ELSEVIER

Journal of Chromatography A, 815 (1998) 213–223

JOURNAL OF CHROMATOGRAPHY A

Determination of antibiotics in different water compartments via liquid chromatography–electrospray tandem mass spectrometry

Roman Hirsch[a,*], Thomas A. Ternes[a], Klaus Haberer[a], Armin Mehlich[b], Frank Ballwanz[b], Karl-Ludwig Kratz[c]

[a]*ESWE Institute for Water Research and Water Technology, Soehnleinstrasse 158, D-65201 Wiesbaden, Germany*
[b]*StVUA Detmold, Westernfeldstrasse 1, D-32758 Detmold, Germany*
[c]*Institute for Nuclear Chemistry, Strassmannweg 2, D-55099 Mainz, Germany*

Received 9 December 1997; received in revised form 17 April 1998; accepted 17 April 1998

Abstract

For the determination of 18 antibiotics in water samples down to the lower ng/l range, an analytical multi method is presented. The analytes belong to different groups of antibiotics such as penicillins, tetracyclines, sulfonamides and macrolid antibiotics. Samples were enriched using a universal freeze–drying procedure or a solid-phase extraction facultatively. Analysis was performed by liquid chromatography with electrospray–tandem MS detection. Chromatography required different columns and eluents. Mean recovery rates were in excess of 70%, however, with one exception and a quantitation limit of 50 ng/l for the tetracyclines and 20 ng/l for all other antibiotics were set.

Keywords: Water analysis; Mass spectrometry; Antibiotics; Tetracyclines; Penicillins; Macrolid antibiotics; Sulfonamides

1. Introduction

One of the most relevant topics in today's environmental analytical chemistry is water quality. In order to characterize water quality, concentrations of polar organic pollutants have to be determined. In addition to pesticides, industrial chemicals and their metabolites, pharmaceutical substances have experienced a fast growing interest. Recent studies have shown that a multitude of drugs are present in aquatic systems [1–3]. The interest in the analysis of antibiotic residues in the environment arises from the fact that they are suspected of being responsible for the appearance of bacterial strains that are resistant to antibiotics which are important drugs for the treatment of many serious infections.

In Germany the annual production of antibiotics is in the range of 2000 tons per year. One such main group are the penicillins, with production rates in Germany of approximately 900 tons/year [4]. Tetracyclines, sulfonamides and macrolid antibiotics are some additional important groups of antibiotics for which a proper analytical method in water down to the ng/l range has to be designed (see Fig. 1). Intake pathways into the aquatic environment result from their applications in both human and veterinary medicine. After consumption the active compounds are often metabolized only partially and are then excreted via urine or feces [5]. Thus, they are

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00335-5

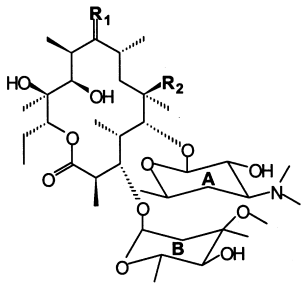
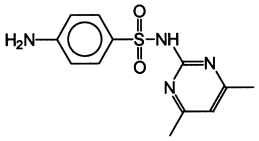
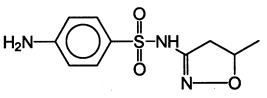
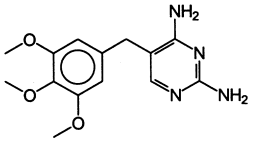
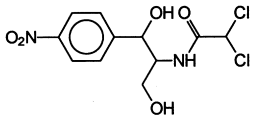
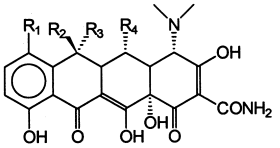
substance	CAS-registry-no.	structure
group ANT		
macrolid antibiotics		
		A = desosamine B = cladinose (3-O-methylmycarose)
clarithromycin	[81103-11-9]	R_1 = O; R_2 = OCH_3
erythromycin	[114-07-8]	R_1 = O; R_2 = OH
roxithromycin	[80214-83-1]	R_1 = N-O-CH_2-CH_2-OCH_3; R_2 = OH
sulfamethazine	[57-68-1]	
sulfamethoxazole	[723-46-6]	
trimethoprim	[738-70-5]	
chloramphenicol	[56-75-7]	
group TET		
tetracyclines		
chlortetracycline	[57-62-5]	R_1 = Cl; R_2 = OH; R_3 = CH_3; R_4 = H
doxycycline	[17086-28-1]	R_1 = H; R_2 = H; R_3 = CH_3; R_4 = OH
oxytetracycline	[79-57-2]	R_1 = H; R_2 = OH; R_3 = CH_3; R_4 = OH
tetracycline	[60-54-8]	R_1 = H; R_2 = OH; R_3 = CH_3; R_4 = H

Fig. 1. Structures of the investigated antibiotics.

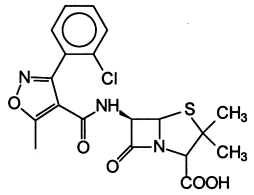
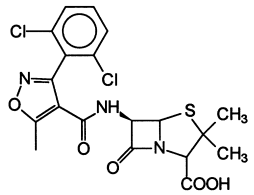
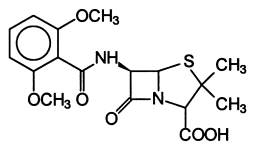
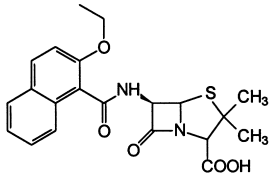
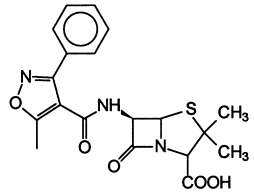
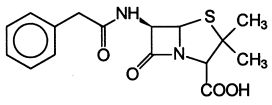
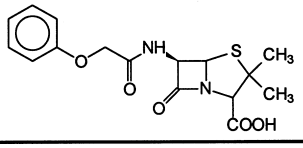
group PEN		
cloxacillin	[61-72-3]	
dicloxacillin	[3116-76-5]	
methicillin	[61-32-5]	
nafcillin	[147-52-4]	
oxacillin	[66-79-5]	
penicillin G	[61-33-6]	
penicillin V	[87-08-1]	

Fig. 1. (*continued*)

presumably present in raw sewage and may even leave the sewage treatment plants (STPs) as they have probably not been fully eliminated [2,3,6].

The use of those substances as feed additives in veterinary medicine underlies only minor legal regimentation. Application amounts are not available. Therefore, no estimation for the environmental intake of these substances can be made. As these compounds are most likely to be found at very low concentrations in the different compartments of the aquatic environment, like STP influents and effluents, surface, ground and drinking water [7,8], it is

necessary to develop a sensitive procedure which has the capability to confirm the presence of antibiotics down to the lower ng/l range.

Most of the continuously monitored water contaminants are determined via gas chromatography–mass spectrometry (GC–MS). A separation of the antibiotics mentioned herein via GC requires derivatization of the polar moieties. In literature some methods for the determination of e.g., chloramphenicol and sulfamethazine via GC–MS have been described [9,10]. However, as the analyte groups show different properties concerning number and kind of functional groups (see Fig. 1), it seems quite difficult to develop a universal derivatization procedure suitable for all the substances. High-performance liquid chromatography (HPLC) coupled to a tandem MS detector is a powerful technique for separation, identification and quantitation of polar compounds and was therefore chosen as a preferable method. As, macrolid antibiotics, which are made up of three structure elements, the erythronolide aglycone and two sugar moieties (desosamine and cladinose as described in Fig. 1), show no measurable UV absorption in the spectrum of conventional deuterium lamps, most publications concerning the determination of these substances describe electrochemical detection [11]. Generally, most of the published methods concerning the determination of antibiotic drugs are designed for complex matrices like meat, milk, blood etc., and achieve only relatively high detection limits in the range of several hundred ng/kg or ng/l, respectively [12–16]. In addition most of the applied extraction procedures are especially designed for only one special class of antibiotics e.g., tetracyclines.

The aim of this work was to establish an effective and simple multi-method in order to determine the amounts of several antibiotic substances via LC–MS–MS down to the lower ng/l range.

2. Experimental

Reference compounds were purchased from Sigma except for roxithromycin and clarithromycin which were supplied courtesy of the manufacturers (Roussel Uclaf, France and Abbott, Germany). Standard solutions were stored in phosphate buffer (see below) at −20°C. They were always renewed after two months. All solvents were utilized in gradient grade or higher quality.

2.1. Preconcentration

For the extraction of the analytes from water two alternative methods have been developed based on solid-phase extraction (SPE) and lyophilization as described below.

2.1.1. Lyophilization

Prior to the lyophilization procedure, water with high natural organic matter (NOM), like STP effluents and surface water samples, were filtered through a 0.45-μm glass fibre filter. Then 100 ml of water was filled into a 250-ml round-bottomed flask. In order to prevent the formation of solid calcium and magnesium carbonate, the cations were complexed by the addition of 100 mg of EDTA (disodium salt, purchased from Riedel-de Haen). Afterwards, the samples were frozen in an ethanol bath at −30°C. During the freezing procedure the flasks were rotated to obtain a large ice surface and thus significantly reduce the overall drying time. Samples were then mounted on a freeze-dryer for about 15 h. Finally, the lyophilized samples were redissolved in 1 ml of phosphate buffer (c=20 mmol/l) and the resulting extracts were stored at −20°C until measurement. Prior injection, the extracts were filtered through 0.45-μm PTFE syringe filters (Schleicher and Schuell) to remove precipitated EDTA. The phosphate buffer was prepared by mixing two solutions of KH_2PO_4 and K_2HPO_4, both 20 mmol/l, until a pH value of 6.0 was obtained.

2.1.2. Solid-phase extraction

As an alternative to the lyophilization method, a SPE method for the antibiotics (except tetracyclines) was developed based on methods that are currently in use for a variety of medium polar substances. One liter of the sample was glass fibre filtered (see above) and the pH was adjusted to 3.0 with H_2SO_4 (c=3 mol/l). The solid phase material (100 mg of Lichrolute EN and 250 mg of Lichrolute C_{18}, both purchased from Merck, Darmstadt) was manually filled into glass cartridges and was consecutively conditioned with 3×2 ml *n*-hexane, 3×2 ml methanol

and 6×2 ml water (pH 3.0). The sample was sucked through the cartridge with a flow rate of approximately 20 ml/min. The cartridges were then dried for 1 h with nitrogen and eluted four times with 1 ml methanol. The extracts were reduced to approximately 20 μl in a gentle nitrogen stream and then filled up to 1 ml with phosphate buffer and stored at −20°C until measured.

2.2. HPLC conditions

The HPLC system consisted of a Merck–Hitachi L-6200 pump connected to an AS-2000a autosampler and a D-6000 interface. The LC conditions required three types of columns and gradients. Tetracyclines were separated using a 125×3 mm Macherey and Nagel Lichrospher 100 RP-8 (end-capped) column (5 μm) with a mobile phase consisting of 20 mmol/l oxalic acid in water–acetonitrile (method TET). Due to the low pH of the gradient buffer, EDTA might precipitate after injection of the freeze-dried extract and clog the capillary of the MS interface. To circumvent such problems, the HPLC solvent should be sent to waste during the first 3 min of the run as the EDTA was hardly retained on the chromatographic columns and therefore elutes before the analytes. Alternatively, the lyophilizate for the tetracycline determination should be reconstituted in the oxalic acid buffer to prevent a precipitation during measurement. Penicillins were chromatographed utilizing a 125×3 mm Merck LiChrospher 100 RP-18 (end-capped) column (5 μm) with a mobile phase consisting of 10 mmol/l ammonium acetate in water–acetonitrile (method PEN). For the remaining antibiotics a 125×3 mm Merck LiChrospher RP-18 column (5 μm) with a mobile phase containing 10 mmol/l ammonia acetate in water–acetonitrile (method ANT) was utilized. The injection volume was 50 μl. Solvents and gradients are shown in Table 1.

Table 1
Mobile phase compositions for the three separation methods

Time (min)	Solvent A[a] (%)	Solvent B[a] (%)
Method TET		
0	90	10
1.7	90	10
2.7	75	25
15	38	62
20	23	77
21	0	100
25	0	100
26	90	10
35	90	10
Method PEN	Solvent C[a] (%)	Solvent D[a] (%)
0	90	10
8	90	10
10	70	30
26	70	30
28	0	100
32	0	100
34	90	10
41	90	10
Method ANT	Solvent C[a] (%)	Solvent E[a] (%)
0	74	26
2	62	38
7	62	38
10	48	52
15	0	100
21	0	100
25	74	26
33	74	26

[a] Solvent A: 1800 ml 10 mmol/l oxalic acid+200 ml acetonitrile; solvent B: 800 ml 10 mmol/l oxalic acid+1200 ml acetonitrile; solvent C: acetic acid was added to 900 ml water containing 20 mmol/l NH_3 until a pH of 5.7 was obtained. To this 100 ml of acetonitrile was added; solvent D: 400 ml of solvent C+600 ml acetonitrile; solvent E: 200 ml of solvent C+800 ml acetonitrile.

2.3. MS–MS parameters

The system utilized was a Perkin-Elmer Sciex API III plus triple stage quadrupole mass spectrometer with electrospray ionization. Except for chloramphenicol, the analysis was performed in positive ion mode. Nitrogen was used as curtain gas with a flow-rate of 1 l/min synthetic air with a pressure of 250 kPa and an approximate flow-rate of 0.7 l/min was utilized as nebulizer gas. The interface had a temperature of 60°C. The HPLC flux was split 1:10 in the interface resulting in an approximate spray flux of 40 μl/min. Orifice voltages varied generally from 60 to 70 V, depending on the best mass signal of the ionization products.

MS–MS parameters were optimized in continuous flow mode as follows: after determination of the best conditions for the isolation of the precursor ion (mostly proton or ammonium adduct of the respective analyte) the ion spray voltage, quadrupole and

Table 2
MS–MS parameters

Substance	Precursor mass	Product ion 1	Product ion 2	Optional product ion 3
Clarithromycin	750.0 $[M+H]^+$	116.1 [cladinose[a] $-OCH_3+H]^+$	591.7 [M−desosamine[a]$+H]^+$	158.2 [desosamine$+H]^+$
Erythromycin	716.2 $[M-H_2O+H]^+$	522.2 [M−desosamine$-2H_2O+H]^+$	558.4 [M−desosamine$-H_2O+H]^+$	158.2 [desosamine$+H]^+$
Roxithromycin	838.2 $[M+H]^+$	158.2 [desosamine$+H]^+$	679.8 [M−desosamine$+H]^+$	116.2 [cladinose$-OCH_3+H]^+$
Sulfamethazine	279.4 $[M+H]^+$	124.1 [aminodimethylpyridine$+H]^+$	186.1 [M−aminophenyl$]^+$	–
Sulfamethoxazole	254.2 $[M+H]^+$	92.1 [aminophenyl$]^+$	108.1	–
Trimethoprim	292.6 $[M+H]^+$	123.1 [M−trimethoxyphenyl$]^+$	231.2 $[M-2CH_3O+H]^+$	–
Chloramphenicol	323.1 $[M-H]^-$	152.0 [nitrobenzylalcohol carbanion$]^-$	176.0 $[194-H_2O]^-$	194.0 [M−dichloroacetamide-H$]^-$
Chlortetracyline	479.1 $[M+H]^+$	444.1 $[M-H_2O-NH_3+H]^+$	462.1 $[M-NH_3+H]^+$	–
Doxycycline	445.1 $[M+H]^+$	428.1 $[M-NH_3+H]^+$	410.1 $[M-H_2O-NH_3+H]^+$	–
Oxytetracyline	461.2 $[M+H]^+$	426.2 $[M-H_2O-NH_3+H]^+$	200.6	–
Tetracycline	445.2 $[M+H]^+$	410.1 $[M-H_2O-NH_3+H]^+$	154.0	–
Cloxacillin	453.1 $[M+NH_4]^+$	160.1 [cleavage in β-lactam$+H]^+$	277.1 [cleavage in β-lactam$+H]^+$	178.1
Dicloxacillin	487.0 $[M+NH_4]^+$	160.2 [cleavage in β-lactam$+H]^+$	311.0 [cleavage in β-lactam$+H]^+$	212.0
Methicillin	381.1 $[M+H]^+$	165.0 [dimethoxybenzaldehyd$]^+$	222.1 [cleavage in β-lactam$+H]^+$	150.1 [165−methyl$]^+$
Nafcillin	432.2 $[M+NH_4]^+$	171.1 [ethoxynaphthyl$]^+$	199.1 [ethoxynaphthylcarbonyl$]^+$	181.1
Oxacillin	419.0 $[M+NH_4]^+$	144.1 [phenylisoxazolyl$+H]^+$	243.0 [M−methylphenylisoxazolyl$]^+$	172.1
Penicillin G	352.2 $[M+NH_4]^+$	160.1 [cleavage in β-lactam$+H]^+$	176.0 [cleavage in β-lactam$+H]^+$	114.0 $[160-CO_2+H]^+$
Penicillin V	368.1 $[M+NH_4]^+$	114.2 $[160-CO_2+H]^+$	160.2 [cleavage in β-lactam$+H]^+$	98.2

For quantification either total ion current or strongest mass signal was used depending on best signal-to-noise ratio.
[a] See Fig. 1.

lens conditions for the argon collision induced dissociation were optimized (precursor scan: 1 Da steps, 10 ms dwell time; product ion scan: 0.1 Da steps, 2 ms dwell time; multiple reaction monitoring (MRM): dwell time >200 ms depending on number of recorded mass traces). Precursor and product ion masses of the single compounds are given in Table 2. Additionally, most likely fragmentation patterns are shown, where plausible. They are in accordance to mass spectra of other LC–MS determinations [13,15,16]. Selective scan procedures were used when analyzing the environmental samples.

3. Results and discussion

Due to the predicted and previously detected low concentrations of antibiotics in the aquatic environment, a preconcentration step was necessary prior to measurement. Therefore, two alternative extraction methods, a freeze-drying and a SPE method, have been developed and tested with regard to their suitability for the enrichment of antibiotics (see below). The obtained extracts were then separated via HPLC using three different gradients and columns according to the three analyte groups as indicated in Fig. 1 and Table 1. For detection electrospray–tandem MS methods were established allowing a sensitive measurement. In the following evaluation data of the methods are presented.

3.1. Recoveries and quantitation limits

Calibration standards were obtained by adding the respective amount of the standards to 100 ml of a mountain spring water which is free of any anthropogenic organic contaminants. Milli-Q water as a standard solvent proved to be not useful as the macrolid antibiotics show decreased recoveries from water which has little or no salt content. They were carried out over the whole procedure. Recovery rates were determined by comparison of 1 μg/l calibration standards ($n=3$) with an unenriched standard solution. Results are shown in Table 3. Except for erythromycin, trimethoprim and tetracycline, recoveries after lyophilization exceeded 80% with relative standard deviations between 2 and 15%. In comparison to SPE, recoveries were generally higher using the lyophilization procedure. Tetracyclines were not solid-phase extractable with the conditions described above, presumably due to irreversible

Table 3
Recoveries for the lyophilization and the SPE method

Substance	Mean recovery[a] of lyophilization in mountain spring water (%, $n=3$)	Recovery of lyophilization in spiked surface water sample (%)	Recovery of SPE in mountain spring water (%)
Clarithromycin	102	85 (91)[b]	90
Erythromycin	54	106 (120)	120
Roxithromycin	100	79 (100)	75
Sulfamethazine	81	40 (88)	15
Sulfamethoxazole	81	60 (84)	75
Trimethoprim	68	78 (103)	87
Chloramphenicol	82	n.d.[c] (98)	90
Chlortetracyline	87	45	n.d.
Doxycycline	80	68	n.d.
Oxytetracyline	108	49	n.d.
Tetracycline	72	53	n.d.
Cloxacillin	89	123	68
Dicloxacillin	88	137	61
Meticillin	88	118	62
Nafcillin	86	121	58
Oxacillin	90	116	66
Penicillin G	94	106	61
Penicillin V	79	114	107

[a] Relative standard deviations ranged between 2 and 15%.
[b] Values in parentheses are from spiked stream water sample with less NOM content.
[c] n.d.=Not determined.

adsorption on the solid-phase material. Addition of EDTA to the water sample would probably allow a SPE of tetracyclines, and hence will be tested in the future.

Limits of quantitation (LOQs) were determined as the second lowest calibration point of the linear correlation (seven calibration points), typically ranging from 10 ng/l to 5000 ng/l. LOQs with the freeze-drying enrichment step were independent of the type of water samples used and were 50 ng/l for the tetracyclines and 20 ng/l (equalling 100 pg per injection) for all others. Except for the tetracyclines quantitation limits for SPE are at least one order of magnitude lower because of the larger sample volume. LOQs could be decreased further by using a smaller final extract volume which was not possible for the freeze-dried extract due to the high EDTA content which has to be solubilized.

Calibration graphs usually exhibit excellent linearity in a range from the LOQ up to 5000 ng/l with correlation coefficients better than 0.99 even without internal standard.

In comparison, both methods can generally be used for the extraction of the analytes with the exception, that the described SPE method is not capable of extracting the tetracyclines. The lyophilization method on the other hand allows the enrichment of all investigated analytes in one single step with slightly better recovery rates. In addition, the method requires less time effort which is advantageous when many samples have to be analyzed. On the other hand, the SPE method enables a more sensitive determination of the antibiotics than the lyophilization due to the larger sample volumes. Therefore, in order to achieve low detection limits for the examination of less contaminated ground and drinking water the more labour-intensive SPE method is recommendable.

For further experiments the lyophilization method was preferred as all analytes can be simultaneously determined. Additionally, the less time consuming sample pretreatment was more appropriate for the routine analysis of a large number of environmental samples.

3.2. Native samples

For the additional evaluation of the freeze-drying method, different surface water samples with high NOM values were spiked with 1 μg/l of the analytes. To simulate first a worst case situation, a

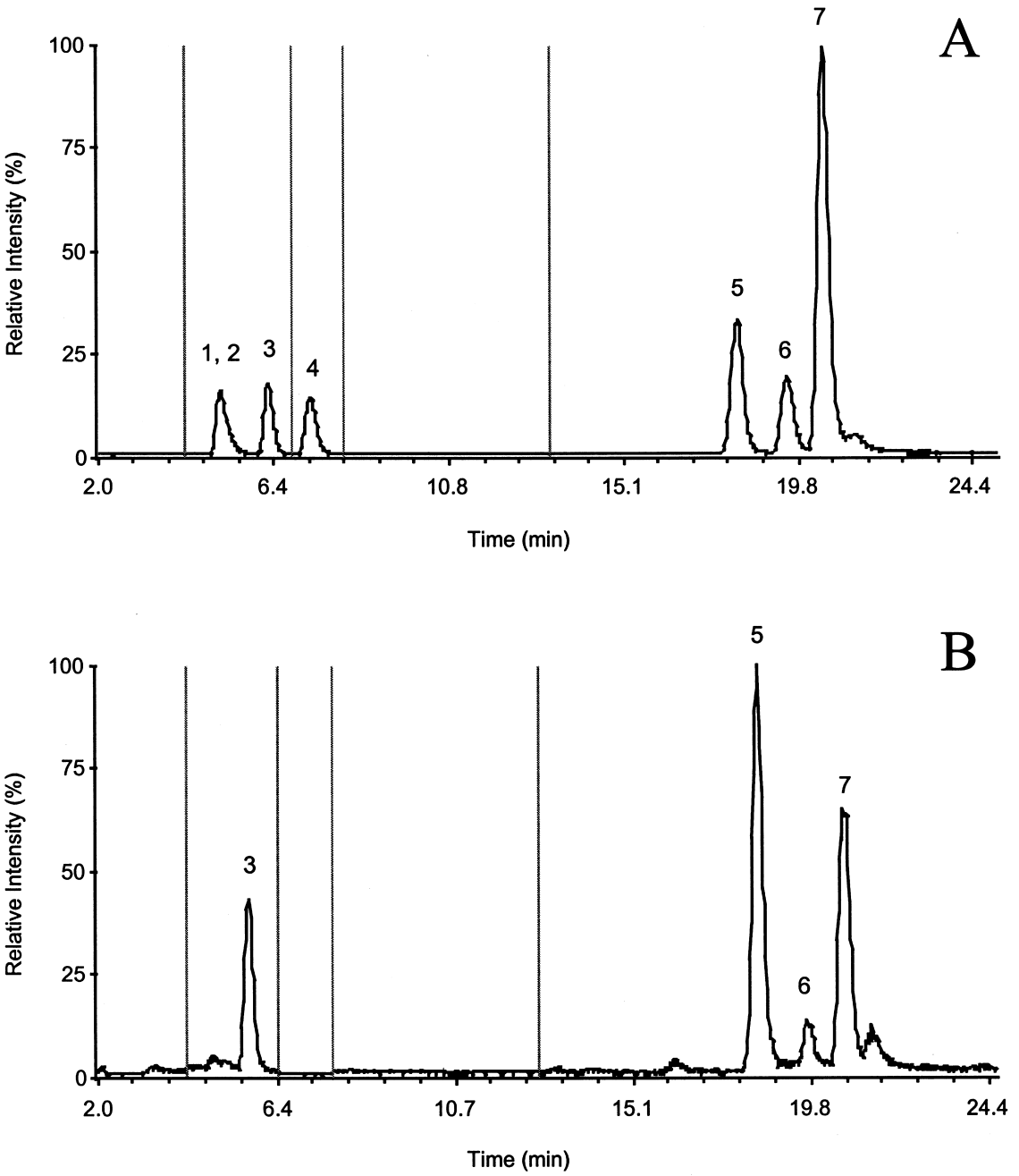


Fig. 2. Total ion chromatogram of a 2 μg/l standard mixture (A) and of an extract of a small river (B) by method ANT. (1=Sulfamethazine, 2=trimethoprim, 3=sulfamethoxazole, 4=chloramphenicol, 5=erythromycin, 6=clarithromycin, 7=roxithromycin).

surface water consisting of about 80% of municipal and industrial STP outflows was used. The extracts were measured and compared with a calibration series (see Table 3). Examples for calibration chromatograms for the three analytical groups are shown in Fig. 2A, Fig. 3A and Fig. 4. The chromatogram of

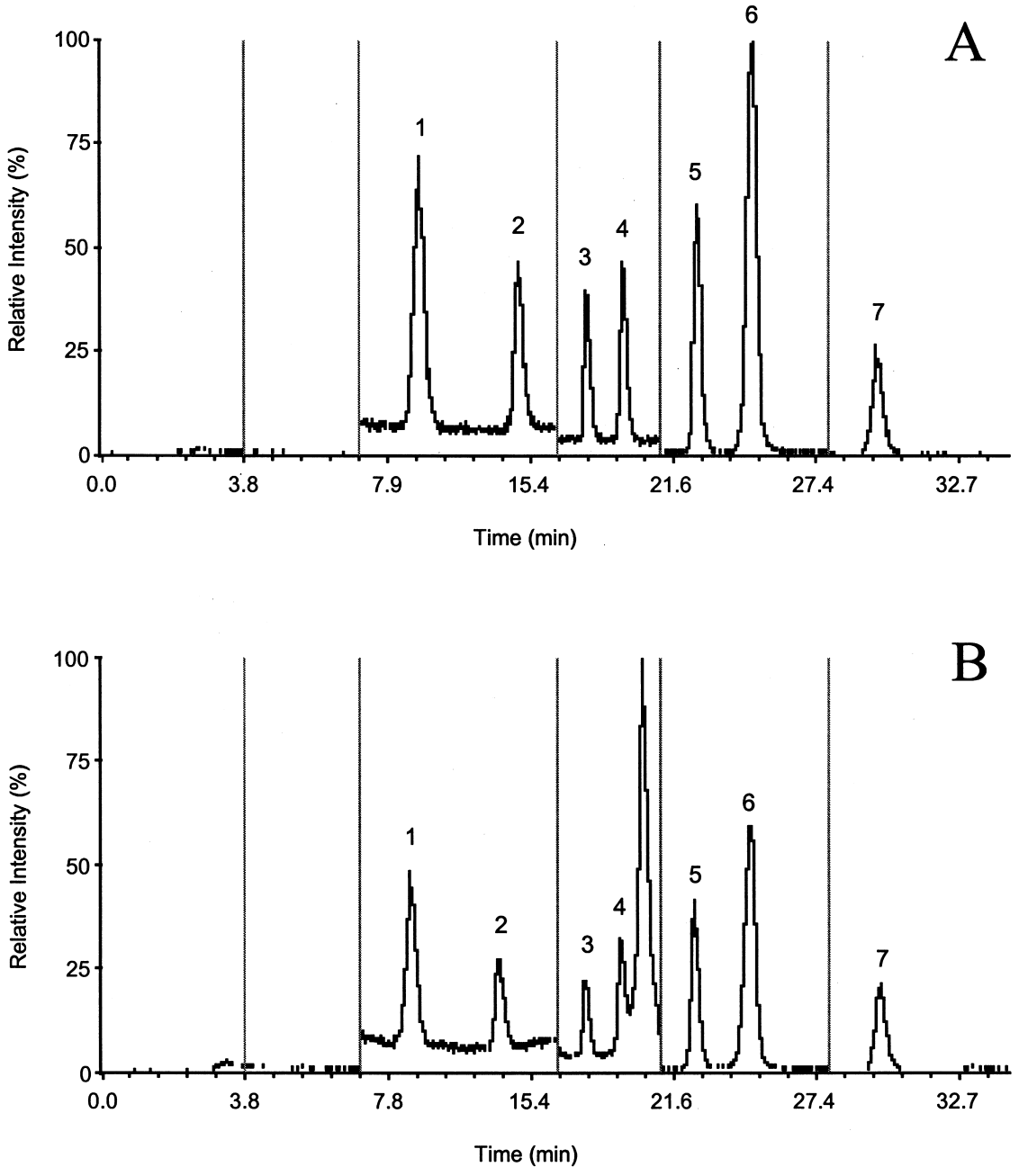


Fig. 3. Total ion chromatogram of a 1 μg/l calibration standard (A) and of an extract of a spiked (1 μg/l) surface water (B) by method PEN. (1=Meticillin, 2=benzylpenicillin, 3=phenoxymethylpenicillin, 4=oxacillin, 5=cloxacillin, 6=nafcillin, 7=dicloxacillin). The unspiked sample did not exhibit any residues of penicillins.

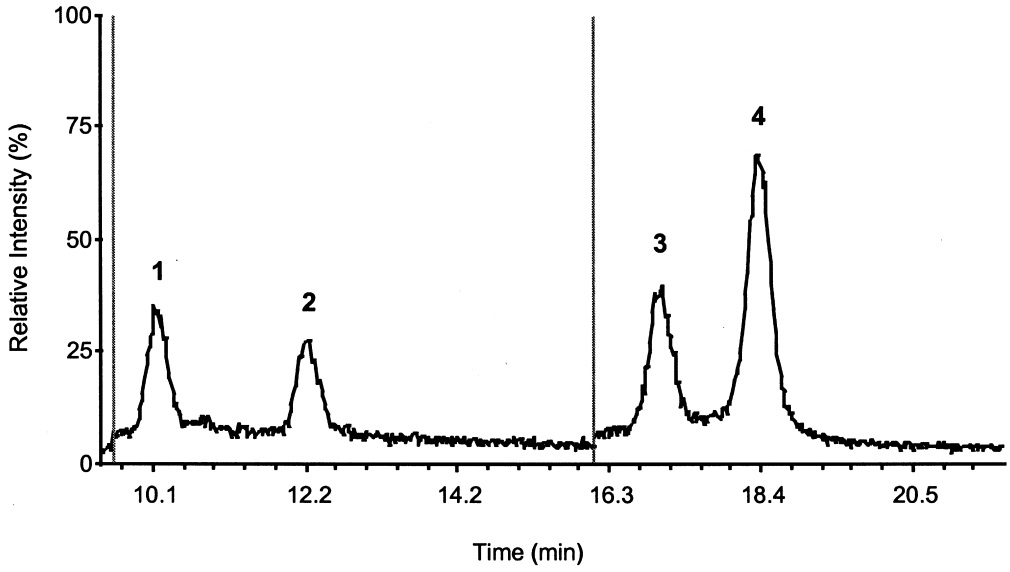


Fig. 4. Total ion chromatogram of a 1 μg/l calibration standard of group TET. (1=Oxytetracycline, 2=tetracycline, 3=chlortetracycline, 4=doxicycline).

a spiked sample for the penicillins is shown in Fig. 3B. In comparison to the calibration standard (Fig. 3A) the chromatograms are very similar indicating that matrix effects are effectively supressed by the MS–MS technique. However, the recoveries exhibited slightly elevated values, demonstrating that in highly contaminated samples residual matrix effects can falsely indicate higher concentrations. The tetracyclines showed recoveries ranging between 45 and 68% of the expected values. Unspiked samples did not show any contamination with neither penicillins nor tetracyclines. The antibiotics of the ANT group exhibited recoveries ranging from 40 to 106%. Considering the fact that generally a freeze drying enrichment preserves all non-volatile substances, decreased recoveries such as for the tetracyclines in a highly contaminated surface water are most likely caused by interactions with solubilized matrix components, which in the following deactivate them for detection.

Using the method for a less contaminated sample should generally result in better recoveries due to smaller amounts of NOM. This was exemplarily shown for the ANT group spiking the analytes to a stream water with a fresh water content of about 75%. The values are also given in Table 3 (in parentheses). As expected, the recoveries increased remarkably, ranging now from 84 to 120%.

The applicability of the described method was demonstrated exemplarily on a sample of the small river Lutter in Bielefeld, Germany. It revealed the presence of five antibiotics. Erythromycin was quantified with 0.62 μg/l whereas the other two macrolid antibiotics roxithromycin and clarithromycin were each detected in a concentration of 0.19 μg/l. In addition, sulfamethoxazole and trimethoprim were determined with concentrations of 0.48 and 0.12 μg/l respectively (see Fig. 2B). All other 13 compounds were not detectable. As the water sample was taken in the vicinity of a municipal sewage treatment plant the origin of the detected antibiotics amounts is probably human medication.

4. Conclusions

LC–electrospray tandem MS is a powerful analytical technique for the sensitive determination of polar micro contaminants in water like antibiotics. The method presented herein offers a simple, effective and sensitive possibility to quantify 18 antibiotics down to the lower ng/l range in different

water matrices. Lyophilization as a universal preconcentration step allows an easy extension of the method to the analysis of various other relevant compounds.

Five of the examined drugs present in a small river stemmed most likely from human medication, as a large amount of its water consisted of municipal sewage treatment plant effluents.

Antibiotics that are used for veterinary purposes and excreted after incomplete metabolization will be present in manure [17,18]. Manure however is often used as top soil dressing and can contaminate river water via run-off or drainage. Through leaching it is possible for ground water to be exposed to pharmaceutical residues. An extended investigation of sewage plant effluents, surface waters, drainages and ground waters from agricultural areas in Germany is presently under way. This will show whether, and if so, to what extent human and veterinary use of those drugs can contaminate the aquatic ecosystem.

Acknowledgements

This work was supported by grants of the German Federal Ministry for Education and Research (BMBF) and the Hessian Ministry for Environment, Energy, Youth, Family and Health.

References

[1] H.-J. Stan, M. Linkerhägner, Vom Wasser 83 (1992) 57–88.
[2] M. Stumpf, T. Ternes, K. Haberer, P. Seel, W. Baumann, Vom Wasser 86 (1996) 291–303.
[3] R. Hirsch, T. Ternes, K. Haberer, K.-L. Kratz, Vom Wasser 87 (1996) 263–274.
[4] Anonymous, Produktion nach Güterarten und Güterklassen 1994, Registration No. 4717, Statistisches Bundesamt Wiesbaden, Germany.
[5] W. Forth, D. Henschler, W. Rummel, K. Starke, Allgemeine und Spezielle Pharmakologie und Toxikologie, Wissenschaftsverlag Mannheim, Leipzig, Vienna, Zurich, 6th ed., 1992.
[6] T.A. Ternes, Water Res. (1998) in press.
[7] M.L. Richardson, J.M. Bowron, J. Pharm. Pharmacol. 37 (1985) 1–12.
[8] C.D. Watts, M. Crathorne, M. Fielding, C.P. Steel, in: G. Angeletti et al. (Eds.), Analysis of Organic Micropollutants in Water, Reidel Publ. Corp., Dordrecht, 1983, pp. 120–131.
[9] P.J. Kijak, J. AOAC Int. 77 (1994) 34–40.
[10] A. Cannavan, S. Armstrong Hewitt, W.J Blanchflower, D.G. Kennedy, Analyst 121 (1996) 1457–1461.
[11] M. Hedenmo, B.-M. Eriksson, J. Chromatogr. A 692 (1995) 161–166.
[12] Anon Bundesgesundheitsblatt 10 (1995) 409–413.
[13] R. Straub, M. Linder, D. Voyksner, Anal. Chem. 66 (1994) 3651–3658.
[14] T.B. Vree, A.J.A.M. Van der Ven, C.P.W.G.M. Verwey-van Wissen, E.W.J. van Eywijk-Beneken Kolmer, A.E.M. Swolws, P.M. van Galen, H. Amatdjais-Groenen, J. Chromatogr. B 658 (1994) 327–340.
[15] J.D. Henion, B.A. Thomson, P.H. Dawson, Anal. Chem. 54 (1982) 451–456.
[16] D. Hurtaud, B. Delepine, P. Sanders, Analyst 119 (1994) 2731–2736.
[17] K. Winterhalder, Untersuchung über den Einfluss von Desinfektionsmitteln, Futterzusatzstoffen und Antibiotika auf die Biogasgewinnung aus Schweinegülle, Dissertation, University of Hohenheim, Germany, 1985.
[18] J.-P. Langhammer, H. Büning-Pfaue, Untersuchungen zum Verbleib centimikrobiell wirksamer Arzneistoffe als Rückstände in Gülle und im landwirtschaftlichen Umfeld, Dissertation, University of Bonn, Germany, 1989.