Analytical methods for the determination of pharmaceuticals in aqueous environmental samples

Thomas A. Ternes*
ESWE-Institute for Water Research and Water Technology, Soehnleinstrasse 158, D-65201 Wiesbaden, Germany

Recently several methods have been developed for the determination of drugs and their metabolites in the lower ng/l range using solid phase extraction (SPE), derivatization, detection and confirmation by gas chromatography/mass spectrometry (GC/MS) and GC/MS/MS or LC–electrospray tandem MS (LC–ES/MS/MS). A wide range of pharmaceuticals from different medicinal classes can be determined down to the lower ng/l range. Due to the basically elevated polarity of pharmaceuticals either analysis by LC–ES/MS/MS or an efficient derivatization prior to measurements by GC/MS are mostly essential. A direct comparison of GC/MS and LC–ES/MS/MS displayed that only the latter allows for the analysis of the extreme polar beta-blockers atenolol and sotalol due to an incomplete derivatization of the functional groups. Further, the relative standard deviation using LC–ES/MS/MS was lower. However, when analyzing highly contaminated samples such as sewage a suppression of the electrospray ionization is likely to occur. Thus, to guarantee accurate and reproducible data either an efficient clean-up step has to be included into the sample preparation or an appropriate surrogate standard has to be spiked prior to SPE enrichment.

Keywords: LC-ES/MS/MS; Pharmaceuticals; GC/MS water; Wastewater; Endocrine disruptors

1. Introduction

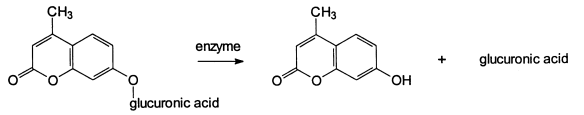
In human medicine annual drug prescriptions amounts to many tons per year, with some 100 tons in Germany alone [1]. This latter amount underestimates the total usage of drugs in the country – it does not include those which can be purchased without a pharmacy prescription and those procured illegally. About 3000 different compounds are used as constituents of medicinal products in human and veterinary medicine and comprise a wide range of different chemical structures. Because of the large number of compounds, to which should be added the huge number of excreted metabolites, it appears to be nearly impossible to develop analytical methods for all these substances in environmental samples. Therefore, a preselection is essential in developing methods for the potential environmentally relevant compounds. To preselect, the following criteria have been considered: (i) elevated annual prescription quantities, (ii) effect doses/concentrations, (iii) pharmacokinetic behavior (e.g. metabolism, urinary/fecal excretion rate).

Some endocrine active drugs possess an extremely high biological potency down to the μg/day doses, therefore it is very likely that they also cause effects at very low concentrations in the environment. For example, the contraceptive 17α-ethinylestradiol adversely effects the reproduction of zebrafish (*Danio rerio*) at concentrations as low as 1 ng/l [2]. The pharmacokinetic behavior directly influences the potential environmental contamination. A drug which is only excreted as metabolites should in general not be found in sewage and the environment. Thus, for those compounds it makes more sense to monitor the stable excreted principal metabolites. Pharmacokinetical data show that human excretion rates of unchanged drugs sometimes even exceed 50%. Additionally, excreted metabolites formed by conjugation with glucuronic acid or other polar compounds are likely to be cleaved by microorganisms into the unchanged pharmaceuticals [3,4], and

*Tel.: +49 (611) 7804343; Fax: +49 (611) 7804375.
E-mail: thomas.ternes@eswe.com

0165-9936/01/$ – see front matter
PII: S0165-9936(01)00078-4

hence the relevant environmental concentration will increase.



MUF-β-glucuronide 4-methylumbelliferone

Due to such high usage levels and excretion rates, detectable concentrations of drugs and their metabolites should not be unexpected in sewage.

This review summarizes those analytical methods which allow for the determination of drugs in different aqueous matrices down to the ng/l range.

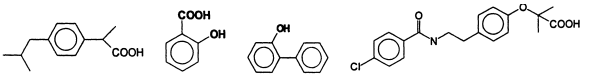
2. Analytical methods

Several analytical methods have been published concerning the determination of pharmaceuticals in biological samples such as serum, blood or urine within the µg/l range [5–13]. The detection was mainly performed by high performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS) or GC/FID, and de Jong et al. [8] have applied GC/MS/MS. Antiepileptic drugs such as carbamazepine have been detected in human serum after a solid phase extraction (SPE) followed by GC/FID at concentration levels down to 14 µg/l [14,15]. However, basically these methods used sample volumes of 10 ml and less. In order to attain quantitation limits within the lower ng/l range for a multitude of drugs in the aqueous environmental matrices, advanced solid phase materials, modified derivatization procedures as well as techniques such as GC/MS and LC–electrospray tandem MS (LC–ES/MS/MS) are essential. In the past, for environmental samples mostly the analysis of individual substances, e.g. clofibric acid, has been described in the literature.

In the following, six different analytical methods from the ESWE institute are described in detail, enabling the analysis of about 80 pharmaceutical compounds and phenolic antiseptics. Further, a selection of analytical methods reported in literature is summarized.

2.1. Acidic drugs

The term 'acidic drugs' comprises pharmaceutical compounds containing carboxylic moieties and one or two phenolic hydroxy groups. All these compounds can be nearly quantitatively enriched on C_{18}-bonded phases at pH 2–3. At the acidic pH no ionic functional groups are present since the hydroxy and carboxylic moieties are protonated. Below, the chemical structures of the antiphlogistic ibuprofen, salicylic acid, the antiseptic biphenylol and the lipid regulator bezafibrate are shown.



ibuprofen salicylic acid biphenylol bezafibrate

A multi-analytical method has been described for the determination of different acidic drugs belonging to medicinal classes such as antiphlogistics, lipid regulators together with compounds such as salicylic acid, the main metabolite of acetylsalicylic acid (ASA), as well as phenolic antiseptics in sewage, river and drinking water [16]. The method consists of SPE using 500 mg RP-C_{18} (Merck), followed by methylation of carboxylic groups with diazomethane, acetylation of phenolic hydroxy groups with acetanhydride/triethylamine (1:1, v/v) and determination by GC/MS (Fig. 1).

The acidic drugs (e.g. antiphlogistics, lipid regulators) and their metabolites were simultaneously

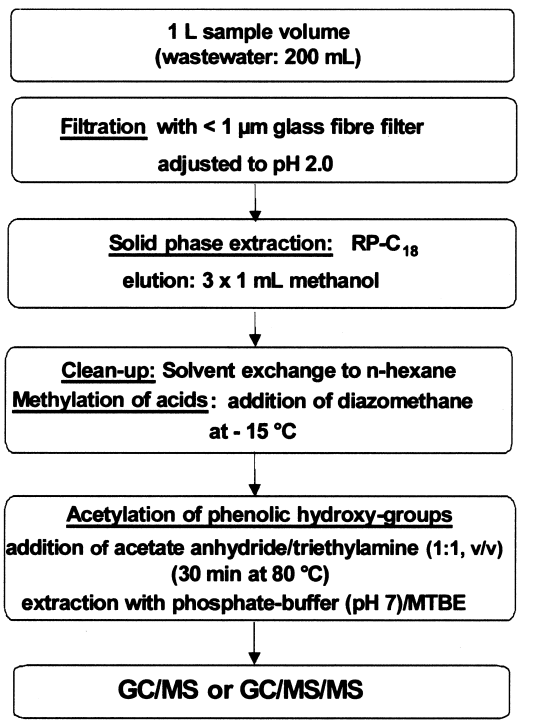


Fig. 1. Scheme of the analytical method for acidic pharmaceuticals and phenolic antiseptics [16].

Table 1
Recoveries (REC) and S.D. (1σ, *n*=3) of acidic pharmaceuticals in spiked ground water, LOQ for different matrices [16]

Analytes	REC ± 1σ RP-C_{18}/EN batch 95 (%)	REC ± 1σ RP-C_{18}/EN batch 97 (%)	REC ± 1σ RP-C_{18} batch 97 (%)	LOQ in STP[a] GC/MS (ng/l)	LOQ in SW[b] GC/MS (ng/l)	LOQ in DW[c] GC/MS/MS (ng/l)
ASA	90 ± 12	52 ± 8	48 ± 18	50	10	10
Clofibric acid	58 ± 3	71 ± 12	82 ± 9	50	5	1
Ibuprofen	71 ± 3	82 ± 13	81 ± 5	50	5	1
Ibuprofen-OH	n.a.[d]	< 15	63 ± 8	50	20	n.a.[d]
Ibuprofen-COOH	n.a.[d]	74 ± 6	75 ± 5	50	20	n.a.[d]
Gemfibrozil	85 ± 4	49 ± 9	89 ± 7	50	5	5
Fenoprofen	79 ± 3	79 ± 9	90 ± 6	50	5	5
Ketoprofen	86 ± 5	77 ± 5	94 ± 5	50	5	5
Diclofenac	75 ± 3	50 ± 11	89 ± 5	50	5	1
Fenofibric acid	77 ± 6	40 ± 9	96 ± 6	50	5	5
Bezafibrate	80 ± 5	70 ± 12	92 ± 14	250	5	25
Indometacine	78 ± 3	11 ± 5	90 ± 11	50	5	5
Naproxen	90 ± 8	54 ± 15	91 ± 6	50	10	n.a.[d]
Tolfenamic acid	74 ± 5	28 ± 7	83 ± 5	50	20	n.a.[d]
Meclofenamic acid	85 ± 12	51 ± 7	89 ± 6	50	20	n.a.[d]

[a]STP effluent.
[b]Surface water.
[c]Drinking water.
[d]Not analyzed.

determined with phenolic antiseptics. Recoveries of pharmaceutical residues frequently exceeded 80% (Table 1) and standard deviations (S.D.) (1σ, *n*=3) varied between 5 and 26%. The average recoveries of the phenolic antiseptics ranged from 49 to 97% (Table 2). Limits of quantification (LOQs) down to 10 ng/l were achieved in sewage treatment plant (STP) effluents as well as in river water using GC/MS, and down to 1 ng/l using GC–ion trap MS/MS. The solvent exchange even

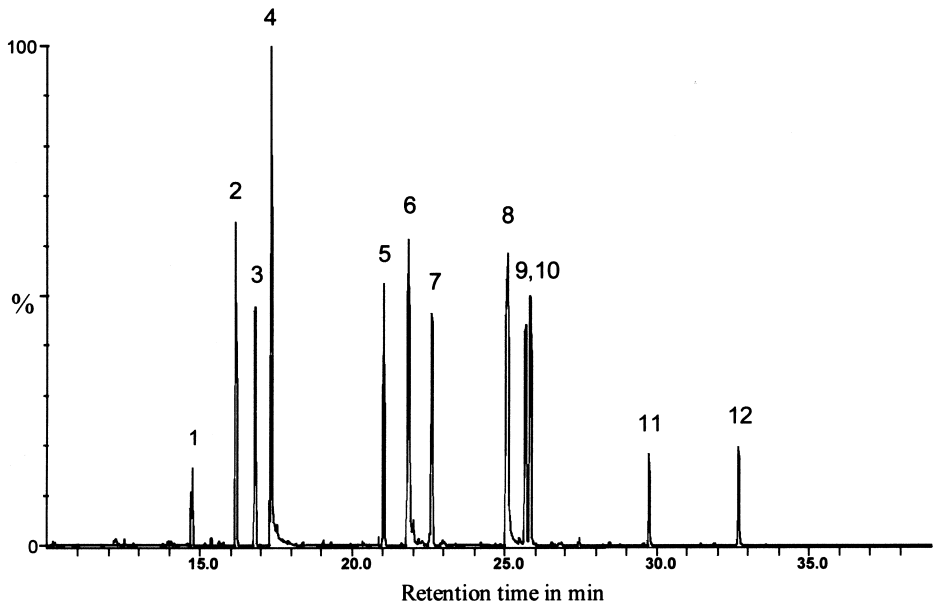


Fig. 2. GC/MS total ion chromatogram of a standard solution. 1: ε-HCH (internal standard); 2: terbutalin; 3: clenbuterol; 4: salbutamol; 5: metoprolol; 6: timolol; 7: propranolol; 8: nadolol; 9: bisoprolol; 10: betaxolol; 11: fenoterol; 12: carazolol.

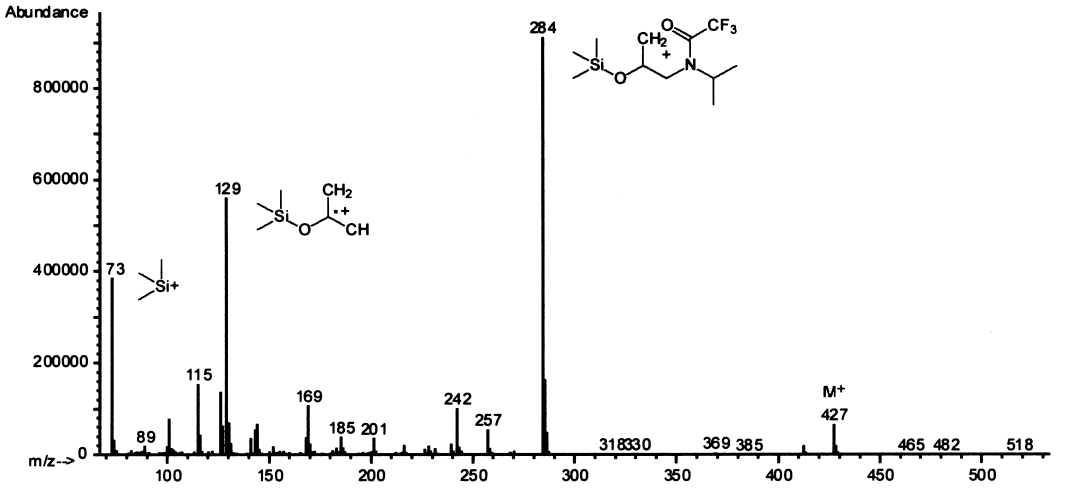


Fig. 3. GC/MS spectrum of propranolol after derivatization with MSTFA and MBTFA.

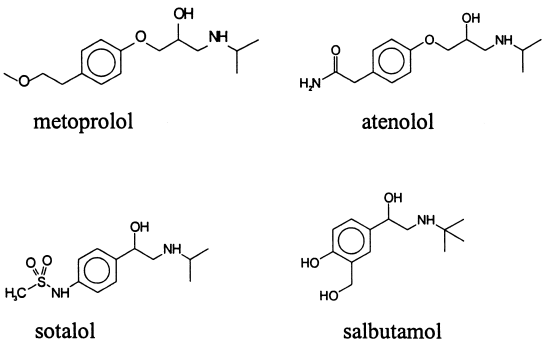
allows for the analysis of pharmaceuticals and antiseptics in treated and raw sewage. The relatively low temperature of −15°C is essential for the simultaneous determination of phenolic antiseptics since otherwise both methylated and trifluoroacetylated phenolic hydroxy groups can be formed. To analyze alternatively acidic drugs or antiseptics the diazomethane methylation or the trifluoroacetylation can be performed solely. As been shown in Table 1 the recoveries of acidic pharmaceuticals can differ dramatically when using different batches even of the same SPE material from the same company (see Table 1).

In the further literature, most methods described, generally allow for the determination of individual or few compounds. For instance, Hignite and Azarnoff [17] and Garrison et al. [18] analyzed simultaneously the metabolites salicylic acid and clofibric acid by GC/MS after derivatization with diazomethane. For enrichment, Hignite and Azarnoff [17] used an anion exchanger and Garrison et al. [18] applied liquid/liquid extraction by dichloromethane. Other authors combined 'reversed phase' (RP) extraction and GC/MS after derivatization with pentafluorobenzylbromide for the determination of clofibric acid [19–21]. Buser et al. [22,23] and Buser and Müller [24] used for the individual determination of clofibric acid, diclofenac and ibuprofen, SPE on a polystyrenedivinylbenzene copolymer (Bio Beads SM 2), a diazomethane methylation and finally enantioselective GC/MS. LOQ was attained down to 1 ng/l for these three compounds. Sacher et al. [25] analyzed eight acidic pharmaceuticals by SPE on RP-C_{18} ec and GC–ion trap MS/MS after on-line methylation in the injector using trimethylsulfoniumhydroxide down to the lower ng/l range.

Thus, a quite high variety of different methodologies are described for enrichment and derivatization of acidic pharmaceuticals, with SPE and GC/MS being the prevailing alternatives.

2.2. Betablocker and β_2-sympathomimetics

Both medicinal classes (betablocker and β_2-sympathomimetics) contain a secondary aminoethanol structure as well as several hydroxy groups. Due to the elevated number of functional groups their polarity is relatively high, thus, for GC analysis an efficient derivatization is essential. Below, the chemical structures of three selected betablockers (metoprolol, atenolol, sotalol) and the β_2-sympathomimetic salbutamol are illustrated.



Two alternative analytical methods have been developed (see Figs. 2–4) which allow the simultaneous determination of betablockers and β_2-sym-

Table 2
Recoveries (REC) and S.D. (1σ, *n*=3) of antiseptics in spiked ground water, LOQ for different matrices [16]

Substance	REC ± 1σ RP-C_{18} / EN batch 95 in %	REC ± 1σ RP-C_{18} batch 97 in %	LOQ in STP[a] GC/MS in ng/l	LOQ in SW[b] GC/MS in ng/l	LOQ in DW[c] GC/MS/MS in ng/l
Antiseptics					
Biphenylol	65 ± 9	90 ± 7	50	10	10
Chlorophene	110 ± 8	97 ± 5	50	10	10
4-Chloro-*m*-cresol	54 ± 6	56 ± 6	50	10	10
Tetrabromo-*o*-cresol	50 ± 4	82 ± 21	50	10	10
4-Chloroxylenol	60 ± 8	96 ± 10	50	10	10
Bromophen	n.a.[d]	67 ± 9	50	10	10
Phenylsalicylate[e]	48 ± 10	74 ± 11	50	10	10
5-Chlorosalicylic acid	54 ± 10	51 ± 6	50	10	10
5-Bromosalicylic acid	54 ± 10	49 ± 7	50	10	10
Metabolites of ASA					
Salicylic acid	56 ± 20	55 ± 10	50	10	10

[a]STP effluent.
[b]Surface water.
[c]Drinking water.
[d]Not analyzed.
[e]The derivatization of these analytes can be incomplete in real samples, thus a LC–ES/MS/MS detection method should preferable be developed.

pathomimetics in sewage, river and drinking water using either GC/MS or LC–ES/MS/MS [26]. For GC/MS the sample preparation includes SPE, a two-step derivatization by silylation of the hydroxy groups and trifluoroacetylation of the secondary amino moieties. Since LC–ES/MS/MS has only been published in a German report [27], the following exact conditions are mentioned. The gradient is

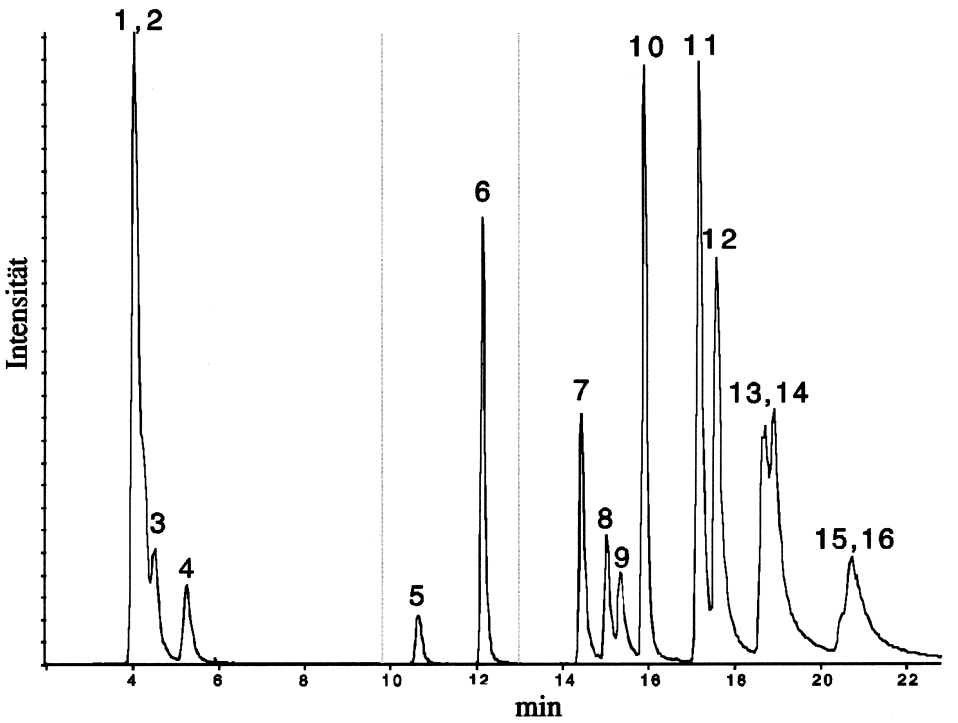


Fig. 4. LC/MS chromatogram of a standard solution. 1: atenolol; 2: salbutamol; 3: terbutalin; 4: sotalol; 5: fenoterol; 6: nadolol; 7: timolol; 8: metoprolol; 9: clenbuterol; 10: celiprolol; 11: bisoprolol; 12: carazolol; 13: propranolol; 14: betaxolol.

Table 3
Gradient conditions for betablockers and β_2-sympathomimetics

Time (min)	Eluent A[a] proportion (%)	Eluent B[b] proportion (%)
0	100	0
1	100	0
17	10	90
18	0	100
22	0	100
24	100	0
34	100	0

[a]Eluent A: 900 ml water containing 20 mmol/l ammonium acetate was adjusted to pH 5.7. Finally 100 ml acetonitrile was added.

given in Table 3 and the precursor and product ions are shown in Table 4. For LC–ES/MS/MS (Perkin Elmer API 365 system) in the positive ion mode after SPE, the volume of the sample extracts was reduced to about 20 µl and diluted with phosphate buffer (20 mmol/l, pH 7) to a final volume of 0.5 ml. 50 µl of the extracts was then injected onto the LC column – a 125×3 mm ID Merck LiChrospher 100 RP-18 column (5 µm) with a flux rate of 0.4 ml/min. The eluent was prepared from two buffers: (i) buffer A: water/acetonitrile containing 5 mmol/l ammonium acetate (pH 7.5) and 10% acetonitrile, (ii) buffer B: 40% buffer A and 60% acetonitrile.

Mean recoveries after extraction and derivatization generally exceeded 70% (Table 5). The S.D. (1σ, n=3) up to 12% exhibited the sufficient precision and reproducibility of the analytical method [26]. The use of an acidic pH would be appropriate for the protonation of acidic phenolic hydroxy groups, but is disadvantageous for the desired deprotonation of the secondary amino moieties. Because of enhanced sorption effects of the betablockers, presumably caused by the secondary amino group, care should be taken to avoid losses by sorption on active glass surfaces as soon as they are dissolved in aqueous matrices. For instance, the usage of glass vials for the final phosphate buffer extraction volume leads to reduced recoveries of the betablockers, while in PTFE vials or in organic solvents no sorption losses were observed.

The efficiency of the method can be shown by mainly comparable recoveries obtained in GC/MS and LC–ES/MS/MS, even at concentrations as low as 10 ng/l (Table 5). Only carazolol and fenoterol

Table 4
MS/MS masses of betablocker and β_2-sympathomimetics

Analytes	Precursor ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)	Optional product ion 3 (m/z)
Atenolol	267.2 $[M+H]^+$	190.2 $[M\text{-}H_2O\text{-}NH_3\text{-isopropyl}]^+$	145.0 $[190\text{-}CO\text{-}NH_3]^+$	–
Salbutamol	240.1 $[M+H]^+$	222.2 $[M\text{-}H_2O+H]^+$	148.0 $[166\text{-}H_2O]^+$	166.1 $[M\text{-}H_2O\text{-tert.butyl}]^+$
Terbutalin	226.1 $[M+H]^+$	152.1 $[M\text{-}H_2O\text{-tert.butyl}]^+$	125.1	107.0 [methylenphenol]$^+$
Sotalol	273.3 $[M+H]^+$	255.2 $[M\text{-}H_2O+H]^+$	213.1	133.0 $[M\text{-}H_2O\text{-isopropyl-}SO_2CH_3+H]^+$
Fenoterol	304.1 $[M+H]^+$	135.1 [propylphenol]$^+$	106.9 [methylenphenol]$^+$	286.2 $[M\text{-}H_2O+H]^+$
Nadolol	310.0 $[M+H]^+$	254.0 $[M\text{-tert.butyl}]^+$	201.1	236.3 $[M\text{-}H_2O\text{-tert.butyl}]^+$
Timolol	317.1 $[M+H]^+$	261.1 $[M\text{-tert.butyl}]^+$	244.1 $[M\text{-tert.butylamine+H}]^+$	188.1 (cleavage of side chain)
Metoprolol	268.3 $[M+H]^+$	116.2 [(*N*-isopropyl-*N*-2-hydroxypropylamine)+H]$^+$	98.0 [(*N*-isopropyl-*N*-propenamine)+H]$^+$	159.0
Clenbuterol	277.2 $[M\text{-H}]^+$	203.0 $[M\text{-}H_2O\text{-tert.butyl}]^+$	259.1 $[M\text{-}H_2O+H]^+$	168.2 [203-Cl]$^+$
Celiprolol	380.2 $[M+H]^+$	251.0 (cleavage of side chain)	307.0 $[M\text{-tert.butylamine+H}]^+$	324.4 $[M\text{- tert.butyl}]^+$
Bisoprolol	326.3 $[M+H]^+$	116.2 [(*N*-isopropyl-*N*-2-hydroxypropylamine)+H]$^+$	74.0	–
Carazolol	299.2 $[M+H]^+$	116.2 [(*N*-isopropyl-*N*-2-hydroxypropylamine)+H]$^+$	222.1	194.0
Propranolol	260.3 $[M+H]^+$	116.2 [(*N*-isopropyl-*N*-2-hydroxypropylamine)+H]$^+$	183.2	–
Betaxolol	308.1 $[M+H]^+$	116.2 [(*N*-isopropyl-*N*-2-hydroxypropylamine)+H]$^+$	98.1 [(*N*-isopropyl-*N*-propenamine)+H]$^+$	72.0 [(*N*-isopropyl-*N*-methylenamine)+H]$^+$

Table 5
Recoveries (REC) of betablockers and β_2-sympathomimetics and LOQs [26]

Analyte	REC LC–MS/MS (n=2) 1 μg/l	REC GC/MS (n=5) 1 μg/l	REC LC–MS/MS (n=2) 10 ng/l	REC GC/MS (n=2) 10 ng/l	LOQ DW, SW[b] GC/MS (ng/l)	LOQ DW, SW[b] LC/MS/MS (ng/l)	LOQ STP[a] LC/MS/MS (ng/l)
Atenolol	98	0	86	0	–	5	50
Sotalol	91	0	87	0	–	5	50
Salbutamol	61	53 ± 9	56	42	5	5	50
Terbutalin	22	22 ± 2	23	30	10	10	50
Fenoterol	51	77 ± 16	23	42	5	5	50
Nadolol	114	97 ± 9	98	125	5	5	50
Timolol	108	95 ± 8	77	109	5	5	50
Metoprolol	98	93 ± 9	71	98	5	5	50
Celiprolol	97	0	80	0	–	5	50
Bisoprolol	94	94 ± 12	67	101	5	5	50
Betaxolol	80	91 ± 12	52	96	5	5	50
Propranolol	–	91 ± 10	–	97	5	5	50
Carazolol	89	97 ± 12	56	26	5	5	50
Clenbuterol	120	86 ± 11	88	87	10	10	50

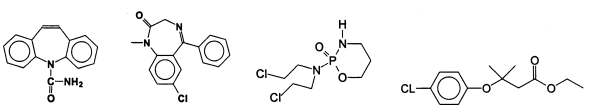
[a]STP effluent.
[b]Surface water.
[c]Drinking water.
[d]Not analyzed.
–: Recoveries were not calculated, since the new batch of a solid phase material was inappropriate for propranolol, exhibiting the importance to check the quality of every batch of SPE material very precisely.

exhibited reduced recoveries at 10 ng/l in comparison to the 1 μg/l spiking level. The LOQ for drinking water was down to 5 ng/l and for sewage water down to 50 ng/l.

A direct comparison of the two detection methods showed that LC–ES/MS/MS has advantages especially for the more polar compounds. For instance, the ubiquitous betablockers atenolol or sotalol could only be determined by LC–ES/MS/MS. Since the derivatization of the hydroxy groups was incomplete, determination by GC/MS was inappropriate.

2.3. Neutral pharmaceuticals

The term 'neutral pharmaceuticals' is used for different compounds from distinct medicinal classes which contain no acidic functional groups and, hence, can be enriched at neutral pH in the reversed phase and sorbents can generally be analyzed by GC/MS without derivatization. The selected different neutral and weakly basic drugs belong to different medicinal classes such as antiphlogistics, lipid regulators, antiepileptic agents, psychiatric drugs and vasodilators (Table 6). Below the chemical structures of a few 'neutral pharmaceuticals' are presented.



An analytical method was developed (Fig. 5) including SPE with 500 mg RP-C_{18} ec at pH 7.5 followed by GC/MS [26]. Prior to enrichment the sample was spiked with 10,11-dihydrocarbamazepine as surrogate standard. However, the determination of phenazon, carbamazepine, cyclophosphamide, ifosfamide and pentoxifylline was frequently subject to interference by organic co-extractants in real samples of rivers and sewage effluents. Therefore, an alternative LC–ES/MS/MS procedure has been developed, allowing the measurement of five neutral drugs in the positive mode. HPLC conditions are similar to those described for the determination of betablockers and β_2-sympathomimetics.

The observed recoveries of the neutral drugs, as measured by GC/MS, generally exceeded 70% [26]. LOQs of 20–50 ng/l were attained in drinking

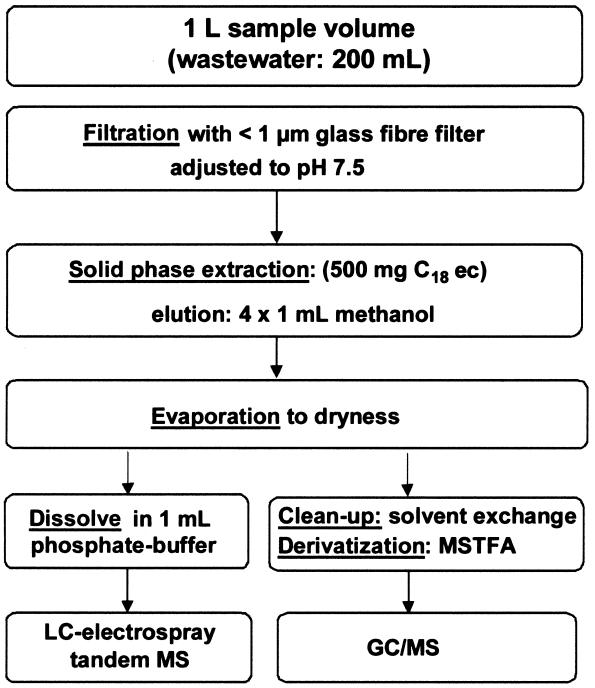


Fig. 5. Scheme of the analytical method for neutral pharmaceuticals [26].

water and of 100–250 ng/l in STP effluents. However, for phenazon, ifosfamide and cyclophosphamide relative low recoveries of 51–57% were observed with S.D. (1σ, $n=3$) of about 35%. The peak widths of cyclophosphamide, ifosfamide, carbamazepine, phenazon and pentoxifylline were sometimes extremely high and seem to be mainly related to an increasing number of samples, especially for STP effluents with high levels of co-extracted organic matter. The peaks of cyclophosphamide and pentoxifylline were subject to interference at the same retention time by co-eluting unknown compounds which even showed the same GC/MS quantitation masses. Furthermore, carbamazepine is decomposed in the injector of the GC/MS forming iminostilbene as degradation product.

Therefore, an alternative procedure using LC–ES/MS/MS was described in parallel which allows the determination of these five drugs (cyclophosphamide, ifosfamide, carbamazepine, phenazon and pentoxifylline). The S.D. (1σ, $n=5$) in LC–ES/MS/MS decreased dramatically and organic impurities did not disturb the peak width. The detection limits were improved to 10 ng/l, independent from the water matrices (Table 6). For the determination of cyclophosphamide, ifosfamide, carbamazepine, phenazon and pentoxifylline LC–ES/MS/MS should be preferred to GC/MS analysis. Meanwhile, five other neutral pharmaceuticals such as diazepam, clofibrate and dimethylaminophenazon were integrated in the method. A determination of carbamazepine by GC/MS in STP discharges is possible if for derivatization, silylation by a mixture of (*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA)/trimethylsilylimidazole (TMSI)/dithioerytrol (DTE) (1000:2:2, v/v/w) is used [28].

Table 6
Recoveries (REC) with S.D. (1σ, $n=5$) of neutral drugs by GC/MS and LC–MS/MS detection in spiked ground water and LOQ for distinct matrices [26]

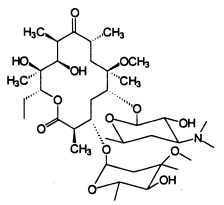
Analyte	REC ± 1σ		LOQ in DW[c], SW[b]		LOQ in STP effl.[a]	
	GC/MS (%)	LC–MS/MS (%)	GC/MS (ng/l)	LC–MS/MS (ng/l)	GC/MS (ng/l)	LC–MS/MS (ng/l)
Clofibrate	71 ± 10	n.a.	20	n.a.	100	n.a.
Phenazon	54 ± 19	45 ± 3	50	10	250	10
Dimethylaminophenazon	93 ± 5	n.a.	20	n.a.	100	n.a.
Ifosfamide	51 ± 20	45 ± 3	50	10	100	10
Cyclophosphamide	57 ± 22	47 ± 3	50	10	250	10
Carbamazepine	99 ± 8	92 ± 1	20	10	100	10
Pentoxifylline	63 ± 7	72 ± 1	50	10	250	10
Diazepam	102 ± 14	n.a.	20	n.a.	100	n.a.
Fenofibrate	91 ± 15	n.a.	20	n.a.	100	n.a.
Etofibrate	82 ± 12	n.a.	20	n.a.	100	n.a.

[a]STP effluent.
[b]Surface water.
[c]Drinking water.
n.a.: not analyzed.

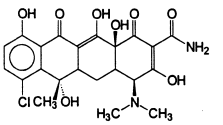
In the literature only a few methods are described which enable the detection of these 'neutral' pharmaceuticals. Phenazon, pentoxifylline and the antiepileptic carbamazepine can be simultaneously determined in the method described for acidic drugs by Sacher et al. [25]. For the enrichment of neutral drugs Möhle et al. [29] used the polystyrenedivinylbenzene copolymer LiChrolute® EN (Merck). An analytical method for the determination of the cytostatic agents cyclophosphamide and ifosfamide in sewage was reported by Steger-Hartmann et al. [30]. The authors used SPE with 500 mg C_{18}-bonded material, a clean-up step on SiOH-SPE cartridges (Fa. Chromabond) and derivatization with trifluoroacetanhydride prior to GC/MS detection. In raw sewage the LOQ was 7 ng/l for ifosfamide and 6 ng/l for cyclophosphamide, the recoveries for ifosfamide and cyclophosphamide were 39% and 30%, respectively. Obviously, the derivatization prevents the increased peak widths and, thus, enables detection limits down to a few ng/l.

2.4. Antibiotics

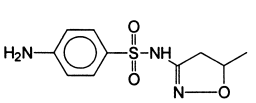
For the determination of 18 antibiotics in water down to the lower ng/l range a multi-analytical method was described [31]. The analytes belong to different groups of antibiotics such as penicillins, tetracyclines, sulfonamides and macrolide antibiotics. Below the chemical structures of a few antibiotics are given.



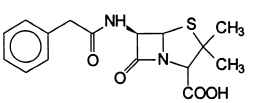
clarithromycin



chlortetracyline



sulphamethoxazole



benzylpenicillin

Samples were enriched using a universal freeze-drying procedure of 100 ml or SPE of 1 l at pH 3.0 with 100 mg of LiChrolute EN and 250 mg of LiChrolute C_{18} (Fig. 6). For freeze-drying the cations were complexed by addition of 100 mg of Na_2EDTA.

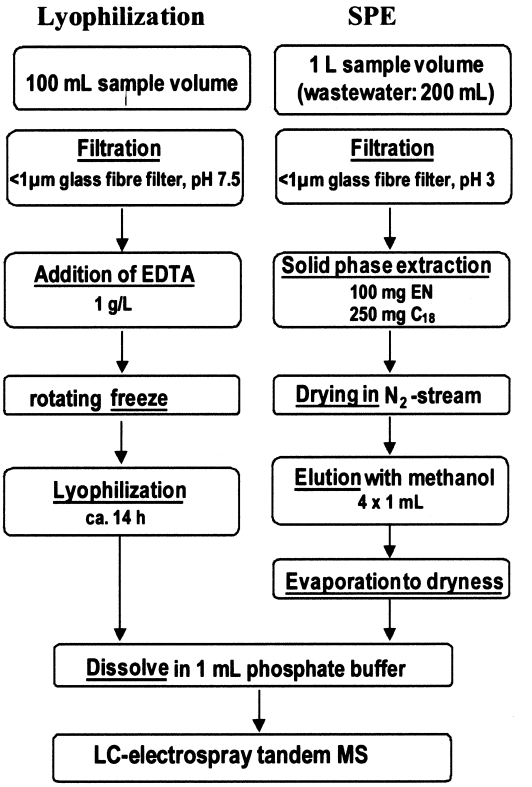


Fig. 6. Scheme of the analytical method for antibiotics [31]. *: For tetracyclines: Na_2EDTA addition.

Analysis was performed by LC–ES/MS/MS, except for chloramphenicol in the positive mode. Chromatography requires the use of C_8- and C_{18}-bonded silica columns and water/acetonitrile mixtures containing ammonium acetate.

Except for dehydrato-erythromycin, trimethoprim and tetracycline, recoveries for the lyophilization were greater than 80% [31] with S.D. (1σ, n=3) between 2 and 15% (Table 7). Recoveries using SPE had a tendency to be slightly lower than for the lyophilization procedure, but is generally sufficient. Tetracyclines are solid phase extractable only after addition of Na_2EDTA to the water sample. Since this was not done, no SPE recoveries are listed in Table 7. LOQs of the antibiotics using the freeze-drying enrichment step are 50 ng/l for the tetracyclines and 20 ng/l (equaling 100 pg per injection) for all others, the results are largely independent of the kind of water matrices. Na_2EDTA addition is essential to quantitatively re-dissolve the freeze-dried sample residues in the phosphate buffer. LOQs using SPE are one magnitude of order lower due to the 1 l sample volume.

Table 7
Recoveries (REC) with S.D. (1σ, *n*=3) of antibiotics spiked in drinking water (DW) and surface water (SW) and LOQ [31]

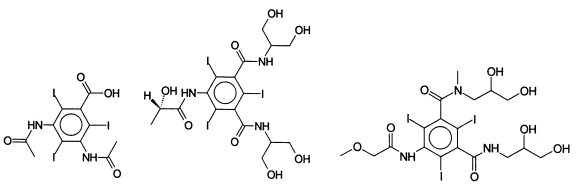
Analytes	REC LY[a] (%)	REC SPE[b] (%)	REC (spiked SW) (%)		LOQ LY[a] (ng/l)	LOQ SPE[b] (ng/l)
			Schwarzbach	Rhine		
Clarithromycin	102	90	85	91	50	5
Dehydrato-erythromycin	54	120	106	120	50	5
Roxithromycin	100	75	79	100	50	5
Sulfamethazine	81	15	40	88	50	5
Sulfamethoxazole	81	75	60	84	50	5
Trimethoprim	68	87	78	103	50	5
Chloramphenicol	82	90	n.a.	98	50	5
Chlortetracycline	87	56[c]	45	n.a.	20	2
Doxycycline	80	86[c]	68	n.a.	20	2
Oxytetracycline	108	74[c]	49	n.a.	20	2
Tetracycline	72	93[c]	53	n.a.	20	2
Cloxacillin	89	68	123	n.a.	50	5
Dicloxacillin	88	61	137	n.a.	50	5
Methicillin	88	62	118	n.a.	50	5
Nafcillin	86	58	121	n.a.	50	5
Oxacillin	90	66	116	n.a.	50	5
Benzylpenicillin	94	61	106	n.a.	50	5
Phenoxymethylpenicillin	79	107	114	n.a.	50	5

[a]Lyophilization.
[b]Solid phase enrichment (*n*=2).
[c]Addition of 1 g/l Na_2EDTA.
n.a.: not analyzed.

In the literature a method using similar SPE enrichment conditions was described by Alder et al. [32]. Instead of LC–ES/MS/MS the authors determined the macrolides and sulfonamides by LC–ES single MS in different water matrices with optimized HPLC conditions. The authors used the surrogate standard josamycine for quantitation.

2.5. *Iodinated X-ray contrast media*

Iodinated X-ray contrast media belong to the most frequently applied compounds in medicine. They exhibit a high polarity and are very persistent against metabolism by organism and environmental degradation. Below the chemical structures of a few contrast media are presented.



diatrizoate iopamidol iopromide

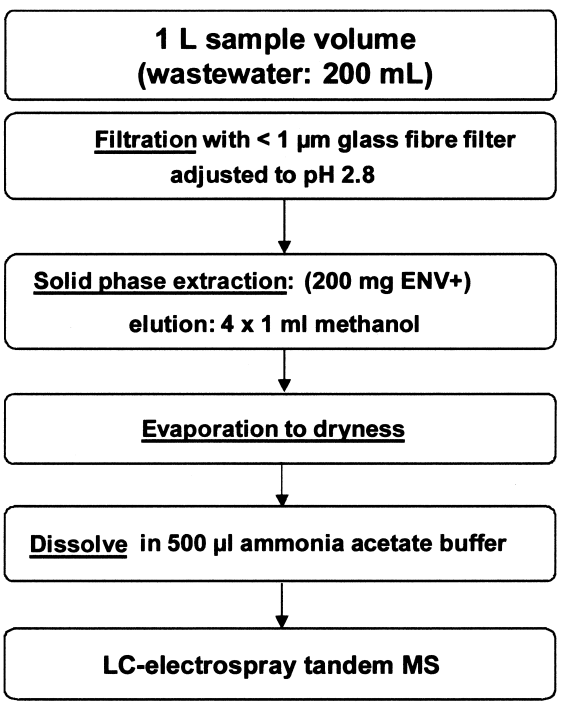


Fig. 7. Scheme of the analytical method for X-ray contrast media [33].

Table 8
Recoveries (REC) of iodinated X-ray contrast media detected by API III plus in ground water (GW) and Rhine water and LOQ of ground water (GW), drinking water (DW), surface water (SW) and STP effluents (STPeffl.) [33]

Analyte	REC GW (n=3) (%)	Rhine unspiked (μg/l)	Rhine 1 μg/l spiked (μg/l)	REC[b] (%)	LOQ GW, DW, SW (ng/l)	LOQ STPeffl. (ng/l)
Iopamidol	51 ± 4	0.30	1.25	96	10	50
Iopromide	89 ± 6	0.15	1.13	98	10	50
Ioxithalamic acid	87 ± 11	0.04	0.75	72	10/30[a]	50
Iothalamic acid	105 ± 16	0.01	0.75	74	10/30[a]	50
Diatrizoate	90 ± 6	0.11	0.70	63	10	50

[a]For surface water.
[b]Difference between spiked and non-spiked Rhine water.

A sensitive method for the determination of iodinated X-ray contrast media in aqueous matrices (Fig. 7) uses SPE on Isolute® ENV+ for sample enrichment [33]. The X-ray contrast media were either partially separated on a 150×2.1 mm ID Intertsil® phenyl-3/GL Sciences (3 μm) column prior to detection by API 365 (Perkin Elmer) or separated by ion pair chromatography on 125×3 mm ID Merck LiChrospher® RP-C_{18} (5 μm) columns, prior to detection by API III plus (Perkin Elmer). Both ES/MS/MS instruments enable the sensitive quantitation of contrast media down to the lower ng/l range.

Mean recoveries of the iodinated X-ray contrast media spiked in ground water exceeded 87% with the exception of iopamidol (51%) (Table 8). The S.D. (1σ, n=3) ranged between 2% and 16%, which shows the efficiency and repeatability of the analytical method [33]. The recoveries in Rhine water (difference between spiked and unspiked sample) for the ionic X-ray contrast media (ioxithalamic acid, iothalamic acid, diatrizoate) were 63–72%, while the non-ionic X-ray contrast media iopamidol and iopromide were recovered nearly quantitatively. The LOQs in ground water, drinking water and surface water were ca. 10 ng/l (only for ioxithalamic acid and iothalamic acid LOQs were in some cases 30 and 20 ng/l, respectively) and 50 ng/l in raw and treated sewage due to the higher proportion of sample impurities. Using the surrogate standard desmethoxy-iopromide (DMI) for quantitation the recoveries in surface water (the original contamination of the water was subtracted from the results of the spiked sample) always exceeded 90%, and were below 70% in STP effluents only for ioxithalamic acid and iothalamic acid (Table 9). Hence, for highly contaminated samples such as sewage the use of a surrogate standard is essential. However, matrix effects can be reduced by in-line introduction of C_{18}-cartridges prior to the SPE extraction, since X-ray contrast media do not sorb on C_{18}-bonded phases.

In the further literature Putschew et al. [34] have described a method for the individual determination of two X-ray contrast media. They determined diatrizoate and iopromide with LC/MS after a combined SPE on LiChrolute EN and Envi-Carb cartridges. Wischnack et al. [35] used adsorbable organic iodine (AOI) detection and showed that

Table 9
Relative recoveries of X-ray contrast media in surface water and STP effluents using API 365 [33]

Analyte	Surface water, unspiked (μg/l)	Surface water, +500 ng/l (μg/l)	Relative[a] recovery (%)	STP effluent, unspiked (μg/l)	STP effluent, +1 μg/l (μg/l)	Relative[a] recovery (%)
Iopamidol	0.18	0.63	90	0.59	1.58	99
Iopromide	0.15	0.70	110	3.07	3.8	73
Ioxithalamic acid	0.04	0.53	98	< 0.05	0.35	35
Diatrizoate	0.14	0.72	116	1.14	2.27	113
Iothalamic acid	0.01	0.51	100	0.09	0.66	57
Iomeprol	0.04	0.51	94	2.06	3.08	102

[a]Relative in comparison to a linear calibration using enriched standards and API 365 detection.

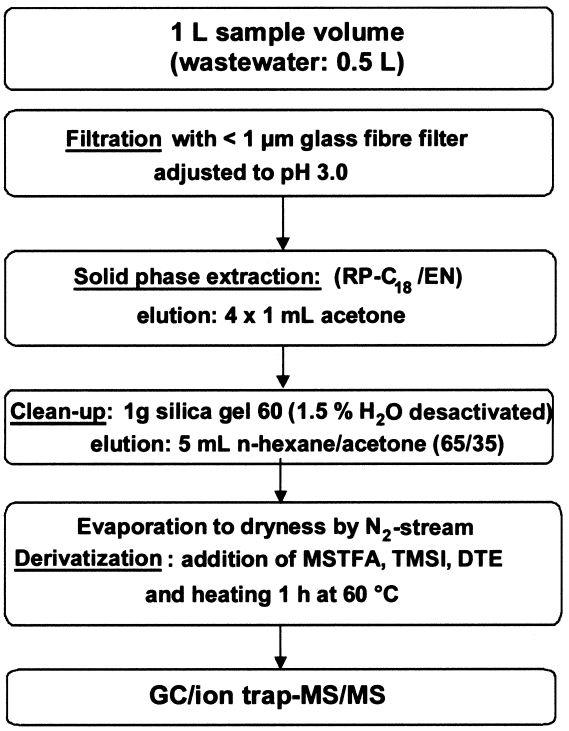
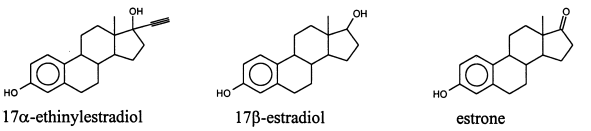


Fig. 8. Scheme of the analytical method for estrogens [36].

organic compounds containing iodine atoms are present in the aquatic environment and in drinking water up to 7 µg/l. They assumed that AOI contamination was primarily due to the presence of iodinated X-ray contrast media.

2.6. Estrogens

Estrogens comprise a group of compounds with steroid structures containing phenolic and sometimes aliphatic hydroxy groups. Natural estrogens (e.g. 17β-estradiol, estrone) and the synthetic contraceptives (e.g. 17α-ethinylestradiol) can be analyzed simultaneously because of their similar physical–chemical properties. Basically, the estrogens possess a higher lipophilicity (log P_{OW} 3.5–4.6) than all the other before mentioned human pharmaceuticals, and their excreted quantities are extremely low. Thus, the prerequisite for an appropriate analytical method for environmental aqueous samples is LOQs down to 1 ng/l. Below, the chemical structures of three estrogens are shown.



Natural estrogens and two selected contraceptives were enriched at pH 3 (Fig. 8) by SPE extraction using a combination of 0.10 g LiChrolut® EN and 0.25 g RP-C_{18} [35]. After clean-up over a 1 g silica gel column, the extracts were derivatized by adding 50 µl of the derivatization mixture MSTFA/TMSI/DTE (1000:2:2, v/v/w) and were detected by GC–ion trap MS/MS.

The described method [36] enables the quantification of estrogens in sewage samples down to 1 ng/l and in river water down to 0.5 ng/l (Table 10). Mean recoveries of the analytes in ground water after SPE extraction, clean-up and derivatiza-

Table 10
Recovery rates (REC) and S.D. (±1σ, *n*=3) of estrogens (spiking with 50 ng/l in ground water (GW) and surface water (SW), and 25 ng/l in STP effluents (STPeffl.) and raw sewage (RWA)) as well as LOQ [36]

Analyte	REC ± 1σ GW (%)	LOQ DW, SW (ng/l)	REC RAW (%)	LOQ RAW[a] (ng/l)	REC STPeffl. (%)	LOQ STPeffl. (ng/l)
Estrone	90 ± 2	0.5	86	1.0	82	1.0
17β-Estradiol	77 ± 11	0.5	84	1.0	76	1.0
Mestranol	90 ± 1	0.5	77	1.0	74	1.0
17α-Ethinylestradiol	85 ± 0	0.5	88	1.0	76	1.0
17β-Estradiol-17-valerate	75 ± 10	1.0	58	2.0	56	2.0
16α-Hydroxyestrone	41 ± 4	0.5	56	1.0	52	1.0
17β-Estradiol-17-acetate (surrogate standard)	88 ± 3	0.5	–	1.0	–	1.0

[a]Only a few samples can be detected facultatively since the MS system has to be cleaned.

Table 11
Concentrations of pharmaceuticals in municipal German STP effluents; results from 1996 to 1998 [36,51,52,58]

Analyte	LOQ (μg/l)	Number STPs	$n >$ LOQ	Median (μg/l)	90-percentile (μg/l)	Maximum (μg/l)
Lipid regulator						
Bezafibrate	0.25	49	48	2.2	3.4	4.6
Gemfibrozil	0.050	49	39	0.40	0.84	1.5
Clofibric acid (metabolite)	0.050	49	47	0.36	0.72	1.6
Fenofibric acid (metabolite)	0.050	49	41	0.38	0.68	1.2
Antiphlogistics						
Diclofenac	0.050	49	49	0.81	1.6	2.1
Ibuprofen	0.050	49	42	0.37	1.2	3.4
Indomethacin	0.050	49	49	0.27	0.40	0.60
Naproxen	0.050	10	10	0.30	0.42	0.52
Ketoprofen	0.050	49	37	0.20	0.25	0.38
Phenazon	0.10	30	28	0.16	0.30	0.41
ASA	0.10	49	22	0.22	0.32	1.5
Salicylic acid (metabolite)	0.050	36	9	< LOQ	0.063	0.14
Betablocker						
Metoprolol	0.025	29	29	0.73	1.3	2.2
Propranolol	0.025	29	28	0.17	0.23	0.29
Betaxolol	0.025	29	17	0.057	0.10	0.19
Bisoprolol	0.025	29	17	0.057	0.13	0.37
β_2-Sympathomimetics						
Terbutalin	0.050	29	11	< LOQ	0.087	0.12
Salbutamol	0.050	29	10	< LOQ	0.072	0.17
Psychiatric drug						
Diazepam	0.030	20	8	< LOQ	0.03	0.04
Antiepileptic						
Carbamazepine	0.050	30	30	2.1	3.7	6.3
Antibiotics						
Clarithromycin	0.020	8	8	0.14	0.24	0.26
Roxithromycin	0.020	10	10	0.68	0.80	1.00
Chloramphenicol	0.020	10	1	< LOQ	< LOQ	0.56
Sulfamethoxazol	0.020	10	10	0.40	0.90	2.00
Trimethoprim	0.020	10	9	0.32	0.62	0.66
Dehydrato-erythromycin (metabolite)	0.020	10	10	2.50	5.10	6.00
X-ray contrast media						
Iopamidol	0.010	25	21	0.66	8.0	15
Iopromide	0.010	24	23	0.75	4.4	11
Diatrizoate	0.010	25	22	0.08	1.5	8.7
Iomeprol	0.010	12	10	0.37	2.8	3.8
Estrogens						
Estrone	0.001	38	20	0.001	0.021	0.070
17β-Estradiol	0.001	38	13	< LOQ	0.002	0.003
17β-Estradiol-17-valerate	0.004	38	0	< LOQ	< LOQ	< LOQ
17α-Ethinylestradiol	0.001	38	9	< LOQ	0.001	0.015
16α-Hydroxyestrone	0.001	15	11	0.001	0.004	0.005

LOQ: limit of quantification. STP: sewage treatment plant effluents (identical with the number of investigated STPs).

tion generally exceeded 75%. The determined S.D. (1σ, n=3) are always below 15% at a spiking level of 50 ng/l. Even in the raw influent and the final effluent from municipal STPs the mean recoveries of estrogens were mostly above 70%.

The improved confirmation using GC/MS/MS is essential for the detection of 17α-ethinylestradiol since an unknown compound exhibited exactly the same retention time [36]. Both EI spectra showed the m/z values of 440 (molecular weight

Table 12
Concentrations of pharmaceuticals in German rivers and streams; results from 1996 to 1998 [36,51,52,58]

Analyte	LOQ (μg/l)	Number STPs	$n >$ LOQ	Median (μg/l)	90-percentile (μg/l)	Maximum (μg/l)
Lipid regulator						
Bezafibrate	0.025	43/22	39	0.35	1.2	3.1
Gemfibrozil	0.010	43/22	28	0.052	0.19	0.51
Clofibric acid (metabolite)	0.010	43/22	35	0.066	0.21	0.55
Fenofibric acid (metabolite)	0.010	43/22	26	0.045	0.17	0.28
Antiphlogistics						
Diclofenac	0.010	43/22	43	0.15	0.80	1.20
Ibuprofen	0.010	43/22	35	0.07	0.28	0.53
Indometacin	0.010	43/22	35	0.04	0.17	0.20
Naproxen	0.010	20/20	20	0.07	0.15	0.39
Ketoprofen	0.010	43/22	5	< LOQ	0.12	0.12
Phenazon	0.020	26/20	21	0.024	0.15	0.95
ASA	0.020	43/22	17	< LOQ	0.16	0.34
Salicylic acid (metabolite)	0.010	35/19	24	0.025	0.13	4.1
Betablocker						
Metoprolol	0.010	45/23	38	0.045	1.2	2.2
Propranolol	0.010	45/23	26	0.012	0.44	0.59
Betaxolol	0.010	45/23	1	< LOQ	< LOQ	0.028
Bisoprolol	0.010	45/23	19	< LOQ	0.19	2.9
β_2-Sympathomimetics						
Terbutalin	0.010	45/23	0	< LOQ	< LOQ	< LOQ
Salbutamol	0.010	45/23	2	< LOQ	< LOQ	0.035
Psychiatric drug						
Diazepam	0.030	30/20	0	< LOQ	< LOQ	< LOQ
Antiepileptic						
Carbamazepine	0.030	26/20	24	0.25	0.82	1.1
Antibiotics						
Clarithromycin	0.020	33/22	7	< LOQ	0.15	0.26
Roxithromycin	0.020	52/40	23	< LOQ	0.20	0.56
Chloramphenicol	0.020	52/40	4	< LOQ	< LOQ	0.06
Sulfamethoxazol	0.020	52/40	26	0.03	0.14	0.48
Trimethoprim	0.020	52/40	10	< LOQ	0.09	0.20
Dehydrato-erythromycin (metabolite)	0.020	52/40	31	0.15	0.63	1.7
X-ray contrast media						
Iopamidol	0.010	25/25	24	0.49	1.6	2.8
Iopromide	0.010	25/25	22	0.10	0.55	0.91
Diatrizoate	0.010	25/25	23	0.23	6.4	ca. 100
Iomeprol	0.010	12/12	12	0.10	0.47	0.89
Estrogens						
Estrone	0.0005	15/15	3	< LOQ	0.001	0.0016
17β-Estradiol	0.0005	15/15	0	< LOQ	< LOQ	< LOQ
17β-Estradiol-17-valerate	0.002	15/15	0	< LOQ	< LOQ	< LOQ
17α-Ethinylestradiol	0.0005	15/15	0	< LOQ	< LOQ	< LOQ
16α-Hydroxyestrone	0.0005	15/15	0	< LOQ	< LOQ	< LOQ

LOQ: limit of quantification.

(MW) of silylated 17α-ethinylestradiol) and 425 (MW 'minus' CH_3), however, with a different ratio. Using MS/MS detection of the target ion m/z 425 a confirmation with regard to identification and quantification of 17α-ethinylestradiol can be carried out. Due to the fact that the MS/MS spectra of the contraceptive and the unknown impurity are different, a precise quantitation is possible using the product ions 193 m/z and 231 m/z of the precursor ion 425 m/z. Using single MS detection the probability of determining excessive concentrations cannot be excluded. There is a distinct possi-

bility that 17α-ethinylestradiol will be overestimated.

In the further literature the methods published for the determination of estrogens in water are also mostly based on SPE, silylation and detection by GC/MS or GC–ion trap MS/MS with LOQs in the lower ng/l range. Different agents are used for silylation such as MSTFA [37], hexamethyldisilazane/trimethylchlorosilane/pyridine, BSTFA and MSTFA/TMCS (e.g. [38–43]). Acetylation with anhydrides (e.g. heptafluorobutyric anhydride) is the other frequently applied derivatization procedure [39,44]. Using LC the derivatization step can be avoided [45,46]. Reversed phase LC coupled on-line to a receptor affinity fluorescence detection based on the human estrogen receptor is reported by Oosterkamp et al. [47]. The detection limit for 17β-estradiol was 5 nmol/l (1 μg/l). A screening of estrogenic compounds in water samples can also be conducted by immuno- and bioassays (e.g. [48–50]).

3. Exposure of the environment to pharmaceuticals

Using the methods discussed above, 36 of 55 pharmaceuticals and five of nine metabolites were quantified in at least one German STP effluent. In general, the removal in the municipal STP exceeded 60% [51]. The highest concentrations of drug residues were measured for the antiepileptic carbamazepine with a maximum of 6.3 μg/l (Table 11). However, X-ray contrast media were found in concentrations as high as 15 μg/l for iopamidol and 11 μg/l for iopromide [52]. In 40 German rivers and streams 31 pharmaceuticals and five metabolites were quantified in at least one sample (Table 12). The highest median values were found for bezafibrate with 0.35 μg/l and carbamazepine with 0.25 μg/l. In Table 11 detailed data are listed of the main pharmaceuticals monitored in municipal German STP effluents from 1996 to 1998; Table 12 summarize the data for German rivers and streams. In ground water samples taken in the same campaign close to the bank of streams, relatively high concentrations of pharmaceuticals up to 2.4 μg/l were sometimes detected. In drinking water only 10 of 69 target pharmaceuticals were found, without exception in the lower ng/l range [53]. However, frequently the pharmaceuticals could not be identified in drinking water.

A literature survey of the exposure, effects, and environmental relevance is presented in the reviews of Halling-Sørensen et al. [54], Daughton and Ternes [55], Jørgensen and Halling-Sørensen [56] and Ternes and Wilken [57].

4. Conclusion

The analytical methods discussed above were used for the determination of pharmaceuticals in different aqueous matrices. Highly polluted sewage was investigated as well as river and drinking waters. Many of the investigated drugs could be detected in influents and effluents of German municipal STPs as well as in rivers and streams. Hence, the applied methods which include GC/MS, GC/MS/MS, and mainly LC–ES/MS/MS are appropriate for the determination of pharmaceuticals and phenolic antiseptics in aqueous environmental samples down to the lower ng/l range.

Acknowledgements

This study was financially supported by grants of the Ministry of Education and Research (BMBF)/ Germany and the Hessian Ministry for Environment, Energy, Youth, Family and Health.

References

[1] U. Schwabe, D. Paffrath, Arzneiverordnungsreport '96 Aktuelle Daten, Kosten, Trends und Kommentare, Gustav Fischer Verlag, Stuttgart, 1999.
[2] A. Wenzel, Ch. Schäfers, A Schmitz, V. Vollmer, Research efforts towards the development and validation of a test method for the identification of endocrine disrupting chemicals, Second Progress Report, EC Contract, B6-7920/98/000015, 2000.
[3] M.L. Richardson, J.M. Bowron, J. Pharm. Pharmacol. 37 (1985) 1.
[4] Th.A. Ternes, P. Kreckel, J. Müller, Sci. Total Environ. 225 (1999) 91.
[5] C. Giachetti, G. Zanolo, P. Poletti, F. Perovanni, J. High Resolut. Chromatogr. 13 (1990) 789.
[6] J.B. Whitlam, J.H. Vine, J. Chromatogr. 181 (1980) 463.
[7] B. Amos, L. Marple, J. Smithers, S.B. Matin, J. Chromatogr. 206 (1981) 151.
[8] E.G. De Jong, J. Kiffers, R.A.A. Maes, J. Pharm. Biomed. Anal. 7 (1989) 1617.
[9] M.S. Leloux, E.G. De Jong, R. Maes, J. Chromatogr. 488 (1989) 357.
[10] C.L. Davies, J. Chromatogr. 531 (1990) 131.

[11] J.G. Leferink, J. Dankers, R.A. Maes, J. Chromatogr. 229 (1982) 217.
[12] I.I. Hewala, Anal. Lett. 25 (1992) 1877.
[13] S.M. Lankford, S. Bai, J. Chromatogr. 663 (1995) 91.
[14] J. Volmut, E. Matisova, P.T. Ha, J. High Resolut. Chromatogr. 12 (1989) 760.
[15] J. Volmut, E. Matisova, M. Melnik, J. High Resolut. Chromatogr. 16 (1993) 27.
[16] Th.A. Ternes, M. Stumpf, B. Schuppert, K. Haberer, Wasser 90 (1998) 295.
[17] C. Hignite, D.L. Azarnoff, Life Sci. 20 (1977) 337.
[18] A.W. Garrison, J.D. Pope, F.R. Allen, in: C.H. Keith (Editor), Identification and Analysis of Organic Pollutants in Water, Ann Arbor Science, Ann Arbor, MI, 1976, p. 517.
[19] Th. Heberer, H.-J. Stan, GIT Fachz. Lab. 39 (1995) 718.
[20] Th. Heberer, H.-J. Stan, Wasser 86 (1996) 19.
[21] H.-J. Stan, M. Linkerhäger, Wasser 83 (1994) 57.
[22] H.-R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 32 (1998) 3449.
[23] H.-R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 33 (1999) 2529.
[24] H.-R. Buser, M.D. Müller, Environ. Sci. Technol. 32 (1998) 188.
[25] F. Sacher, E. Lochow, D. Bethmann, H.-J. Brauch, Wasser 90 (1998) 233.
[26] Th.A. Ternes, R. Hirsch, J. Müller, K. Haberer, Fres. J. Anal. Chem. 362 (1998) 329.
[27] Th.A. Ternes, R. Hirsch, M. Stumpf, T. Eggert, B. Schuppert, K. Haberer, Nachweis und Screening von Arzneimittelrückständen, Diagnostika und Antiseptika in der aquatischen Umwelt. Abschlußbericht des Forschungsvorhabens 02WU95667/3, BMBF und Hessisches Ministeriums für Umwelt, Energie, Jugend, Familie und Gesundheit, 1999.
[28] M. Meisenheimer, Th.A. Ternes, Wasser 94 (2000) 203.
[29] E. Möhle, S. Horvath, W. Merz, J.W. Metzger, Wasser 92 (1999) 207.
[30] Th. Steger-Hartmann, K. Kümmerer, A. Hartmann, Environ. Saf. 36 (1997) 174.
[31] R. Hirsch, Th.A. Ternes, A. Mehlig, F. Ballwanz, K.-L. Kratz, K. Haberer, J. Chromatogr. 815 (1998) 213.
[32] A.C. Alder, C.S. McArdell, E.M. Golet, S. Ibric, E. Molnar, N.S. Nipales, W. Giger, in: Ch. Daughton, T. Jones-Lepp (Editors), Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issue, ACS-Symposium Series, 2001, in press.
[33] R. Hirsch, Th.A. Ternes, A. Merz, K. Haberer, R.-D. Wilken, Fres. J. Anal. Chem. 366 (2000) 835–841.
[34] A. Putschew, S. Wischnack, M. Jekel, Sci. Total Environ. 255 (2000) 129.
[35] S. Wischnack, J. Oleksy-Frenzel, M. Jekel, Abbauverhalten und Vorkommen organischer Iodverbindungen im Raum Berlin, Vortrag auf der Jahrestagung 1998 der Fachgruppe Wasserchemie in der GDCh, Lübeck, 1998.
[36] Th.A. Ternes, M. Stumpf, J. Müller, K. Haberer, R.-D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81.
[37] G. Sturm, I. Loof, L. Spätling, K. Mohr, R. Buchholz, Anal. Chem. Symp. Ser. 10 (1981) 315.
[38] S. Fukushima, A. Akane, K. Matsubara, H. Shiono, H. Morishita, F. Nakada, J. Chromatogr. 565 (1991) 35.
[39] E. Daeseleire, K. Vanoosthuyze, C. Van Peteghem, J. Chromatogr. A674 (1991) 247.
[40] G. Wegener, J. Persin, F. Karrenbrock, O. Rörden, I. Hübner, Wasser 92 (1999) 347.
[41] P. Spengler, W. Körner, J.W. Metzger, Wasser 93 (1999) 141.
[42] A.C. Belfroid, A. Van der Horst, A.D. Vethaak, A.J. Schäfer, G.B.J. Rijs, J. Wegener, W.P. Cofino, Sci. Total Environ. 225 (1999) 101.
[43] S.S. Snyder, T.L. Keith, D.A. Verbruegge, E. Snyder, T.S. Gross, K. Kannan, J.P. Giesy, Environ. Sci. Technol. 33 (1999) 2814.
[44] H.B. Lee, T.E. Pert, J. Assoc. Off. Anal. Chem. Int. 81 (1999) 1209.
[45] G.R. Cannell, R.H. Mortimer, D.J. Maguire, R.S. Addison, J. Chromatogr. 563 (1991) 341.
[46] G. Brenner-Weiss, M. Nusser, U. Obst, Wirkungsbezogene Analytik am Beispiel der Östrogene, Essener Tagung, 1999.
[47] A.J. Oosterkamp, M.T. Villaverde Herraiz, H. Irth, U.R. Tjaden, J. van der Greef, Anal. Chem. 68 (1996) 1201.
[48] A.M. Soto, C. Sonnenschein, K.L. Chung, M.F. Fernandez, N. Olea, M.F. Olea-Serrano, Environ. Health Perspect. 103 (1995) 113.
[49] L.S. Shore, M. Gurevitz, M. Shemesh, Bull. Environ. Contam. Toxicol. 51 (1993) 361.
[50] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (1998) 1549.
[51] Th.A. Ternes, Water Res. 32 (1998) 3245.
[52] Th.A. Ternes, R. Hirsch, Environ. Sci. Technol. 34 (2000) 2741.
[53] Th.A. Ternes, Pharmaceuticals and metabolites as contaminants of the aquatic, in: Ch. Daughton, T. Jones-Lepp (Editors), Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issue, ACS-Symposium Series, 2001, in press.
[54] B. Halling-Sørensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C. Holten Lützhøft, S.E. Jørgensen, Chemosphere 36 (1998) 357.
[55] Ch. Daughton, Th.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[56] S.E. Jørgensen, B. Halling-Sørensen, O. Hutzinger, Chemosphere 40 (2000) 691.
[57] Th.A. Ternes, R.-D. Wilken, Sci. Total Environ. 225 (1999) 176.
[58] R. Hirsch, Th.A. Ternes, K.-L. Kratz, K. Haberer, Sci. Total Environ. 225 (1999) 109.

Thomas Ternes graduated with a diploma in Chemistry 1989 and received his PhD on the analysis of organic contaminants in water and fish by GC/MS at the University of Mainz in 1993. Since 1994 he is coordinating and managing research projects at the ESWE-Institute for Water Research and Water Technology. He is a lecturer at the University of Mainz for the analysis of organic contaminants in the environment. Since 1994 he is dealing with the analysis of organic compounds especially pharmaceuticals and personal care products in wastewater, river and drinking water.