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Determination of Polar Drug Residues in Sewage and Surface Water Applying Liquid Chromatography−Tandem Mass Spectrometry

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A simple and rapid method is presented for the trace-level analysis of 10 polar pharmaceutical residues in various types of water samples from the aquatic environment. Using this method, the pharmaceuticals and several drug metabolites can be analyzed in drinking and surface waters and in wastewater (treated and untreated sewage) at concentrations down to 0.01 μg/L. Samples are prepared by a simple in situ derivatization enabling the preconcentration of very polar metabolites by automated solid-phase extraction. The analytes were separated by liquid chromatography with tandem mass spectrometric detection and quantified by comparison with an internal standard. Limits of quantification were between 0.01 and 0.02 μg/L for three phenazone-type pharmaceuticals, six of their metabolites, and the antiepileptic drug carbamazepine. Except for dimethylaminophenazone, recoveries for all analytes were between 87 and 117% for raw and purified sewage, groundwater, and surface and drinking water. Investigations of some environmental samples revealed that sewage and surface water treatment causes a slight reduction of the concentrations of some analytes whereas other compounds were persistent during water treatment. Thus, some compounds were detected at the low-microgram per liter level in sewage effluents of wastewater treatment plants in Berlin (Germany) and were also found at high-nanogram per liter concentrations in Berlin surface water samples.

The fate of residues of pharmaceuticals, personal care products, and endocrine disrupting compounds has been recognized as an emerging issue of environmental research.[1−4] Wastewater treatment plants (WWTPs) receive a large spectrum of organic contaminants, which are in part eliminated during treatment.[1,4−6] Nevertheless, several persistent organic chemicals, such as some persistent pharmaceutical residues, are not completely removed during conventional sewage treatment. Discharges of municipal sewage effluents are therefore considered as a main source of environmental pollution by pharmaceutical residues,[1,3] and residues of pharmaceuticals have been detected up to the microgram per liter level in municipal sewage.[1,3,7] Due to dilution, degradation, and sorption, lower concentrations of such contaminants can also be expected and have already been detected in treated sewage and surface waters and groundwater.[1]

Today's instrumental analytical methods apply gas chromatography (GC)[8,9] or liquid chromatography (LC)[7,10,11] in combination with detection by mass spectrometry (MS). Especially for polar pharmaceuticals, LC−MS is the method of choice for the detection.[7,11] In comparison to GC analysis, run times and sample preparation times are reduced due to the fact that no derivatization prior to analysis with LC is necessary. LC with single mass spectrometric analysis offers good sensitivity, but when very complex matrixes such as sewage are investigated, insufficient selectivity often impairs the unequivocal identification and quantification of the analytes. LC with tandem mass spectrometric detection (LC−MS/MS) promises both high sensitivity and much better selectivity for the unambiguous identification and quantification of environmental contaminants at trace-level concentrations.[12−14] Recently published methods for the determination of pharmaceutical residues in environmental samples use electrospray ionization (ESI). In general, ESI provides lower limits of detection than atmospheric pressure chemical ionization (APCI), but in case of

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(1) Heberer, Th. *Toxicol. Lett.* **2002**, *131*, 5−17.
(2) Stan, H. J.; Heberer, Th. Pharmaceuticals in the aquatic environment, In *Dossier Water Analysis*; Suter, M. J. F., Ed.; *Analusis* **1997**, *25*, M20−M23.
(3) Kümmerer, K. *Chemosphere* **2001**, *45*, 957−969.
(4) Daughton, C. G.; Ternes, T. A. *Environ. Health Perspect.* **1999**, *107* (Suppl 6), 907−938.
(5) Ternes, T. A.; Stumpf, M.; Mueller, J.; Haberer, K.; Wilken, R.-D.; Servos, M. *Sci. Total Environ.* **1999**, *225*, 81−90.
(6) Ternes, T. A. *Water Res.* **1998**, *32*, 3254−3260.
(7) Ternes, T.; Bonerz, M.; Schmidt, T. *J. Chromatogr., A* **2001**, *938*, 175−185.
(8) Ternes, T. *Trends Anal. Chem.* **2001**, *20*, 419−434.
(9) Zwiener, C.; Glauner, T.; Frimmel, F. H. *J. High Resolut. Chromatogr.* **2000**, *23*, 474−478.
(10) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.
(11) Lopez de Alda, M.; Diaz-Cruz, S.; Petrovic, M.; Barcelo, D. *J. Chromatogr., A* **2003**, *1000*, 503−526.
(12) Baronti, C.; Curini, R.; D'Ascenzo, G.; Di Corcia, A.; Gentili, A.; Saperi, R. *Environ. Sci. Technol.* **2000**, *34*, 5059−5066.
(13) Lagana, A.; Bacaloni, A.; Fago, G.; Marino, A. *Rapid Commun. Mass Spectrom.* **2000**, *14*(6), 401−407.
(14) Sole, M.; Lopez de Alda, M.; Castillo, M.; Porte, C.; Ladegaard-Pedersen, K.; Barcelo, D. *Environ. Sci. Technol.* **2000**, *34*, 5076−5083.

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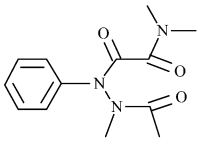
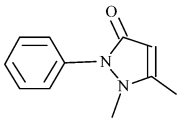
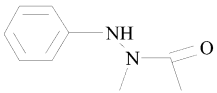
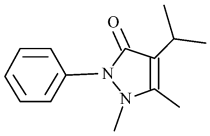
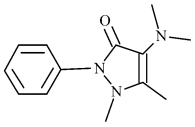
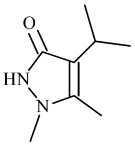
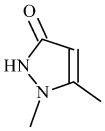
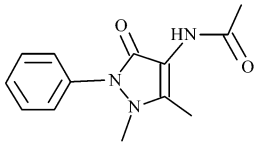
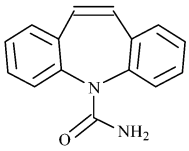
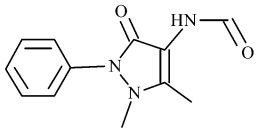
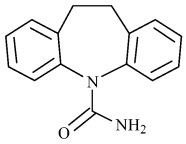
Table 1. Structures and Origin/Use of the Selected Pharmaceuticals and Metabolites

Structure	compound *Origin/use* Mol. weight CAS-Nr.	compound *Origin/use* Mol. weight CAS-Nr	Structure
	AMDOPH *Metabolite* 263.29 519-65-3	Phenazone *Analgesic* 188.23 60-80-0	
	AMPH *Metabolite* 164.2 38604-70-5	Propyphenazone *Analgesic* 230.31 479-92-5	
	DMAA *Analgesic* 231.29 58-15-1	PDP *Metabolite* 154.21 --------	
	DP *Metabolite* 112.13 3201-28-3	AAA *Metabolite* 245.28 83-15-8	
	Carbamazepine *Anti-seizure* 236.27 298-46-4	FAA *Metabolite* 231.26 1672-5-8-8	
	DHC *Surrogate* 238.28 3564-73-6		

complex matrixes, such as sewage, false negative results due to matrix ionization suppression effects might be obtained.[7,15−17] APCI could in such cases act as a suitable tool for sensitive and precise quantification even if appropriate surrogates for matrix compensation are not available or additional cleanup steps shall be omitted.

Analgesic and antipyretic pharmaceuticals, namely, dimethylaminophenazone (DMAA), phenazone, propyphenazone, and metamizole (*N*-methyl-*N*-(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)aminomethansulfonic acid; sodium salt also known as dipyrone) have widely been used in German medical care. After ingestion, they are partly excreted in their native form or after biotransformation as metabolites. Some of these pharmaceuticals have already been detected in sewage and surface water samples.[1,18,25] Recently, oxidative products of dimethylaminophenazone, 1-acetyl-1-methyl-2-dimethyloxamoyl-2-phenylhydrazide (AMDOPH), and 1-acetyl-1-methyl-2-phenylhydrazide (AMPH) have been identified in groundwater samples.[19] After human metabolism of metamizole, acetoaminoantipyrine (AAA) and formylaminoantipyrine (FAA) are excreted[20,21] and the microbiological metabolites 1,5-dimethyl-1,2-dehydro-3-pyrazolone (DP)[22] and 4-(2-methylethyl)-1,5-dimethyl-1,2-dehydro-3-pyrazolone (PDP)[23] could also appear in the aquatic environment (see Table 1).

In this study, the development and performance of a robust, reproducible, reliable, and sensitive analytical method for the analysis of pharmaceuticals and their metabolites in waste and

(15) Miao, X.-S.; Koenig, B. G.; Metcalfe, C. D. *J. Chromatogr., A* **2002**, *952*, 139−147.
(16) Zuehlke, S.; Duennbier, U.; Heberer, Th. *J. Sep. Sci.*, in press.
(17) Christian, T.; Schneider, R. L.; Färber, H. A.; Skutlarek, D.; Meyer, M. T.; Goldbach, H. E. *Acta Hydrochim. Hydrobiol.* **2003**, *1*, 36−44.
(18) Heberer, Th.; Duennbier, U.; Reilich, Ch.; Stan, H. J. *Fresenius Environ. Bull.* **1997**, *6*, 438−443.
(19) Reddersen, K.; Heberer, T.; Duennbier, U. *Chemosphere* **2002**, *49*, 539−544
(20) Carretero, I.; Vadillo, M.; Laserna, J. J. *Analyst* **1995**, *120*, 1729−1732
(21) Schmidt, R.; Brockmeyer, R.; *Vom Wasser* **2002**, *98*, 37−54.
(22) Sauber, K.; Müller, R.; Keller, E.; Eberspaecher, J.; Lingens, F. *Z. Naturforsch.* **1977**, *32c*, 557.
(23) Lingens, F.; Blecher, R.; Blecher, H.; Blobel, F.; Eberspaecher, J. *Int. J. Syst. Bacteriol.* **1985**, *35*, 26.

surface water and groundwater samples applying LC−APCI-MS/MS is reported. The method enables the evaluation of the removal of these pharmaceutical residues by conventional and novel sewage treatment processes[24] and the investigation of their fate in the environment.

EXPERIMENTAL SECTION

Materials. Reference substances and the surrogate standard compound dehydrocarbamazepine (DHC) were obtained from Sigma-Aldrich (Steinheim, Germany) and Ferak (Berlin, Germany). AMDOPH and DP were commercially synthesized by Witega Laboratorien Berlin-Adlershof GmbH (Berlin, Germany), and PDP was obtained after microbial degradation of propyphenazone.[26] Standard solutions in 2-propanol (0.1−100 μg/mL) were stored at 4 °C. Bakerbond Octadecyl (C18) extraction columns (6 mL) packed with 1 g of adsorbent, methanol, acetonitrile, potassium carbonate, and formic acid were obtained from Baker (Deventer, The Netherlands). All solvents utilized were HPLC grade or of higher quality.

Sample Collection and Storage. Water samples were collected in glass bottles and, as far as possible, analyzed within 24 h or stored at 4 °C for less than 2 days. Larger numbers of samples might not always be analyzed immediately, necessitating a safe storage of the samples to exclude significant analyte losses. Despite sample cooling, the extraction should be carried out within a few days to avoid analyte losses. Sewage samples were analyzed as soon as possible to avoid further microbial degradation. Storing of the methanolic eluates after solid-phase extraction (SPE) at 4 °C for 1−2 weeks still yielded recoveries of ~100% for all analytes and the surrogate for all tested sample types.

Daily composite samples were taken from the influents and the effluents of the municipal WWTP in Ruhleben located in the central western districts of Berlin, Germany. This plant receives the wastewater from a large combined sewer. It is therefore prone to stormwater impact and also receives limited amounts of industrial effluents. The aerated biological process of the conventional WWTP is operated with a primary sedimentation. The sewage is purified by mechanical and an extended biological (activated sludge) sewage treatment for biological phosphorus and nitrogen removal. Thus, the treatment train of the WWTP also includes a nitrification and a denitrification step with sludge recirculation. During dry weather conditions, more than 200 000 m^3 of sewage per day are processed by this WWTP with an approximate residence time of up to 32 h. The 24-h composite samples of the raw and treated sewage water were taken every 5 days from June 4 to July 14, 2002. The influent and the effluent samples were collected with a time shift taking into account the residence time of the sewage in the WWTP.

Surface water sampling sites were located at a surface water treatment plant (SWTP) used for phosphate elimination of surface water under the strong influence of municipal sewage discharges from the WWTP in Berlin−Schönerlinde. Per year, ~80 million m^3 of surface water is treated by flocculation with iron sulfate. After passing the SWTP, the water flows into Lake Tegel. Water was collected from the influents and the effluents of the SWTP by daily or weekly composite sampling (September 23, 2001 to August 18, 2002) again taking into account the residence time of the water inside of the plant.

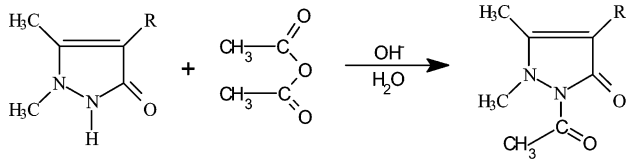


Figure 1. Reaction pathway of the pyrazolones with acetic anhydride.

Additionally, nine stream or lake samples were taken (November 23 to December 4, 2001) to get a first overview on the degree of the surface water concentrations of these analytes in Berlin. All of these samples were taken as grab samples.

Preparation of Samples and Standards. For external standard calibration, samples of purified water (250 mL) were fortified with a multicompound standard solution resulting in individual concentrations of 0.02, 0.04, 0.1, 0.4, 1, 5, and 10 μg/L. Sample analysis was done using 250 mL of the filtered WWTP influent or 250 mL of nonfiltered water samples (groundwater, sewage effluents, and surface or drinking water). For quantification, an internal standard solution (25 μL of 10 μg/mL DHC in 2-propanol) was added to all samples, blanks, and fortified samples, giving a concentration of 1 μg/L.

Solid-phase extraction of DP and PDP was not satisfactory. Therefore, an in situ derivatization was found to be necessary. One gram of potassium carbonate was added to the sample, and then 2.5 mL of acetic anhydride was dissolved by shaking the solution for 30 s. DP and PDP were quantitatively acetylated (Figure 1) and then extractable by SPE. AMDOPH, AMPH, FAA, AAA, carbamazepine, phenazone, propyphenazone, and DMAA were not acetylated by this in situ derivatization but are extractable without transformation. Sample extraction was performed using RP-C18 cartridges and an automated extraction system (Autotrace SPE Workstation) from Zymark (Hopkinton, MA). After conditioning the cartridges twice with 10 mL of methanol and a buffer solution (1 L of distilled water with 4 g of potassium carbonate and 10 mL of acetic acid anhydride; pH ~5.4), respectively, 250 mL of the samples was percolated through the cartridges at a flow rate of 10 mL/min. Then the cartridges were washed with 10 mL of the above-mentioned buffer solution. After drying for 50 min by nitrogen flush, the cartridges were eluted directly into 1.5-mL sample vials, using 1.0 and 0.6 mL of methanol. Thus, ~0.7 mL of the eluate was collected in the vials. Finally, 10 μL of the eluate was injected and analyzed by LC−MS/MS.

LC−MS/MS Analysis. The LC measurements were performed using a Waters 2690 LC instrument (Waters, Milford, MA). Liquid chromatographic separation was carried out at room temperature using a SymmetryShield RP8 column (2.1 × 150 mm; 3,5 μm, Waters) and a RP1 gard column (2 × 10 mm UltraSep ES; SEPSERV, Berlin, Germany). For the separation of the analytes, isocratic liquid chromatography was applied. Thus, the total time of the LC−MS/MS method could be reduced to 9 min, enabling a high sample throughput. The composition of the mobile phase was 15% acetonitrile, 38% methanol, and 47% water. The

(24) Zuehlke, S.; Duennbier, U.; Gnirss, R.; Lesjean, B.; Buisson, H. Long-term comparison of trace organics removal performances between conventional and membrane activated sludge processes. Proceedings Water Environment Federation Technical Conference (WEFTEC) 2003, Los Angeles, CA, October 11−15, 2003.

(25) Heberer, T.; Reddersen, K.; Mechlinski, A. *Water Sci. Technol.* **2002**, *46*, 81−88.

(26) Zuehlke, S.; Duennbier, U.; Heberer, Th. *J. Chromatogr., A* **2004**, *1050*, 201−209.

Table 2. Retention Times, Precursor Ions, Product Ions, and MRM Conditions Used for LC−MS/MS Measurements

compound	retention time (min)	precursor ion (m/z)[a]	cone voltage (V)	collision energy (V)[b]	product ions (m/z)
AMDOPH	3.06	264.0	22	20	72.0 $(C_3H_6NO)^+$
				9	222.0 $(M-C_2OH_3+2H)^+$
AMPH	4.07	164.9	22	17	91.9 $(M-C_3NOH_7+H)^+$
				28	65.1 $(C_3N_2H)^+$
DMAA	2.30	232.1	27	12	113.0 $(M-C_6H_5-C_2H_6N+H)^+$
				12	111.0 $(M-C_6H_5-C_2H_6N-2H+H)^+$
DP (acetylated)	3.06	155.0	18	10	112.9 $(M-C_2OH_3+2H)^+$
				30	68.9 $(C_4OH_5)^+$
carbamazepine	5.25	237.1	33	18	194.0 $(M-CNOH_2+H)^+$
				40	165.0 $(M-CNOH_2-NH-CH_2+2H)^+$
phenazone	2.87	189.1	40	30	56.0 $(C_3NH_6)^+$
				20	147.0 $(M-C_2OH_2+H)^+$
propyphenazone	5.53	231.1	45	32	56.0 $(C_3NH_6)^+$
				22	189.0 $(M-C_3H_6+H)^+$
PDP (acetylated)	5.23	197.0	19	12	155.0 $(M-C_2OH_3+2H)^+$
				24	112.9 $(M-C_2OH_3-C_3H_6+H)^+$
AAA	2.56	246.1	30	15	228.1 $(M-H_2O+H)^+$
				28	82.9 $(C_4NOH_5)^+$
FAA	2.56	232.1	30	12	214.2 $(M-H_2O+H)^+$
				18	82.9 $(C_4NOH_5)^+$
IS DHC	5.60	239.1	33	18	194.0 $(M-CNOH_2-2H+H)^+$
				40	165.0 $(M-CNOH_2-NH-CH_2-2H+2H)^+$

[a] All precursor ions $(M + H)^+$. [b] Collision energies for the individual product ions.

flow rate was set to 0.2 mL/min without any split before entering the source. The addition of buffers (ammonium acetate or ammonium hydroxide in varying concentrations) to the mobile phase caused a dramatic decrease of the responses of the analytes due to lower ionization ratios and was therefore omitted.

Mass spectrometric measurements were performed using a Quattro micro tandem mass spectrometer (Micromass, Altincham, U.K.) equipped with an APCI source operated in positive ionization mode. High-purity nitrogen was used for desolvation and nebulization. Collision gas was argon (99.998%). Desolvation was performed at 250 °C with a desolvation gas flow of 520 L/h and a cone gas flow of 45 L/h. The source temperature was set to 105 °C to avoid solvent recondensation. MS/MS parameters were optimized in continuous flow mode injecting 5 pg/μL standard solutions in water/acetonitrile (1:1) at 200 μL/min. For all of the analytes, the precursor ions used for selected reaction monitoring (SRM) were the corresponding protonated molecular ions (Table 2). After recording the individual product ion spectra for all analytes (example for PDP is shown in Figure 2), suitable SRM transition ions were chosen for multiple reaction monitoring (MRM) analysis. Precursor and product ion masses as well as the individual cone and collision energy voltages applied for the analysis of the pharmaceutical residues are compiled in Table 2. Data acquisition was carried out using different retention time windows. Dwell times for SRM were set to 0.05−0.25 s, depending on the intensity of the product ions for each transition.

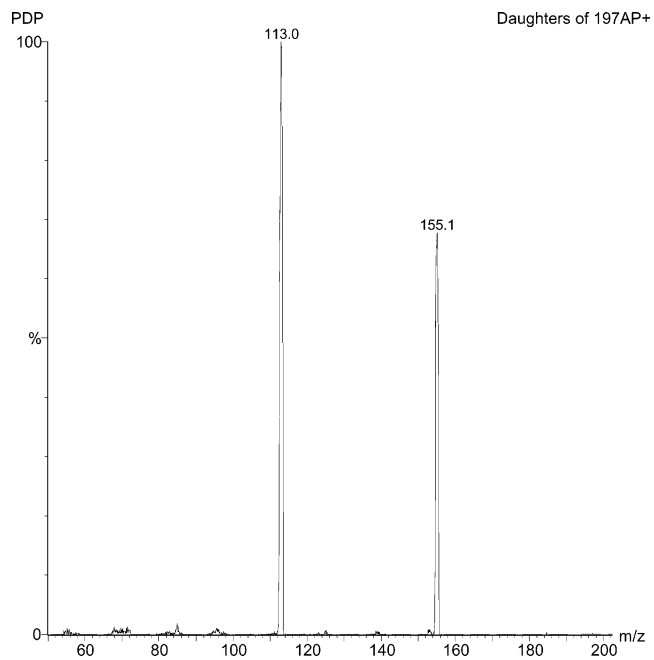


Figure 2. Product ion spectrum of acetylated PDP. Collision energy, 20 V.

RESULTS AND DISCUSSION

Selection of the Ionization Mode. ESI-MS-based methods have been commonly reported as being suitable for the analysis of pharmaceutical compounds in environmental water samples.[7,11,15] Hereby often internal quantification is necessary since matrix-dependent peak suppression or enhancement occurs if matrix-containing samples are analyzed.[7,17] For almost all of the investigated substances, ESI led to higher peak intensities than APCI. Nevertheless, ESI signals of the very polar compounds investigated in this study were strongly influenced by matrix compounds occurring in the samples. A reliable ESI method applicable for the accurate quantification of all contaminants in all possible types of environmental water samples would require suitable (coeluting) surrogates for every single analyte to compensate for signal suppression caused by coeluting matrix compounds. Especially for treated and untreated sewage, recoveries far below 100% were observed due to matrix interference problems in the ESI source.

Table 3. LOQs, Correlation Coefficients (0.02−10 μg/L), and SPE Column Recoveries (%)

compound	correlation coefficient	SPE column recovery	LOQs (μg/L)
AMDOPH	0.999	108	0.01
AMPH	0.997	119	0.02
DMAA	0.995	104	0.02
DP	0.997	nd[a]	0.02
carbamazepine	0.999	96	0.01
phenazone	0.999	107	0.02
propyphenazone	0.999	103	0.02
PDP	0.995	nd[a]	0.02
AAA	0.999	119	0.02
FAA	0.999	110	0.02

[a] nd, not determined because derivatized reference compounds are not available.

Table 4. Analyte Recoveries (%)[a] and RSDs (%) of All Analytes Added to Four Different Types of Aqueous Matrixes ($n \geq 5$)

	drinking water		surface water		WWTP effluent		WWTP influent	
compound	rec	RSD	rec	RSD	rec	RSD	rec	RSD
AMDOPH	95	5	105	10	98	8	99	2
AMPH	87	7	86	8	92	10	106	5
DMAA	103	9	17	4	72	18	133	4
DP	99	5	100	8	101	8	115	15
carbamazepine	100	5	100	8	100	11	106	5
phenazone	99	5	92	9	101	11	107	2
propyphenazone	94	6	91	8	94	8	106	4
PDP	107	5	99	7	114	8	117	9
AAA	101	6	95	12	99	11	106	11
FAA	108	3	92	12	102	9	100	5

[a] Adjusted by DHC values to compensate variations of the volume of the SPE extracts.

On the other hand, APCI provides a matrix-independent ionization resulting in recoveries of ∼100% (Table 4). Thus, sensitive detection and reliable external quantification could also be achieved for complex matrixes such as raw and treated sewage.

In Situ Derivatization. Sensitive and selective methods for the determination of pharmaceutical residues in environmental water samples, in particular sewage, are based on GC/MS or LC−MS following concentration using SPE. The compounds selected for this study are very polar and would at least require derivatization prior to their sensitive analysis by GC/MS. Such procedures are often very time-consuming. In contrast, LC with mass spectrometric detection provides sufficient sensitivity and selectivity for most of the analytes without derivatization. Nevertheless, very polar analytes or compounds with low molecular weights, such as DP and PDP, may not be extractable from water samples. Therefore, an in situ derivatization is necessary to decrease the polarity and to increase the molecular weights of these substances by acetylation (shown in Figure 1 and described in section Preparation of Samples and Standards). Derivatized DP and PDP are easily extractable at pH <5.5, and due to the higher masses of the molecules, detection is more sensitive.

Aminoantipyrine (AA), another minor metabolite of the pharmaceutical metamizole, is also influenced by this derivatization resulting in the formation of AAA. Thus, all results for AAA also include AA determined as AAA. Derivatization of AA, DP, and PDP is complete because equivalent amounts of AAA were formed from AA and neither native DP nor PDP was found after acetylation.

Limits of Quantification (LOQs). For each of the investigated compounds, the determination of the LOQ was based on the signal-to-noise (S/N) ratio of the analyte peak and the baseline close to this peak obtained from analyses of field samples (Tables 3). The LOQ was determined as the concentration with a minimum S/N ratio of 10. This procedure was performed for each type of aqueous matrix to be analyzed. For the determination of the LOQs, noncontaminated drinking water and river water samples were spiked at concentrations in the range of 0.001−0.1 μg/L using a mixture of standard compounds. LOQs in WWTP influent and effluent samples could not be measured for most of the compounds because of their ubiquitous occurrence in all of these samples. The LOQs for sewage were calculated based on the signal intensities and noise levels near the peaks and were identical to those determined for drinking and surface water samples. Thus, for all types of water samples, LOQs were between 0.01 and 0.02 μg/L for all analytes (Table 3). All calibration graphs were linear (Table 3) from the LOQs up to concentrations of 10 μg/L.

Method Recoveries and Accuracy. Analyte recoveries were determined by adding a multicompound standard mixture to previously analyzed samples at environmental concentrations. Thus, noncontaminated drinking water and river water samples were spiked at individual concentrations of 1 μg/L using a mixture of standard compounds (Table 4). Recoveries for sewage influent and effluent samples were determined in spiking experiments carried out with original samples after adding the standard compounds (standard addition method) resulting in concentrations 1 μg/L higher than those detected in the nonspiked samples. For sewage, the final recoveries were calculated by subtracting the amounts of the analytes detected in the nonspiked samples from those measured for the fortified recovery samples (Table 4). Not less than five recovery experiments were performed for each type of aqueous matrix. External calibration over a range of 0.02−10 μg/L was used alongside the internal standard compound DHC. Thus, slight deviations in sample extract volumes could be compensated by this surrogate. Even for different types of water samples, the recoveries were always ∼100% for all analytes (Table 4). Unacceptable recovery for DMAA in surface water samples might have resulted from fast degradation processes occurring during sample preparation. Much better recoveries (72−133%) were observed for drinking water and sewage. The use of DMAA in Germany was already prohibited in 1978; therefore, it was never detected in the investigated WWTP influent and effluent samples as well as in surface water. Thus, the low recovery for this compound in surface water samples will not lead to any problems in quantitation. Residues of DMAA were in the past caused by spills from a former production plant. DMAA and its metabolites reached the Berlin groundwater by infiltration of contaminated surface water and are still relevant for some drinking water production plants today.[19,26]

Analytical Application. The fate of the pharmaceutical residues in a municipal wastewater treatment plant and an enhanced effluent treatment facility (SWTP) was investigated to

Table 5. Summary of Analytical Results Obtained from the Investigations at the WWTP Ruhleben (Berlin, Germany) (June 4 to July 14, 2002; *n* = 12)

		influent			effluent		
compound	LOQs (μg/L)	values > LOQ	max (μg/L)	med (μg/L)	values > LOQ	max (μg/L)	med (μg/L)
AMDOPH	0.01	12	0.71	0.55	12	0.73	0.60
AMPH	0.02	12	0.15	0.11	12	0.19	0.11
DMAA	0.02	0			0		
carbamazepin	0.01	12	2.00	1.45	12	2.10	1.65
phenazone	0.02	12	0.45	0.35	12	0.41	0.33
propyphenazone	0.02	12	0.18	0.12	12	0.17	0.13
PDP	0.02	9	0.04	0.02	9	0.03	0.02
AAA	0.02	12	8.80	6.65	12	7.00	5.30
FAA	0.02	12	1.90	1.30	12	2.00	1.40

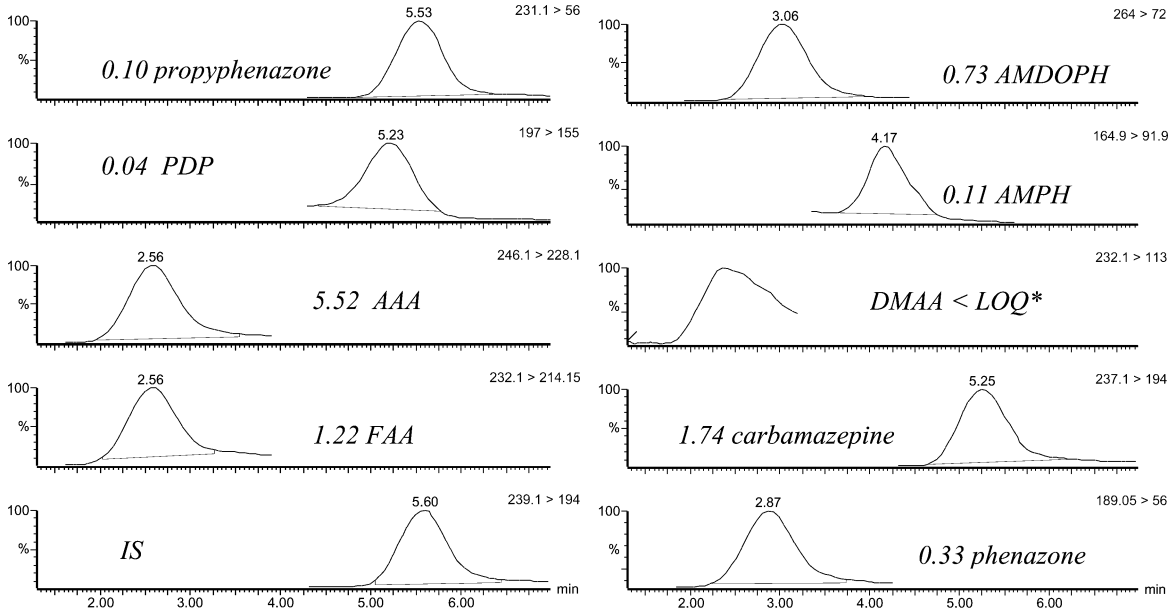


Figure 3. LC−MS/MS SRM chromatograms for all analytes and the surrogates in a WWTP influent sample. Concentrations, μg/L; only the most intense trace is shown. * baseline shift normalized to 100%, no distinct analyte peak.

Table 6. Summary of Analytical Results Obtained from the Investigations of Samples Collected from the SWTP Tegel (Berlin, Germany) (September 23, 2001 to August 18, 2002; *n* = 13)

		influent			effluent		
compound	LOQs (μg/L)	values > LOQ	max (μg/L)	med (μg/L)	values > LOQ	max (μg/L)	med (μg/L)
AMDOPH	0.01	13	0.39	0.27	13	0.35	0.27
AMPH	0.02	11	0.15	0.04	10	0.13	0.05
DMAA	0.02	0			0		
DP	0.02	10	0.07	0.03	12	0.11	0.06
carbamazepine	0.01	13	1.25	0.57	13	1.37	0.73
phenazone	0.02	13	0.50	0.26	13	0.48	0.15
propyphenazone	0.02	13	0.14	0.09	10	0.08	0.06
PDP	0.02	0			0		
AAA	0.02	13	0.83	0.63	13	1.01	0.47
FAA	0.02	13	0.67	0.32	13	0.51	0.30

prove the performance of this method. In the influent and effluent of the WWTP Ruhleben, eight target compounds were detected (Table 5). Concentrations above 1 μg/L were determined for AAA, FAA, and carbamazepine. DMAA was not detected above its LOQ. Figure 3 shows a LC−MS/MS chromatogram of a WWTP effluent sample. DP could not be determined in the investigated sewage samples due to uncertainties in quantification caused by peak impurities.

The effluents from another WWTP in Berlin−Schönerlinde are discharged into a canal and reach the SWTP in Berlin−Tegel designed to remove residual phosphate from the sewage-prone surface water. In the effluent from this enhanced effluent treatment facility, median concentrations of AMDOPH (0.27 μg/L), AMPH (0.05 μg/L), FAA (0.30 μg/L), and carbamazepine (0.73 μg/L) were comparable to their influent concentrations. On the other hand, the concentrations of phenazone, propyphenazone, and AAA

Table 7. Summary of Analytical Results of Berlin Stream and Surface Water Samples; November 23 to December 4, 2001; N = 9

compound	LOQs (μg/L)	values > LOQ	max (μg/L)	med (μg/L)
AMDOPH	0.01	8	0.30	0.13
AMPH	0.02	5	0.16	0.02
DMAA	0.02	0		
DP	0.02	2	0.04	
carbamazepine	0.01	8	1.30	0.23
phenazone	0.02	7	0.37	0.08
propyphenazone	0.02	6	0.11	0.03
PDP	0.02	0		
AAA	0.02	7	1.0	0.12
FAA	0.02	8	1.0	0.07

were up to 40% lower after surface water treatment. DP, a metabolite of phenazone, was measured in the effluents at slightly higher concentrations than in the influents of the SWTP (Table 6).

In streams and lakes of Berlin, maximum concentration levels up to 1.3 μg/L were found for carbamazepine, AAA, and FAA (see Table 7). Additionally, AMDOPH, AMPH, DP, phenazone, and propyphenazone were detected at the nanogram per liter level. Results in the nanogram per liter range have previously also been reported for propyphenazone[7,25] or phenazone[21,25]

CONCLUSION

A simple and rapid method has been developed for the trace-level analysis of 10 polar pharmaceutical residues. This method applies automated SPE, liquid chromatographic separation, and measurement applying tandem MS for the unequivocal identification and quantification of these residues in complex environmental samples. The analytes were detected by APCI-MS/MS, which provides good selectivity and sensitivity. The method provides high recoveries for all analytes (87−117%), allowing the accurate quantification of the pharmaceutical residues in raw and purified sewage, groundwater, and surface and drinking water. Although a sample volume of only 0.25 L is used for SPE and isocratic liquid chromatographic separation is applied, low LOQs between 0.01 and 0.02 μg/L are obtained. The use of a surrogate for volume control and optimization of sample preparation enabled the application of this method for long-term investigations of groundwater and sewage, surface, and drinking water in Berlin, Germany. Eight of the 10 polar pharmaceutical residues could be detected in both influent and effluent samples from a WWTP. Seven of these compounds were present in effluents from the SWTP in Berlin—Tegel and were also detected in Berlin's streams and lakes.

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