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Research Article

Determination of Endocrine Disrupting Compounds and Acidic Drugs in Water by Coupling of Derivatization, Gas Chromatography and Negative-Chemical Ionization Mass Spectrometry

Dedicated to Prof. Dr. mult. Dr. h. c. Müfit Bahadir on the occasion of his 60th birthday

The determination of acidic pharmaceutical and endocrine disrupting compounds at low ng/L levels in surface and wastewater requires highly selective and sensitive analytical procedures. Therefore, the water samples under study were prepared by means of solid phase extraction (SPE) for analyte enrichment and clean up. Prior to GC-NCI-MS, the polar analytes were derivatized using pentafluorobenzyl bromide. The performance of this analytical method has been revealed in fortification experiments by limits of detection ranging from 0.01 to 0.2 ng/L for, e. g., ibuprofen and 17α-ethinylestradiol, respectively. SPE may be the most rate determining step of the analytical procedure. Therefore, the performance of SPE disks and cartridges was additionally compared. Method evaluation demonstrated that even complex sample matrices, such as model wastewater or even synthetic humic acids, did not interfere with the quantification and identification of target analytes. The performance of the analytical method developed for water monitoring was further emphasized by the investigation of surface water of the River Saale and effluents of the wastewater treatment plant (WWTP) at Halle, Saxony-Anhalt, Germany. In those samples, acidic pharmaceuticals and corresponding metabolites occurred at 0.1 ng/L for clofibric acid up to 498 ng/L for bezafibrate. Technical nonylphenol and bisphenol A were found in every water sample. Meanwhile, 17α-ethinylestradiol was determined only in one WWTP effluent sample at 1 ng/L.

Keywords: Acidic pharmaceutical residues; Endocrine disrupting compounds; Derivatization; Pentafluorobenzyl bromide; Negative chemical ionization mass spectrometry (GC-NCI-MS)

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1 Introduction

Environmental pollution by bioactive substances such as endocrine disrupting compounds and pharmaceutical residues became apparent in the seventies [1]. Since that time, the environmental behavior of particularly endocrine disruptors (EDs) has been investigated in detail. This group of compounds includes natural and synthetic hormones (17α-ethinylestradiol, EE2) as well as industrial products such as technical mixtures of 4-nonylphenols (t-NP) and bisphenol A (BPA) with clear estrogenic effects on aquatic organisms [2, 3]. Otherwise, little is known about the ecotoxicological effects and health consequences of low-dose and long-term exposure of organisms to drug residues. Permanent introduction of these substances into the environment via municipal wastewater needs to be monitored by highly sensitive analytical methods. Current analytical protocols for EDs and drug monitoring use separate methods for the analysis of polar compounds of different chemical classes often associated with special usage [4 – 7]. For the analysis of polar drugs such as the β-blocker metoprolol, atenolol or propranolol, LC-MS-MS is the method of choice after enrichment of the analytes by solid phase extraction. Limits of quantification (LOQs) range from 5 to 50 ng/L, depending on the matrix [5]. For the detection of acidic drugs such as the analgetics ibuprofen and naproxen, LC-MS-MS under negative electrospray ionization conditions is increasingly used with LOQs between 0.15 and 500 ng/L. When LC-MS techniques are used for environmental monitoring, an appropriate control of the matrix influence during ionization is a quite crucial factor necessary to achieve the concentration range needed [8]. In particular, the detection of analytes at low concentrations in ground, tap, and drinking water requires either analyte enrichment from a larger sample volume, which consumes additional time, or a highly sensitive LC-MS-MS instrument.

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Neutral drugs such as the neuroleptics carbamazepine, diazepam or the polycyclic musk compounds Galaxolide® and Tonalide® can sensitively be detected by GC-MS [9, 10]. However, acidic drugs and endocrine disrupting compounds containing carboxyl and hydroxyl groups often demand a derivatization prior to GC-MS. Trialkylsilylation of OH-functionalized compounds with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [11] or N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [12] and esterification [13] of carboxylated compounds are most favored. However, silylation procedures are not always quantitative, particularly in cases of multiple functionalized analytes. Furthermore, trimethylsilyl derivatives (TMS) have a temporary stability due to hydrolyses and possible intramolecular rearrangements. Residues of the silylation agents can also affect the stationary phase of the GC capillary. Derivatization of phenolic compounds with pentafluorobenzoyl bromide and analysis by GC with an electron-capture detector or with negative chemical ionization mass spectrometry (GC-NCI-MS) proved to be highly sensitive to detect estrogens and phenolic xenoestrogens such as BPA, 17α- and 17β-estradiol, and nonylphenols at limits of detection (LODs) of 0.02, 0.10, and 0.05 ng/L, respectively [14].

Derivatization of both R-OH and R-COOH compounds is quantitatively possible using pentafluorobenzyl bromide (PFBBr). The PFB-ether and -ester can be detected sensitively by GC-ECD, GC-NCI-MS and LC-atmospheric electron capture ionization mass spectrometry, as has been shown for amino acids and DNA adducts from biological fluids [15].

Due to the high selectivity of this method, the efforts for sample preparation can be reduced to some extent [14], and even analytes from a complex soil matrix can be determined as reported for a fungicide metabolite by Dieckmann et al. [16]. Fenpropimorphic acid could be detected as a PFB derivative by GC-NCI-MS with ten times more sensitivity than methylester in a GC-MS analysis using electron impact ionization. Additionally, the high stability of the PFB derivative during the storage of analytical solutions was emphasized.

Reports on environmental monitoring of pharmaceutical residues using this promising method combination are rare. Therefore, in this study, a GC-NCI-MS based multi-component protocol for the trace determination of selected acidic drugs and endocrine disrupting compounds including analyte enrichment by SPE (disks or cartridges) and a derivatization using pentafluorobenzyl bromide is presented and discussed in detail.

2 Experimental

2.1 Chemicals and Materials

Bezafibrate, clofibric acid, diclofenac monosodium salt, ibuprofen, naproxen and β-estradiol diacetate (purity = 95%) were purchased from ICN Biomedicals (Aurora, Ohio, USA). Gemfibrozil was obtained from Promochem (Wesel, Germany). Technical 4-nonylphenol (Pestanal, purity = 94%) was purchased from Riedel-de Haën (Seelze, Germany), bisphenol A (purity grade = 97%) and 17α-ethinylestradiol (EE2) with a purity = 85% were obtained from Fluka (Zwijndrecht, The Netherlands). Pentafluorobenzyl bromide (PFBBr), sodium sulphate (Na_2SO_4), potassium carbonate (K_2CO_3), 85% sulphuric acid (H_2SO_4), acetone, hexane and methanol (SupraSolv) were all purchased from Merck (Darmstadt, Germany). 4n-Nonylphenol (4n-NP) was obtained from Dr. Ehrenstorfer (Augsburg, Germany); deuterium labeled bisphenol A (BPA d_{12}; purity: 95%) was synthesized at the University of Leipzig. 1,2,3,4-tetrachlorobenzene, used as syringe standard, was purchased from Sigma (Deisenhofen, Germany).

Pure water was obtained from the Modulab® Analytical purification system produced by Christ (Stuttgart, Germany). A model wastewater was prepared in accordance with DIN 38412 T24 (German Industry Norm, 1981) and contained casein, meat extract, urea and K_2HPO_4. Model wastewater as well as synthetic humic acids obtained from Roth (Karlsruhe, Germany), were used to study matrix effects. Dissolved organic carbon (DOC) was measured with a "HighTOC II" analyzer (Elementar Analysensysteme, Hanau, Germany).

The ENVI™-18 DSK SPE disks (octadecyl (C18) bonded phase) were obtained from Supelco (Bellefonte, PA, USA). Additionally, Empore™ SDB-XC (styrene divinylbenzene copolymer, diameter: 47 mm) extraction disks were kindly donated by 3M (St. Paul, Minnesota, USA). The disk-holder and the corresponding 6-port holder manifold as well as 4 mL vials with PTFE/neoprene septa were supplied by Supelco. Silanized 200 µL inserts and 2 mL vials were purchased from Agilent Technologies (Waldbronn, Germany). GF 52 glass-fiber filters were obtained from Schleicher and Schüll (Dassel, Germany).

Stock standard solutions of bezafibrate, clofibric acid, diclofenac monosodium salt, ibuprofen, gemifibrozil, naproxen, BPA and t-NP were prepared in methanol at a concentration of 1 µg/mL. Standard mixtures were obtained by dilution with methanol. Every sample was spiked before SPE with the internal standards 4n-nonylphenol (4n-NP), BPA d_{12} and estradiol diacetate in methanol to get concentrations of 100 ng/L each. These internal standards were used for quantification of technical NP, BPA, and EE2. The other target analytes were quantified by external calibration or standard addition, respectively, when real samples were analyzed.

2.2 Sampling and SPE Procedure

Ten water samples of the Saale river (DOC = 8 mg/L) were taken at urban sites in the city of Halle, located in the middle east of Germany on May 25, 2004. After acidification to pH 3 with concentrated H_2SO_4 the samples were filtered (GF52, 52 mesh glass-fiber filter) and extracted on the same day or kept at 4°C in the dark overnight. The stock solution of the model wastewater was prepared in accordance to DIN 38412 T24 (German Industry Norm, 1981. Contents per liter: 1.28 g peptone from casein, 0.88 g meat extract, 240 mg urea, 14 mg NaCl, 8 mg $CaCl_2 \cdot 2H_2O$, 0.06 g $MgSO_4 \cdot 6H_2O$ and 11.5 ml phosphoric acid (85%), final pH of 3.0). The medium was stored under sterile conditions in the dark at 4°C overnight. It was never stored longer than two weeks. A dilution with bidistilled water (50/50 v/v) gives a DOC of 125 mg/L, simulating the matrix of a wastewater treatment plant.

A solution of synthetic humic acid (10 mg/L; Roth, Karlsruhe, Germany) was prepared in bidistilled water. The resulting DOC of 3.9 mg/L is typical for natural waters where DOC varies widely from <1 to >50 mg/L, where the lowest values apply to ground water [17, 18].

For optimization and examination of different SPE materials, half a liter of model wastewater and water containing humic acids, respectively were spiked with 100 ng of each analyte and the internal standard mixture. The SPE disks were conditioned with 50 mL methanol and 30 mL bidistilled water acidified to pH 3 using diluted H_2SO_4. After sample extraction at a flow rate of 50 mL/min, a washing step with 10 mL water followed before the disk was dried

and eluted with 40 mL methanol. Before derivatization, methanol extracts were evaporated to dryness and redissolved in 1 mL acetone.

In comparison to the SPE disks, SPE glass cartridges packed with 100 mg LiChrolut® EN and 250 mg LiChrolut® C18 (Merck, Darmstadt, Germany) were applied in accordance with the procedure described previously [19]. The SPE cartridges were used to extract wastewater and river water.

2.3 Derivatization with Pentafluorobenzyl Bromide

All samples were derivatized with PFBBr following Nakamura et al. [20]. The 1 mL acetone solutions were treated with 100 µL of 10% aqueous potassium carbonate solution and subsequently with 100 µL of 5% PFBBr in acetone. After keeping the mixture at 60°C for 1 h, 1 mL of *n*-hexane and 0.5 mL of bidistilled water were added to remove the excessive reagent. Afterwards, the hexane layer was separated, dried with sodium sulphate, filtered and concentrated to a final volume of 200 µL. Prior to analysis, 2 µL 1,2,3,4-tetrachlorobenzene was added to every sample as a syringe standard to check the performance of the GC/MS instrument.

2.4 Instrument and Analytical Parameters

GC was performed using a 6890 A series gas chromatograph equipped with a mass-selective detector 5973 (Agilent Technologies, San Jose, USA). Injections (1 µL) were carried out in pulsed splitless mode at a temperature of 280°C at 172 kPa. The pulse time was 1.5 min. The injector was coupled to a retention gap (2.5 m 6 0.32 mm I.D.) and a HP-5MS capillary column (30 m 6 0.25 mm I.D., 0.25 µm film thickness, J&W Scientific Inc., Folsom, USA). Helium was used as carrier gas at a column flow of 1 mL/min. The GC oven temperature program started at 50°C. The temperature was maintained for 1 min and then increased at a rate of 10 K/min to 280°C, and held for 13 min. The MS parameters were as follows: interface temperature 280°C, source temperature 150°C, quadrupole temperature 100°C, solvent delay 11 min. The MS was operated in negative chemical ionization mode (NCI). The optimum flow rate of methane as the reagent gas was 4 mL/min (adjusted with octafluoronaphthalene). The multiplier voltage was 2200 V. For quantification, the GC-MS was operated in selected ion monitoring (SIM) mode. The target ions are listed in Tab. 1. Fragmentation pathways and matrix effects were studied using full scan mode in the mass range from 50 to 650 amu.

For the quantification of target analytes in real water samples, internal standardization or standard addition were applied. The linearity of the standard addition curves was demonstrated by correlation coefficients of $r^2 = 0.994$.

3 Results and Discussion

3.1 GC-NCI-MS Analysis

The negative chemical ionization is either based on an electron capture process or proton abstraction from the neutral molecule. In the case of the pentafluorobenzyl (PFB) derivatives of the target analytes, the dominant ion (equal to $[M-H]^-$; M = molecular mass of analyte) was found in nearly all NCI mass spectra as the result of a preferred loss of the pentafluorobenzyl radical. As an example, the

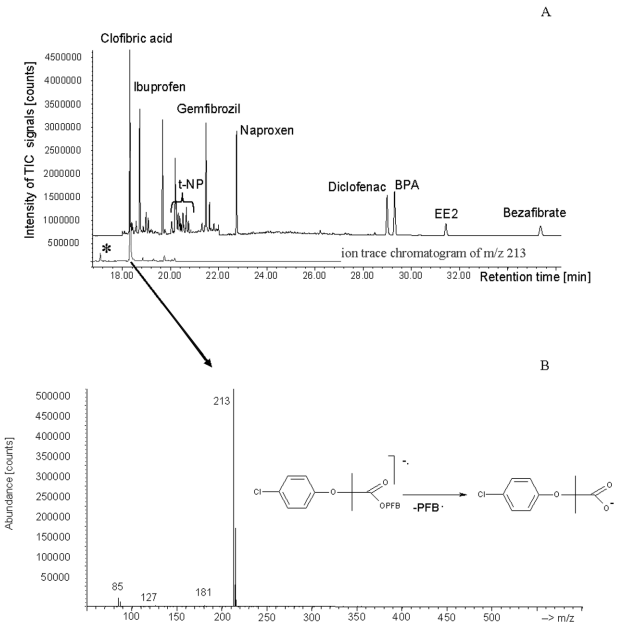
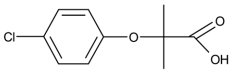
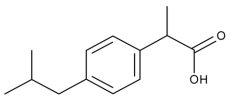
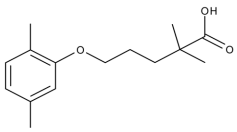
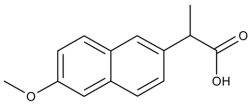
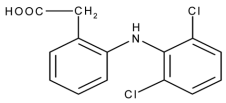
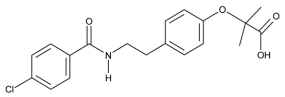
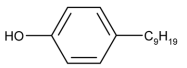
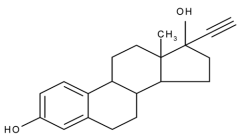
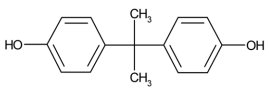


Figure 1. A: Total ion current chromatogram of the GC-NCI-MS analysis in full scan mode of water samples from the Saale river spiked with 50 ng/L of each analyte. The ion trace chromatogram at *m/z* 213 is characteristic of clofibric acid PFB ester. *: Accompanying signals at *m/z* 213 are caused by matrix components. Molecular ions of the unknown compounds are at *m/z* 374, 460, and 407. B: Full scan spectrum of clofibric acid after pentafluorobenzylation and fragmentation pathway.

NCI mass spectrum of the clofibric acid PFB ester is shown in Fig. 1. The characteristic, most abundant ion, $[M-H]^-$, is chosen for quantification by selected ion monitoring (SIM). The corresponding ^{13}C-ion $[M-H+1]^-$ or the chlorine isotope ion $[M-H+2]^-$ (in case of the chloro-substituted target analytes) was used as the qualifier ion together with some less abundant fragments (see Tab. 1). In order to ensure the identification of the analytes, additional substance characteristics as GC retention times and ion ratios were used, supported by isotope labeled internal standards if available (see Tab. 1). In the case of BPA, both hydroxyl groups were quantitatively derivatized. Under NCI conditions, the resulting elimination of one pentafluorobenzyl radical gives abundant ions at *m/z* 407 and 408.

In comparison to the other target analytes under study, the diclofenac pentafluorobenzyl ester showed a different fragmentation pattern under GC-NCI-MS conditions. Neither the theoretical pseudomolecular ion of the diclofenac derivative at *m/z* 475, nor the loss of the PFB moiety could be detected. Instead, the dominating ion was recorded at *m/z* 456, reflecting a loss of 19 amu. The isotopic pattern at *m/z* 456 revealed a dichlorinated compound (see Fig. 2). Since an abundant fluorine radical elimination is rather unlikely due to the high stability of F-C bonds, an intra-molecular cyclization forming 1-(2,6-dichlorophenyl)indoline-2-one is proposed as the favored reaction (see Fig. 2). Reddersen and Heberer [21] have already proved the formation of the PFB derivative of diclofenac by recording the intact molecular ion at *m/z* 475 by means of GC-MS with electron impact ionization (EI). The most abundant fragment was formed by elimination of the OCOPFB moiety. In that study, the less abundant ion at *m/z* 276 (3% relative intensity) already pointed to a probable intra-molecular cyclization during mass spectrometric decomposition. The ion at *m/z* 276, representing the dichlorophenyl indolineone structure [21], was found in the NCI mass spectrum of the

Table 1. Structures of analytes, GC retention times, target ions (a: quantifier, b: qualifier) and ion ratios (a:b) used for GC-NCI-MS analysis in selected ion monitoring mode (SIM) after derivatization with pentafluorobenzyl bromide.

Analyte	Structure	GC retention time [min]	NCI-MS(SIM) target ion [*m*/*z*]	Ion ratios of quantifier/ qualifier ions
Clofibric acid		17.12	213[a] 215[b] 214 85	1:0.33
Ibuprofen		17.53	205[a] 177[b] 206	1: 0.53
Gemfibrozil		20.27	249[a] 250[b] 125	1 : 0.16
Naproxen		21.54	229[a] 230[b]	1: 0.16
Diclofenac		27.57	456[a] 458[b] 276	1: 0.65
Bezafibrate		33.72	360[a] 362[b] 196	1: 0.35
t-NP and 4n-NP		18.56 – 19.54 (t-NP) 20.59 (4n-NP)	219[a] 220[b] 93	1: 0.19
EE2		29.90	Mono derivative 295[a] 296[b] 277	1: 0.17
BPA and BPA d_{12}		27.89 (BPA) 27.79 (BPA$d_{11/12}$)	bi derivative 407[a] , 408[b] 135, 93 (BPA) 418[a]/419[b] 141, 97 (BPA $d_{11/12}$)	1: 0.3 1: 0.9

diclofenac PFB derivative in this study as well. The isotopic pattern at *m*/*z* 456, 458, and 460 and their corresponding ion ratios of 1 : 0.6 : 0.1 (see Fig. 2) supported the suggestion that the dichlorophenyl moiety remains intact during fragmentation. Finally, it was proposed that the mass difference of 19 amu can be generated only from an intra-molecular water elimination and subsequent proton abstraction during the process of negative chemical ionization. By all means, the intra-molecular cyclization of diclofenac also seems to be a preferred reaction in solution and is probably responsible for decreasing the extraction yield of diclofenac under acidic conditions [21]. Even in the environment, Reddersen and Heberer [21] observed 1-(2,6-dichlorophenyl)indoline-2-one as a metabolite of diclofenac. In the actual study, an intra-molecular reaction of diclofenac in solution due to acidic conditions can be absolutely excluded since GC-NCI-MS studies were performed using the target analyte dissolved in acetone.

The performance of the entire method is depicted in Tab. 2. The reproducibility was verified by duplicates of three parallel samples prepared separately. The derivatives were stable for at least six days stored at 4°C in the dark, which was confirmed by repeated analysis. The appropriate chromatographic behavior of the PFB derivatives was demonstrated by the narrow peaks shown in Figs. 1 and 4. The excellent separation characteristic enables a reproducible determination of peak areas. The highly selective detection by GC-NCI-MS in

Table 2. Method performance parameters in dependence on the extraction medium used. LODs and LOQs were determined in accordance to [37]. Recoveries were determined at analyte concentrations of 100 ng/L each.

	LOD GC-NCI-MS analysis spiked pure water [ng/L]	SPE disks ENVI C18 DSK recovery [%]	% RSD (n = 3)	LOQ [ng/L]	SPE disks SDB-XC recovery [%]	% RSD (n = 3)	LOQ [ng/L]	SPE cartridge LiChrolut C18/ LiChrolut EN recovery [%]	% RSD (n = 3)	LOQ [ng/L]
Clofibric acid	0.01	83	8	0.1	65	6	0.1	62	17	0.1
Ibuprofen	0.01	62	6	0.2	50	4	0.3	88	19	0.1
Gemfibrozil	0.04	104	7	0.1	85	8	0.1	85	11	0.1
Naproxen	0.05	87	1	0.2	50	4	0.5	78	10	0.3
Diclofenac	0.06	14	28	0.8	28	12	0.5	15	10	0.6
Bezafibric acid	0.15	89	11	0.6	71	9	1.0	91	12	0.6
t-NP/4n-NP	25/0.1	115/87	13/10	40/0.1	83/77	12/11	45/0.5	119/44	15/17	40/0.2
EE2	0.2	83	8	0.5	78	7	0.5	75	10	0.5
BPA	0.05	45	10	0.5	57	12	0.5	71	18	0.2

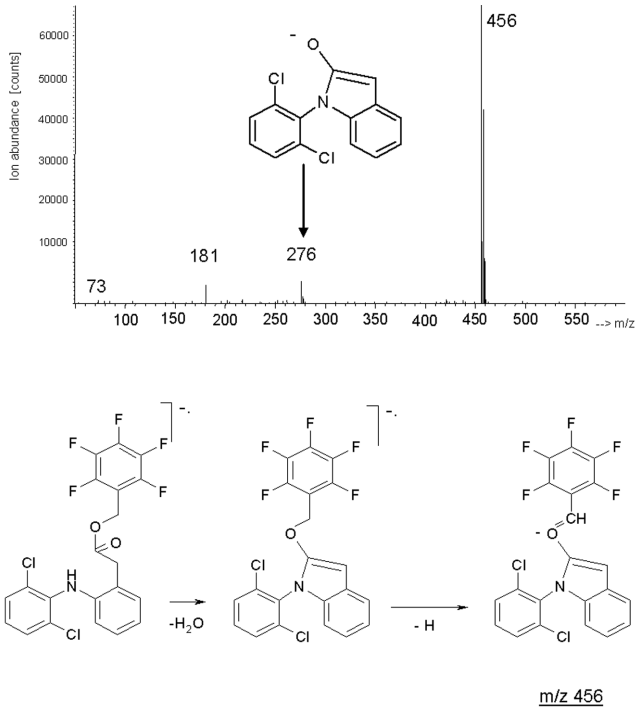


Figure 2. NCI-MS mass spectrum of the pentafluorobenzyl derivative of diclofenac and the fragmentation pathway proposed for the ion at *m/z* 456.

selected ion monitoring mode was reflected by signal-to-noise ratios of, e.g., 1200 for bezafibrate and 24000 for clofibric acid at 0.25 pg injected of each analyte. The instrumental limits of detection obtained for the derivatized target analytes were in the pg/L range (see Tab. 2, LOD). They allowed the trace detection of the target analytes even in complex matrices of fortification experiments and in real water samples. In recent studies, similar LODs of selected estrogens and xenoestrogens proved the high sensitivity of the applied analytical method. Nakamura et al. [20] reported a LOD of 4.6 ng/L for BPA. Kuch et al. [14] and Xiao et al. [22] determined a LOD of 0.2 ng/L for EE2 detected as a pentafluorobenzoyl derivative in drinking water and groundwater. This value is similar to the LOD obtained for the corresponding pentafluorobenzyl derivative presented in Tab. 2.

3.2 Performance of SPE Procedures

In spite of highly sensitive and selective detection by NCI-MS, the analysis of drugs in water samples requires the enrichment of the analytes. Apart from appropriate SPE cartridges, SPE disks are known as time-saving devices with high analyte capacity, and are suited for the extraction of large sample volumes, as reported for the determination of explosives and nitrosamines, xenoestrogens, and alkaloids from water as well as from biological fluids [23 – 24]. Current protocols for the determination of drugs and EDs use SPE cartridges based on octadecyl coated silica, divinylbenzene/N-vinylpyrrolidone or polystyrene/divinylbenzene adsorbents [7, 15].

In the present study, SPE disks, Empore™SDB-XC (polystyrene-divinylbenzene on a PTFE membrane) as well as C18-bonded silica on a porous glass fiber matrix (ENVI™-18 DSK) were examined in comparison to SPE cartridges [19]. Previous studies on EDs in water [25 – 29] were used as an initial point for the optimization of analyte enrichment with SPE disks. The target analytes (each at a concentration of 100 ng/L) were extracted after acidification of the model wastewater to pH 3. Table 2 shows the resulting recoveries of the target analytes from model wastewater determined by the entire protocol, including SPE, derivatization with PFBBr, and GC-NCI-MS analysis. In most cases, Empore™SDB-XC disks resulted in lower recoveries (mean recovery of 67%) compared to ENVI™ – 18 DSK disks (mean recovery of 77%), achieved with 40 mL elution solvent each. Relative standard deviations of both disks varied about 10%. Hence, the ENVI™-18 DSK disk seems well suited for routine analysis, particularly when compared with the mean recovery of 74% determined with the SPE cartridge. Remaining advantages of the SPE cartridge use are: the lower elution volume (10 mL), the reduced clogging tendency and the yield of cleaner extracts.

Diclofenac was not efficiently extracted from the model wastewater samples, neither by the SPE disks (recoveries of 14% and 28%) nor by the SPE cartridges filled with the sorbent mixture (recovery of 15%). In order to examine if the matrix is responsible for an inefficient extraction, the influence of humic acids was studied. For this purpose, synthetic humic acids were suspended in bidistilled water to give a DOC of 3.9 mg/L in order to simulate the matrix of ground or surface water [17, 18]. The effect of this matrix on the entire protocol was compared to bidistilled water spiked with the target analytes. Both samples were spiked with 100 ng/L of each analyte before applying SPE mixed bed cartridges. Thus, recoveries of diclofenac in humic acid matrix and pure water of 59% and 75% were deter-

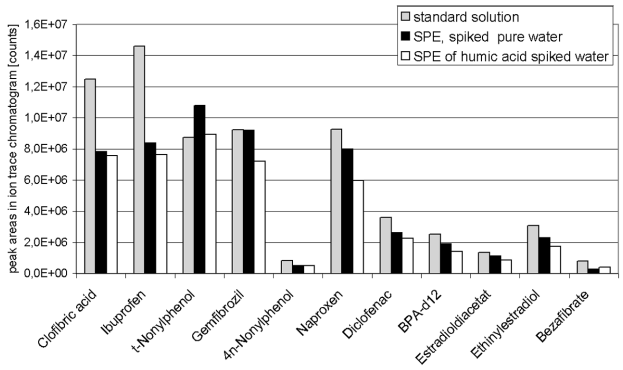


Figure 3. Influence of humic acids on the analyte recovery using SPE, derivatization and GC-NCI-MS (SIM) for analysis; standard solution: standard mixture in acetone derivatized and analyzed by GC-NCI-MS(SIM).

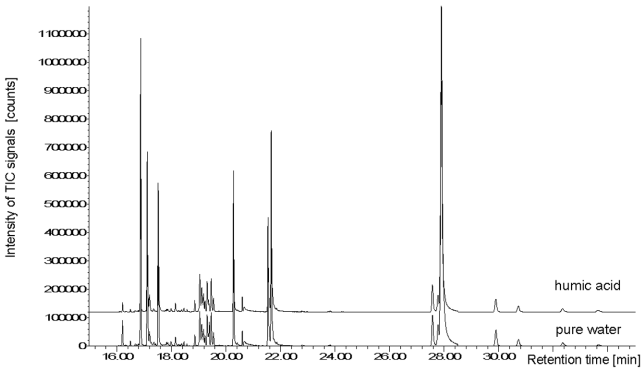


Figure 4. GC-NCI-MS (SIM) total ion current chromatograms of pure water samples and water samples spiked with synthetic humic acids. Both were spiked with 100 ng/L of each analyte (relative offset between both traces = 10%).

mined, respectively (see Fig. 3). Analyte loss may be also caused by intramolecular water elimination during extraction under acidic conditions, as suggested by Reddersen and Heberer [21].

In principle, co-derivatization of humic acids is possible. However, separation and identification of the target analytes were not interfered with because of the highly selective NCI-MS analysis in SIM mode. As shown in Fig. 4, no additional signals could be detected when compared to the pure water sample spiked with the target analytes.

As a consequence of the ubiquitous presence of t-NP in the environment [14, 19], recoveries above 100% resulted (see Tab. 2), probably caused by a contamination during the SPE procedure. The background concentration of tNP, permanently checked by analysis of blanks, was reproducible and allowed a LOD of 27 ng/L. Uncontrolled contaminations by t-NP, and partially by BPA, can occur when the samples encounter plastic material during sampling, storage or sample preparation.

3.3 Method Evaluation

The robustness of the entire procedure was clearly shown. The calibration curves obtained with this protocol were linear over a concentration range of 1 to 100 ng/L, with correlation coefficients (r^2) between 0.949 and 0.998. By means of the entire optimized protocol, LOQs of the analytes in model wastewater were lower than values recently published by Koutsouba et al. [31]. In order to compare LOQs determined in model wastewater with LODs determined in less complex matrices of other studies, an approximate relationship of LOQ $\cong 3 \times$ LOD [36] can be used. LODs reported for the determination of pentafluorobenzyl derivatives of several acidic pharmaceuticals in sewage were about 2 ng/L using SPE-GC-MS with electron impact ionization (EI). For a similar procedure, Sacher et al. [32] obtained LODs in tap water between 3.5 and 8.7 ng/L. Cathum et al. [33], operating in electron impact ionization mode, described LODs of about 2 ng/L for the PFB derivatives of natural hormones. Increasingly, LC-MS/MS techniques achieve LOQs between 5 and 25 ng/L [34]. Most problems in LC-MS arise from matrix molecules interfering with the ionization of target analytes. Ion suppression is a known effect since matrix components compete for ion formation. In contrast, the analytical protocol presented here has principally proved to be free from matrix interferences and to be sensitive and selective for the trace analysis of acidic pharmaceuticals and endocrine disruptors in water samples at a pg/L level.

Table 3. Concentration levels of selected acidic drugs and endocrine disruptors in water samples ($n = 10$) of the Saale river at Halle, Germany.

Target analyte	$n >$ LOQ	Min	Max	Mean
Clofibric acid	9	0.1	118.5	14.7
Ibuprofen	10	0.1	33.6	6.8
t-NP	10	118.0	433.0	234.0
Gemfibrozil	8	0.2	34.5	5.9
Naproxen	7	0.3	92.9	15.8
Diclofenac	10	0.4	245.3	32.4
BPA	10	42.0	417.0	156.0
Bezafibrate	5	1.0	498.1	130.2

3.4 Investigations on Surface Water Samples

Xenoestrogens and pharmaceuticals in the water of the Saale river were determined at average concentrations ranging from 6 to 130 ng/L (see Tab. 3). EE2 was detected only in one sample at 0.8 ng/L, whereas all the other target analytes were found in nearly every surface water sample. The maximum levels for nonylphenols at 433 ng/L and for BPA at 417 ng/L were found at the effluent of the wastewater treatment plant in Halle (Saxony-Anhalt, Germany, 250 000 inhabitants). In the Saale river, diclofenac concentrations ranged from 0.4 to 245 ng/L (see Tab. 3). Average concentrations of clofibric acid and ibuprofen were 14.7 and 6.8 ng/L, respectively. The contamination of surface water depends strongly on the performance of river-adjoining water treatment plants and the amounts of drugs released into wastewater. These results matched those found in water samples of the Danube and Amper rivers [35]. There, diclofenac concentrations ranged from 20 to 360 ng/L. For ibuprofen and clofibric acid, concentrations below 10 ng/L were observed [35]. Investigations of the Detroit river in Canada [36] indicated higher concentrations of drugs, with median values of 141 ng/L for ibuprofen, 26 ng/L for diclofenac and 207 ng/L for naproxen. Maximum concentrations were detected at 790, 42, and 551 ng/L, respectively.

The pollution profile of clofibric acid, diclofenac and bezafibrate downstream the Saale river (see Fig. 5) indicated at sampling site B, the estuary of the Saale and Weisse Elster rivers, indicates a noticeable input of drug residues originating from the city of Leipzig (Sax-

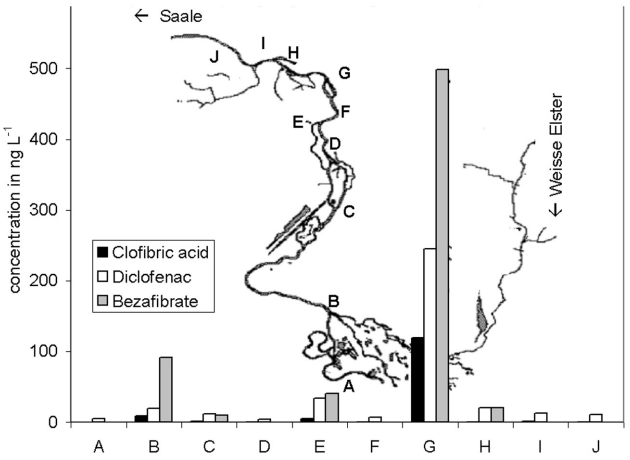


Figure 5. Distribution of bezafibrate, diclofenac and clofibric acid downstream the Saale river. Sampling sites B: Estuary of the Saale and Weisse Elster rivers, G: Effluent of the WWTP at Halle (Saxony-Anhalt, Germany). Concentration values of sites A, C, D, F, I, and J were magnified by factor 10. (Background map GIS+ by IT-Consult Halle, 2002 – 2007).

ony, Germany, 500 000 inhabitants). The remarkable concentrations at site G were caused by the effluent of the wastewater treatment plant of the city of Halle.

The investigation of real water samples proved the feasibility of the entire protocol for water analysis. Particularly, in combination with the selective NCI-MS detection, additional clean-up can be reduced or even avoided when ground water and surface water with DOC < 1 mg/L are to be analyzed.

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