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Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

An analytical method is presented enabling the determination of nine neutral pharmaceuticals in groundwater, and for most of the compounds, in rivers and wastewater down to the lower ng/l range. The analytes belong to different medicinal groups such as antiphlogistics, psychiatric drugs and antidiabetics. Samples are enriched using solid-phase extraction (SPE) with RP-C_{18}ec material. Analysis is performed by liquid chromatography with detection by electrospray tandem MS. Mean recoveries generally exceed 80% in groundwater, and the quantification limits are down to 50 ng/l in wastewater and down to 10 ng/l in groundwater. Losses were observed to occur either from ion suppression in the electrospray ionisation or SPE. Losses for all compounds could not be compensated for by the surrogate standard dihydrocarbamazepine. In raw municipal wastewater, concentration levels were detected for caffeine up to 147 μg/l and for propyphenazone up to 1.3 μg/l.

Keywords: Water analysis; Surrogate standard; Propyphenazone; Glibenclamide; 4-Aminoantipyrene

1. Introduction

The analytical determination of pharmaceuticals is mainly limited to clinical studies dealing with matrices of blood serum, tissue and urine. An overview of the detection methods of more than 100 individual drugs is published by Neill et al. [1]. For determination in the μg/l to mg/l (kg) range, high-performance liquid chromatography (HPLC) has been used in combination with DAD, UV and fluorescence detection. However, due to dilution and degradation, much lower concentration levels would be expected if we were to examine drugs after they enter the aquatic environment. To attain detection limits down to the lower ng/l range, high enrichment factors (100–10 000) and sensitive detection methods such as GC–MS, GC–MS–MS or LC–electrospray tandem MS (LC–ES-MS–MS) are essential. Furthermore, elevated levels of humic substances often have to be separated when analysing environmental samples. Therefore, a mere modification of the analytical methods from clinical studies may not be appropriate for environmental samples. The analysis of pharmaceuticals in municipal wastewater, river water, groundwater and drinking water requires the development of new methods enabling detection limits in the lower ng/l range.

The first report concerning the occurrence of pharmaceutical residues in the environment was published by Garrison et al. [2] from the Environmental Protection Agency (US EPA), who found clofibric acid and salicylic acid in a municipal sewage treatment plant (STP) in concentrations of 1–2 μg/l. They used liquid–liquid extraction with dichloromethane, methylation by diazomethane and

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detection by GC–MS. These two compounds were also found by Hignite and Azarnoff [3] in a STP of Kansas City, KS (USA) with relatively high concentration levels of 1–95 μg/l using an anion exchanger for extraction and the same derivatisation and detection method as described by Garrison et al. [2]. In Canada, the pharmaceuticals ibuprofen, clofibric acid and naproxen were identified in wastewater by Rogers et al. [4] using XAD extraction with diazomethane methylation and GC–MS detection. However, these publications did not lead to further investigations in the area of the environmental occurrence and relevance of pharmaceuticals in the environment. In Europe, the first comprehensive studies on the occurrence of pharmaceuticals in the environment were reported by Watts et al. [5], Waggott [6] and Richardson and Bowron [7]. These investigations in the United Kingdom revealed that drugs were present in the aquatic environment at concentrations up to ~1 μg/l, but the exact concentrations for the individual drugs were not always determined. The authors used liquid–liquid-extraction and XAD-extraction, detection by GC–MS, HPLC, field desorption MS and immunoassays. Concentration levels of pharmaceuticals up to the μg/l range were also predicted by Van der Heide and Hueck-Van der Plas [8]. Considering prescription quantities and pharmcokinetic data, they calculated pharmaceutical concentrations in Dutch wastewater. For example, they predicted a concentration of up to 40 μg/l for iodinated X-ray contrast media, up to 70 μg/l for acetylsalicylic acid and corresponding metabolites, and up to 4.5 μg/l for tetracyclines. In Germany, clofibric acid has been identified in river and groundwater and even in drinking water with concentration levels ranging up to 0.165 μg/l [9,10] using liquid–liquid extraction with dichloromethane, pentafluorobenzylbromide, GC–AED and GC–MS. Using GC–MS and different derivatisation procedures (diazomethane, silylation, trifluoroacetylation), we identified a range of lipid regulators and antiphlogistics in STP effluents and river waters [11,12] as well as betablockers and β_2-sympathomimetics [13,14]. In total 36 of 55 pharmaceuticals and five of nine corresponding metabolites were detected in at least one sample of treated wastewater [15,16].

As outlined above, the establishment of GC–MS analysis in the 1970s enabled the first identification and determination of pharmaceutical residues in the environment. However, gas chromatography is limited to compounds with high vapor pressure and to those whose vapor pressure can be elevated by derivatisation. Nevertheless, due to their high polarity, many pharmaceuticals are not very suitable for determination by GC–MS. Hence, the current paper intends to exhibit the advantages and limitations in the use of LC–ES tandem MS for the analysis of pharmaceuticals in environmental samples. A total of nine pharmaceuticals and metabolites were selected for determining an analytical method (Table 1).

2. Materials and methods

Reference compounds were purchased from Sigma (Germany), except for glibenclamide, propyphenazone (Synopharm, Germany), omeprazole (Promochem, Germany) and dihydrocarbamazepine (Alltech, Germany). Standard solutions were stored at −20°C. All solvents utilised were HPLC grade or higher quality.

2.1. Method

Due to the predicted low concentrations of pharmaceuticals in the aquatic environment, a pre-concentration step is essential prior to measurement. An effective and time saving method was established which allows the simultaneous enrichment of all selected pharmaceuticals.

2.2. Solid phase extraction (SPE)

A 1-litre glass fibre filtered sample was adjusted, if necessary, to a neutral pH (7.0–7.5) with H_2SO_4 (3 mol/l). Afterwards, the sample was passed through a glass cartridge filled with 500 mg of Isolute C_{18} (both purchased from IST, Bad Homburg) with a flux of ~20 ml/min, assisted by vacuum. The cartridges were then dried for 1 h with nitrogen and eluted three times with 1 ml methanol. The extracts were reduced to ~20 μl in a gentle nitrogen stream and were then brought to 1 ml with phosphate buffer and stored at −20°C until analysis.

Table 1
Pharmaceuticals and metabolites

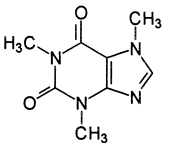
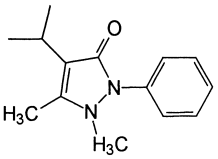
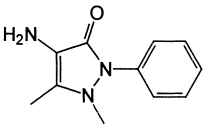
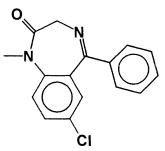
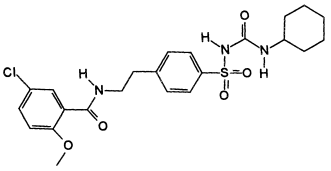
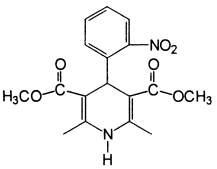
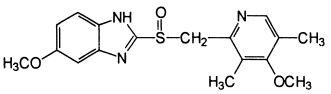
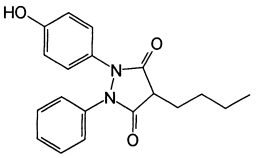
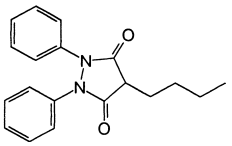
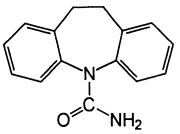
Substance	CAS-No.	Chemical structure	Application
---	---		
Caffeine	58-08-2		Constituent of coffee, psychostimulation
Propyphenazone	479-92-5		Antiphlogistic
4-Aminoantipyrine	83-07-8		Metabolite of metamizole
Diazepam	439-14-5		Psychiatric drug
Glibenclamide	10238-21-8		Antidiabetic
Nifedipine	21829-25-4		Calcium antagonist
Omeprazole	73590-58-6		Ulcer therapeutics
Oxyphenbutazone	129-20-40		Metabolite of phenylbutazone

Table 1. Continued

Substance	CAS-No.	Chemical structure	Application
Phenylbutazone	50-33-0		Antiinflammatory agent
10,11-Dihydro-carbamazepine	3564-73-6		Surrogate standard

2.3. HPLC conditions

The HPLC system consisted of a Perkin-Elmer L-6200 pump connected to an AS-2000a autosampler and a D-6000 interface. The analytes were chromatographed at room temperature utilising a 125×3 mm Merck LiChrospher® 100 RP-18 (end capped) column (5 μm) with a mobile phase consisting of 20 mmol/l ammonia acetate in water–acetonitrile. The injection volume was 50 μl and the flow-rate was 0.4 ml/min. Solvents and gradients are shown in Table 2.

2.4. MS–MS parameters

The system utilised was a Perkin-Elmer Sciex API 365 triple stage quadrupole mass spectrometer with turbo electrospray ionisation. The analysis was performed in positive ion mode. Nitrogen was used as curtain gas and nebulizer gas with a flow-rate of 1 l/min. Since the interface allows at maximum a flow-rate of 200 μl/min, the LC effluent was split 1:1, resulting in an spray flux of ~200 μl/min. Nitrogen was used as turbo gas with a flow-rate of 6 l/min and a temperature of 400°C. Orifice voltages varied generally from 10 to 50 V, depending on the best signal of the ionisation products.

MS–MS parameters were optimised in continuous flow mode as follows. After determination of the best conditions for the isolation of the precursor ion (mostly due to proton adduct of the respective analyte), the ion spray voltage, quadrupole and lens conditions for the argon induced collision dissociation were optimised (parent scan: 1-Da steps, 10-ms dwell time; product-ion scan: 0.1-Da steps, 10-ms dwell time; multiple reaction monitoring (MRM): dwell time >200 ms depending on number of recorded mass traces). Precursor and product ion

Table 2
LC–MS–MS parameters

Column	125×3 mm Merck LiChrospher 100RP C_{18} (ec); 5 μm		
Eluent	Buffer A1: 90% 0.02 mol/l NH_4Ac-buffer pH 5.7+10% CH_3CN Buffer B1: 40% buffer A1+60% CH_3CN		
Gradient	Time (min)	Buffer A1 (%)	Buffer B1 (%)
	0	100	0
	1	100	0
	3	88.8	11.2
	17	10	90
	18	0	100
	22	100	0
	30	100	0

Table 3
Retention time, precursor ions and product ions

Substance	Retention time (min)	Precursor ion (m/z) $[M+H]^+$	Product ion 1 (m/z)	Product ion 2 (m/z)
Caffeine	8.91	195.1	138.0 $[M-H_3C-N-CO+H]^+$	110.0 $[M-CO-N(CH_3)-CO+H]^+$
4-Aminoantipyrine	13.43	204.0	187.0 $[M-NH_2]^+$	159.1 $[M-NH_2-CO]^+$
Propyphenazone	19.51	231.1	189.2 $[M-C_3H_7+H]^+$	200.8 $[M-2CH_3+H]^+$
Diazepam	22.87	285.0	257.0 $[M-CO+H]^+$	154.1
Glibenclamide	22.50	493.7	369.1 $[M-C_6H_{10}-NH-CO+H]^+$	169.1 $[CO-C_6H_3Cl-OCH_3]^+$
Nifedipine	22.03	347.3	315.1 $[M-OCH_3]^+$	253.9
Omeprazole	17.82	346.2	136.2 $[M-H_3CO-(C_7H_4N_2)-SO-CH_2]^+$	197.8 $[M-H_3CO-C_7H_4N_2]^+$
Oxyphenbutazone	17.64	325.2	160.3 $[M-(HO-C_6H_4-N)-(C_4H_9)]^+$	204.1 $[M-(C_6H_5-N)-C_2H_5]^+$
Phenylbutazone	19.39	309.3	160.2 $[M-(C_6H_5-N)-(C_4H_9)]^+$	188.1 $[M-(C_6H_5-N)-C_2H_5]^+$
10,11-Dihydro -carbamazepine	18.74	239.1	194.2 $[M-CO-NH_2]^+$	181.2 $[M-N-CO-NH_2+H]^+$

masses of the individual compounds are given in Table 3.

2.5. Limit of quantification and calibration

The LOQ was calculated according DIN 32645 [17], with a confidence interval of 99% using the standard deviation of a linear regression curve for a detection range from 0.005 to 1 μg/l, with at least seven concentrations by spiking groundwater. LOQ is another term for limit of determination (LOD) mentioned in DIN 32645. Since the calculated LOQ is basically between the first and the second calibration point, the LOQ used were set as the second lowest calibration point of the linear correlation to ensure a precise quantitation. The calibration was performed over the whole procedure after spiking groundwater with the standard mixture of the analytes to attain seven different calibration concentrations. Analysing wastewater, the LOQ determined for groundwater was multiplied for all analytes by a factor of five, which is an empirical worst-case value appropriate even for extremely polluted waters. The use of surface water or even wastewater instead of groundwater for setting up a calibration series cannot be recommended for calibration, as these waters are known to be at least sometimes contaminated by the analytes under investigation. The calculation of the concentrations in native samples was carried out using the surrogate standard dihydrocarbamazepine and the linear seven point calibration curve. For quantitation in each series a blank sample and a recovery sample were included.

2.6. Determination of recoveries and corrected recoveries

A 1-litre sample of groundwater from the highlands of Taunus (middle of Germany), which is not appreciably influenced by man-made organic compounds, was spiked with the analytes. Additionally, 1 litre of river water, 1 litre of treated and 0.2 litre of untreated municipal wastewater were spiked with the analytes. Extraction procedure by SPE enrichment and detection by LC–ES tandem MS were performed as described above. The recoveries were calculated externally in comparison to a non-enriched standard solution by using the peak areas for quantitation. Adjusted recoveries were corrected for using the surrogate standard which was spiked at the beginning to the water sample.

2.7. Sampling procedure

Water samples were collected in brown glass bottles that had been pre-washed with successive rinses of Milli-Q water and acetone and dried for 8 h at 250°C. Samples were either extracted immediately or stored at 4°C for a maximum period of 1 week.

Composite samples of raw influent and final effluent were taken daily from a German municipal STP during the period June 26 to June 30, 2000. Sampling was carried out by a flow proportional

automatic sampler, whereby the composite samples of the final effluent were taken time-related to the influent. The municipal STP near Frankfurt/Main is connected to a sewage system servicing a city with ~312 000 population equivalents. It consists of three commonly used main treatment steps: preliminary clarification followed by an aeration tank with addition of Fe(II)chloride for phosphate elimination and a final end point clarification. All cooled sewage samples (4°C) were analysed as soon as possible (at the latest on the 2nd day), in order to keep microbial degradation to a minimum.

2.8. Sampling of STP effluents and rivers

Effluents of 14 municipal STPs, treating mainly household discharges, were sampled to analyze neutral pharmaceuticals. In general, grab samples of the STP effluents were taken. All STPs utilize three commonly used treatment steps: preliminary and final clarification and an aeration tank. The STPs investigated are located in rural areas connected to a small number of households as well as in cities. The municipalities are not individually mentioned because they prefer to remain anonymous.

Daily composite samples (time proportional) were taken from the rivers Main (Bischofsheim) and Nidda (Nied), as well as from the creek Schwarzbach (Trebur). Additionally, grab samples were taken from the rivers Main (Seligenstadt) and Rhine (Wiesbaden), and from six small rivers and creeks mostly located in the Hessian Ried and greater Frankfurt area. All sampling sites of the rivers and creeks were at least 1 km, but generally between 5 and 10 km, away from the next up-stream STP. Thus, the STP effluents were mixed thoroughly with the running water at the sampling site.

3. Results and discussion

3.1. Method validation

Most recoveries of the analytes in groundwater spiked with 200 ng/l exceeded 80%. Only 4-aminoantipyrine and caffeine showed appreciable lower values with 64 and 66%, respectively (Table 4). In Rhine water some recoveries were slightly reduced in comparison to the spiked groundwater. However, for oxyphenbutazone and phenylbutazone substantial attenuations were observed down to 12 and 30%, respectively. In treated and untreated wastewater the recoveries were lowered for most of the analytes. These losses were caused by matrix impurities which either reduced the sorption efficiencies at the C_{18}ec materials or led to signal suppression in the electrospray interface. To elucidate which of the two effects occurred STP extracts prepared as described above were spiked with seven analytes prior to injection onto the LC column. Except for caffeine the re-

Table 4
Recoveries of selected neutral pharmaceuticals in groundwater, surface water (Rhine), STP effluent ($n=3$) and raw sewage ($n=2$)

Substance	Groundwater, %	Surface water (Rhine), %	STP effluent, %	Raw sewage ($n=2$)[a], %
Caffeine	66±6	48±16	41±1	–[b]
Propyphenazone	97±2	91±0	68±2	52±2
4-Aminoantipyrene	64±10	43±1	12±1	26±4
Diazepam	93±0	92±2	75±2	55±5
Glibenclamide	105±5	97±2	87±2	76±8
Nifedipine	85±7	75±2	51±3	49±5
Omeprazole	88±16	81±4	57±0	64±6
Oxyphenbutazone	80±5	12±1	11±1	16±2
Phenylbutazone	78±6	30±2	9±2	24±6
10,11-Dihydro-carbamazepine	97±3	83±2	61±3	53±5

[a] Due to $n=2$, the statistical error was set as the deviation of the mean from maximum and minimum value.
[b] The recovery determination was not possible, due to the original high concentration in raw sewage.

Table 5
Recoveries of selected neutral pharmaceuticals over the total method (spiked in treated municipal wastewater) and recoveries only for the detection by LC electrospray tandem MS (spiked to the extract after C_{18}ec enrichment)

Substance	Recoveries total method ($n=4$), %	RSD, %	Recoveries LC–ES-MS–MS ($n=3$), %	RSD, %
Caffeine	41	3	75	2
Propyphenazone	63	14	50	3
4-Aminoantipyrine	11	13	16	6
Diazepam	78	9	97	5
Omeprazole	59	7	67	2
Oxyphenbutazone	9	41	9	6
Phenylbutazone	9	15	13	8
10,11-Dihydro-carbamazepine	54	8	51	3

coveries were not appreciably increased in comparison to the recoveries over the total method (Table 5). Thus, the signal suppression in the electrospray plays a crucial role for the losses of 4-aminoantipyrine, omeprazole, oxyphenbutazone, phenylbuatzone and propyphenazone. The spiked extracts for caffeine exhibited a significant increase from 41 to 75%, indicating that its recovery could be enhanced by using another more appropriate SPE material.

For most of the compounds, compensation for the losses was attained by addition of the surrogate standard dihydrocarbamazepine. As illustrated in Table 6, the corrected recoveries with regard to the surrogate standard were significantly higher. This is to be expected, since dihydrocarbamazepine frequently experiences comparable losses for suppression in the electrospray interface in treated and untreated wastewater (Tables 5 and 6). Without using a surrogate standard an underestimation would occur when the data attained in treated and untreated wastewater samples are not corrected. The corrected recoveries for caffeine of ~70% were not optimal, thus the calculated concentrations of caffeine were corrected with the losses attained in the recovery experiments. However, the low corrected recoveries of oxyphenbutazone, phenylbutazone and 4-aminoantipyrine indicate that the determination of these compounds is still rather semi-quantitative. Thus, an appropriate compensation could only be performed by using the more time consuming standard addition method.

Table 6
Corrected recoveries (with surrogate standard) of selected neutral pharmaceuticals in groundwater, surface water (Rhine), STP effluent ($n=3$) and raw sewage ($n=2$)

Substance	Groundwater, %	Surface water (Rhine), %	STP effluent, %	Raw sewage ($n=2$)[a], %
Caffeine	68±8	57±19	68±1	–[b]
Propyphenazone	100±2	109±1	111±4	98±3
4-Aminoantipyrene	66±10	52±1	19±2	49±10
Diazepam	96±0	110±2	122±3	104±2
Glibenclamide	109±2	117±2	142±3	142±13
Nifedipine	88±8	91±3	84±7	93±2
Omeprazole	91±17	97±5	94±1	120±12
Oxyphenbutazone	82±6	15±1	18±2	30±3
Phenylbutazone	80±8	37±1	15±3	46±11

[a] Due to $n=2$, the statistical error was set as the deviation of the mean from maximum and minimum value.
[b] The recovery determination was not possible, due to the original high concentration in raw sewage.

Corrected recoveries of 142% for glibenclamide in treated and raw wastewater indicate that the use of the surrogate standard led to an overestimation when the recovery of the analyte significantly exceeded the recovery of the surrogate standard (Tables 4 and 6). Therefore, it was more appropriate to quantify glibenclamide without using the surrogate standard, even for raw wastewater. As outlined before, a general rule cannot be given for confirmation and quantitation of pharmaceutical analysis in complex matrices such as raw and treated wastewater. The behavior and losses of individual compounds during sample preparation and detection have to be very carefully studied, as sometimes only standard addition may be appropriate for solving problems of higher matrix effects.

The quantification limits for the analytes in groundwater were 10 ng/l and varied in treated wastewater from 25 to 50 ng/l (Table 7) and in raw wastewater from 100 to 250 ng/l. Calibration graphs showed an excellent linearity for all analytes in a range from the LOQ up to 1000 ng/l, with correlation coefficients better than >0.99. For higher concentrations a quadratic correlation was frequently found.

3.2. Removal in a municipal STP

In the influent of a municipal Hessian STP only caffeine, propyphenazone and 4-aminoantipyrine were identified (Table 7). Caffeine was found in the raw wastewater with a mean concentration of 147 μg/l, whereas propyphenazone and 4-aminoantipyrine (semi-quantitative result) exhibited only 0.12 and 0.78 μg/l, respectively. However, in the effluent of the STP the levels were reduced to 0.19 μg/l for caffeine and 0.36 μg/l for 4-aminoantipyrine, thus resulting in elimination efficiencies of >99 and 54%, respectively. Due to its high influent levels, caffeine probably undergoes a rapid biodegradation during the wastewater treatment. In contrast, propyphenazone exhibited no removal within the standard deviation after passing through the STP, rather a small increase of the loads, within the standard deviation, were detected. All the other selected pharmaceuticals were neither identified in raw wastewater nor in treated wastewater. The contaminations by propyphenazone and 4-aminoantipyrine are probably caused by the excreta of patients administered these pharmaceuticals, while the caffeine contamination should be mainly caused by excretions of coffee consumers; the quantities of caffeine used in clinical medicine should be negligible in comparison to coffee consumption.

3.3. Occurrence in treated wastewater and rivers

In the effluents of 14 municipal STPs, maximum concentration levels were found up to 1.9 μg/l for caffeine and up to 0.48 μg/l for propyphenazone (Table 8). Further, 4-aminoantipyrene and diazepam were detected up to 0.36 and 0.053 μg/l, respective-

Table 7
Mean concentrations, loads and elimination for the analytes in a municipal Hessian sewage treatment plant between 26/06/00 and 30/06/00: flow-rate (STP): 35 500–37 000 m^3/day[a]

Substance	LOQ influent, μg/l	Mean conc. influent, μg/l	LOQ effluent, μg/l	Mean conc. effluent, μg/l	Load influent, g/day	Load effluent, g/day	Elimination, %
Caffeine[b]	0.10	147±76	0.025	0.19±0.09	5390±2800	6.9±3.6	>99
Propyphenazone	0.10	0.12±0.09	0.025	0.18±0.02	4.1±3.2	6.4±0.4	None
4-Aminoantipyrine[b]	0.25	0.78±0.27	0.050	0.36±0.09	28±10	13±3	54
Diazepam	0.20	n.d.	0.050	n.d.	–	–	–
Glibenclamide	0.10	n.d.	0.025	n.d.	–	–	–
Nifedipine	0.10	n.d.	0.025	n.d.	–	–	–
Omeprazole	0.10	n.d.	0.025	n.d.	–	–	–
Oxyphenbutazone	0.25	n.d.	0.050	n.d.	–	–	–
Phenylbutazone	0.25	n.d.	0.050	n.d.	–	–	–

[a] n.d., not detected above the limit of quantification (LOQ).
[b] Results are corrected according to recoveries.

Table 8
Concentrations of neutral pharmaceuticals and metabolites in 14 municipal STP effluents[a]

Substance	LOQ, μg/l	n>LOQ	Median, μg/l	90-Percentile, μg/l	Maximum, μg/l
Caffeine[b]	0.025	14	0.072	0.94	1.9
Propyphenazone	0.025	11	0.095	0.16	0.48
4-Aminoantipyrine[b]	0.050	3	n.d.	0.26	0.36
Diazepam	0.050	1	n.d.	n.d.	0.053
Glibenclamide	0.025	0	n.d.	n.d.	n.d.
Nifedipine	0.025	1	n.d.	n.d.	0.089
Omeprazole	0.025	0	n.d.	n.d.	n.d.
Oxyphenbutazone	0.050	0	n.d.	n.d.	n.d.
Phenylbutazone	0.050	0	n.d.	n.d.	n.d.

[a] n.d., not detected above the limit of quantification (LOQ).
[b] Results are corrected according to recoveries.

ly. The other analytes were not identified above the limit of quantification. In rivers and streams, caffeine and propyphenazone were predominantly found with lowered concentration levels up to 0.88 and 0.10 μg/l, respectively (Table 9). The discharge of municipal STPs should be the prevailing source for the contamination of most receiving rivers and streams containing caffeine and propyphenazone. Similar results have already been found for many other pharmaceuticals administered by humans [15,18–25]. Further, the psychiatric drug diazepam was found in two streams up to 0.033 μg/l.

To study the influence of the point source by a pharmaceutical manufacturer, the river Main was sampled directly before (Seligenstadt) and after (Bischofsheim) the respective industrial discharge. As shown in Table 10 elevated concentrations up to 0.63 and 0.13 μg/l could be detected for 4-aminoantipyrene and glibenclamide, both of which are medicinal products of the pharmaceutical company. Since in the other rivers and creeks these two compounds were not found, the discharge of the pharmaceutical company is probably be responsible for the contamination detected in the Main. Thus, as expected and known for many other so-called ''industrial chemicals'', point source contamination of the receiving waters from discharges of industrial treated wastewater can also occur for pharmaceuticals.

In our laboratory, many different pharmaceuticals (e.g. betablockers, antiepileptics, X-ray contrast media) were found in municipal treated wastewater and in German river water using detection by LC–ES-MS–MS [14,26–28]. For many of these medicinal classes the analysis was performed using appropriate surrogate to compensate losses in the electrospray interface and during sample enrichment. Those examples clearly showed the power of the LC–ES-

Table 9
Concentrations of neutral pharmaceuticals in 11 German rivers and streams[a]

Substance	LOQ, μg/l	n>LOQ	Median, μg/l	90-Percentile, μg/l	Maximum, μg/l
Caffeine	0.010	11	0.53	0.81	0.88
Propyphenazone	0.010	9	0.043	0.099	0.10
4-Aminoantipyrene	0.025	1	n.d.	n.d.	0.63
Diazepam	0.010	2	n.d.	0.017	0.033
Glibenclamide	0.010	1	n.d.	n.d.	0.013
Nifedipine	0.010	0	n.d.	n.d.	n.d.
Omeprazole	0.010	0	n.d.	n.d.	n.d.
Oxyphenbutazone	0.010	0	n.d.	n.d.	n.d.
Phenylbutazone	0.010	0	n.d.	n.d.	n.d.

[a] n.d., not detected above the limit of quantification (LOQ).

Table 10
Concentrations of selected neutral pharmaceuticals in German rivers and streams[a]

Substance	Main (Seligenstadt), μg/l	Main (Bischofsheim), μg/l	Rhein (Schierstein), μg/l	Gersprenz (Babenhausen), μg/l	Nidda (Nied), μg/l	Rodau (Mühlheim), μg/l	Schwarzbach (Astheim), μg/l
4-Aminoantipyrine	n.d.	0.63	n.d.	n.d.	n.d.	0.037	n.d.
Glibenclamide	n.d.	0.012	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeine	0.15	0.53	0.35	0.61	0.88	0.70	0.15
Propyphenazone	0.014	0.042	n.d.	0.033	0.051	0.10	0.10

[a] n.d., not detected above the limit of quantification (LOQ).

MS–MS technique. A comparison of two analytical methods for the determination of betablockers in water [26] exhibited that LC–ES-MS–MS leads, in comparison to GC–MS, to reduced relative standard deviations, to a larger variety of polar betablockers which can be analysed and to comparable LOQs. Thus, in any case the method of LC–ES-MS–MS analysis should be preferably used for the study of such environmental samples.

4. Conclusion

LC–ES-MS–MS can be successfully used in environmental analysis of drugs and their metabolites in water resources. The novel technique opens the window for polar, unstable and high molecular mass compounds to be assayed. Especially for relatively polar pharmaceuticals, LC–ES-MS–MS is the method of choice for detection. However, for highly polar organic compounds the enrichment step is frequently also a difficult part of the analytical procedure, a problem which has to be solved. In the MRM mode the sensitivity is sufficient when selected precursor ions are isolated and fragmented into definite product ions. Further advantages in comparison to GC–MS are the reduced run times and the fact that no derivatisation is necessary. However, lower resolution and especially the suppression of signals in the electrospray interface by matrix impurities are responsible for limitations in the application. The latter has to be compensated for to obtain quantitative results by the addition of an appropriate surrogate standard or by a clean-up step introduced in the sample preparation, otherwise, it may be assumed that the results of real samples will be underestimated.

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