124 (1997).
[8] T. Heberer, K. Schmidt-Bäumler and H.-J. Stan, *Acta Hydrochim. Hydrobiol.*, **26**, 272–278 (1998).
[9] G.G. Rimkus, *Toxicol. Lett.*, **111**, 37–56 (1999).
[10] M. Winkler, G. Knopf, C. Hauptvogel and T. Neu, *Chemosphere*, **37**, 1139–1156 (1998).