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Review

Liquid chromatography–tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review

Mira Petrović*, Maria Dolores Hernando, M. Silvia Díaz-Cruz, Damià Barceló

Department of Environmental Chemistry, IIQAB-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain

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Abstract

Pharmaceutical residues are environmental contaminants of recent concern and the requirements for analytical methods are mainly dictated by low concentrations found in aqueous and solid environmental samples. In the current article, a review of the liquid chromatography–tandem mass spectrometry (LC–MS/MS) based methods published so far for the determination of pharmaceuticals in the environment is presented. Pharmaceuticals included in this review are antibiotics, non-steroidal anti-inflammatory drugs, β-blockers, lipid regulating agents and psychiatric drugs. Advanced aspects of current LC–MS/MS methodology, including sample preparation and matrix effects, are discussed.

Keywords: Liquid chromatography–tandem mass spectrometry; Non-steroidal anti-inflammatory drugs; β-Blockers; Antibiotica: lipid regulating agents

Contents

1. Introduction

Pharmaceutically active substances are a class of new, so-called “emerging” contaminants that have raised great concern in the last years [1]. Human and veterinary drugs are continuously being released in the environment mainly as a result of the manufacturing processes, the disposal of unused or expired products, and the excreta. The amount of pharmaceuticals and their bioactive metabolites being introduced into the environment is likely low. However, their continuous environmental input may lead to a high long-term concentration and promote continual, but unnoticed adverse effects on

* Corresponding author. Tel.: +34 93 400 6172; fax: +34 93 204 5904.
E-mail address: mpeqam@cid.csic.es (M. Petrović).

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Table 1
Occurrence of pharmaceutical residues in the STP effluents

Compounds	Concentrations (μg/l) median (maximum)			
Antiphlogistics/anti-inflammatory drugs				
Ibuprofen	0.05 (7.11)[a]	0.37 (3.4)[b]	3.09 (27.3)[c]	4.0 (24.6)[d]
Naproxen	1.12 (5.22)	0.30 (0.52)	–	12.5 (33.9)
Ketoprofen	n.d (1.62)	0.2 (0.38)	–	n.d.
Diclofenac	0.68 (5.45)	0.81 (2.1)	0.42 (2.35)	n.d.
β-Blockers				
Propanolol	0.01 (0.09)	0.17 (0.29)	0.08 (0.28)	–
Metoprolol	0.08 (0.39)	0.73 (2.2)	–	–
Acebutolol	0.06 (0.13)	–	–	–
Oxprenolol	0.02 (0.05)	–	–	–
Lipid regulators				
Gemofibrozil	0.84 (4.76)	0.40 (1.5)	–	1.3 (1.3)
Fenofibrate	0.14 (0.16)	n.d. (0.03)	–	–
Bezafibrate	n.d. (1.07)	2.2 (4.6)	–	–
Clofibric acid	n.d. (0.68)	0.36 (1.6)	–	n.d.
Antiepileptic				
Carbamazepine	0.87 (1.20)	2.1 (6.3)	–	0.7 (2.3)
Antibiotics				
Trimetroprim	0.04 (0.13)	–	0.07 (1.29)	–
Sulfamethoxazole	0.05 (0.09)	–	<0.05 (0.13)	0.24 (0.87)
Erythromycin	–	–	<0.01 (1.84)	0.08 (0.84)
Reference	[22]	[23]	[24]	[25,26]

[a] Seven STP in France, Greece, Italy and Sweden.
[b] Forty-nine STP in Germany.
[c] Five STP in the UK.
[d] Fourteen STP in Canada (eight STP for antibiotics).

aquatic and terrestrial organisms. Numerous papers reported the levels of pharmaceuticals in wastewaters and aqueous and solid environmental matrices. Table 1 gives an overview of concentrations of several main classes of pharmaceuticals in sewage treatment plants (STP) effluents reported in several comprehensive papers and reports.

Many believe that of all the emerging contaminants, antibiotics are the biggest concern because of the potential for antibiotic resistance [2]. The increasing use of these drugs in livestock, poultry production, and fish farming during the last five decades has caused a genetic selection of more harmful bacteria, which is a matter of great concern. However, other pharmaceutical compounds, especially polar one, such as acidic anti-inflammatory drugs and lipid regulators also deserve particular attention. Elimination of acidic pharmaceuticals in STPs was found to be rather low [3] and consequently sewage effluents are one of the main sources of these compounds and their recalcitrant metabolites. Due to their physico-chemical properties (high water solubility and often poor degradability) they are able to penetrate through all natural filtration steps and enter groundwater as well as drinking water [4–7].

Because of the recent awareness of the potentially dangerous consequences of the presence of pharmaceuticals in the environment, the analytical methodology for their determination in complex environmental matrices is still evolving and the number of methods described in the literature has grown considerably. So far, most of the analytical methods reported in the literature for pharmaceutical residue analysis were based on gas chromatography–mass spectrometry (GC–MS) [8], which often requires derivatization of acidic compounds. However, in the last decades, liquid chromatography–mass spectrometry (LC–MS) and LC–tandem MS have experienced an impressive progress, both in terms of technology development and application. LC–MS/MS is indicated as the technique of choice to assay polar pharmaceuticals and their metabolites, and is especially suitable for environmental analysis because of its selectivity.

This paper reviews the state-of-the-art in the LC–tandem MS analysis of main classes of pharmaceutically active substances (listed in Table 2), including antibiotics, anti-inflammatory/analgesics, lipid regulating agents, β-blockers, psychiatric drugs and other human pharmaceuticals in aqueous and solid environmental samples. Various aspects of current LC–MS/MS methodology, including sample preparation and matrix effects, are discussed.

2. Sample preparation

A survey of LC–MS/MS methods developed for the determination of regularly used pharmaceuticals in environmental aqueous and solid matrices is given in Table 3.

Table 2
Pharmaceuticals analyzed in environmental samples by LC tandem MS

Pharmaceutical class	Compound
Analgesic/anti-inflammatory/ antiphlogistic	Diclofenac [27–33]
	Ibuprofen [27–34]
	Ibuprofen metabolite (2-hydroxy-ibuprofen) [28]
	Acetominophen [27]
	Fenoprofen [28,30,31]
	Hydrocodone [27]
	Ketoprofen [28,30–32]
	Naproxen [27,28,30–32]
	Indomethacin [28,30,32]
	Phenazone [35,36]
	Phenylbutazone [37]
	Propylphenazone [37]
	Paracetamol [33]
Lipid regulators	Bezafibrate [28,30,32–34]
	Clofibric acid [28,30,32–34]
	Gemfibrozil [28,30]
	Fenofibrate [33]
	Atorvastatin [38,39]
	Simvastatin [,39,40]
	Lovastatin [39]
	Pravastatin [39]
	Mevastatin [39]
β-Blockers	Atenolol [34,35,40,41]
	Bisoprolol [33,35,40]
	Metoprolol [33,35,40]
	Propanolol [29,35,40,41]
	Sotalol [35,40]
	Pindolol [35,40]
	Betaxolol [35,40]
	Nadolol [35]
	Timolol [35]
	Carazolol [35]
Antibiotics	Tetracyclines
	Tetracycline [26,42–45]
	Oxytetracycline [26,42–46]
	Doxycycline [26,42,43]
	Chlortetracycline [26,42–47]
	β-Lactams (penicillins)
	Cloxacillin [42,47]
	Dicloxacillin [42,47]
	Methicillin [42]
	Nafcillin [42,47]
	Oxacillin [42,47]
	Amoxicillin [42,47]
	Ampicillin [42]
	Penicillin G and V [42,47]
	Macrolides
	Clarithromycin [26,42,43,47]
	Erythromycin [26,42,43,46–49]
	Roxithromycin [26,38,42,43,47–49]
	Oleandomycin [49]
	Ivermectin [49]
	Tylosin [44,46–49]
	Sulphonamides
	Sulfadiazine [26,47]
	Sulfamethoxazole [26,42,43,47]
	Sulfamethazine [26,42,43]
	Sulfamerazine [26,47]
	Sulfadimidine [26,47]
	Fluoroquinolones
	Ciprofloxacin [26]
	Enrofloxacin [26]
	Norfloxacin [26]
	Ofloxacin [26]
	Ionophores
	Salinomycin [49]
	Monensin [49]
	Miscellanius
	Novobiocin [38]
	Chloramphenicol [42,43,47]
	Trimethoprim [42,43,47]
	Tiamulin [49]
Psychiatric drugs (anti-seizure, anti-convulsant, anti-depresant, anti-axiety)	Carbamazepine [27,33,35,36,50]
	Diazepam [27,34,37]
	Dilatin [27]
	Fluoxetine [27]
	Meprobamate [27]
Other human pharmaceuticals	Trimethoprim (chemotherapeutic agent) [28]
	Phentoxifyline (vasodilator) [27,35,36]
	Ranitidine, omeprazole (ulcer healing) [34,37]
	Furosemide, hydrochlorothiazide (diuretics) [34]
	Glibenzlamide (antidiabetic) [37]

Several papers reported on the evaluation of a number of stationary phases for solid phase extraction (SPE) of the selected pharmaceuticals [9,29], however reaching, in some cases, opposite conclusions with respect to the best sorbent material for the extraction of the same group of pharmaceutical compounds. For example, for acidic non-steroidal anti-inflammatory drugs some authors indicated that C18 silica sorbents yield superior results than the polymeric sorbents [9], while other reported higher recoveries by the polymeric Oasis HLB cartridges [31,32]. For the polar to medium-polar pharmaceuticals several authors used the Oasis MCX mixed-mode sorbent, which has both cation-exchange and reversed-phase characteristics.

Most of the non-steroidal anti-inflammatory drugs are acidic in nature with pK_a values between 4 and 4.5, and at neutral pH exist largely in their ionised form, in which they are poorly retained by a lipophilic sorbents. Therefore, to ensure more complete retention of this group of compounds

Table 3
Survey of LC–tandem MS methods used for the quantitative determination of pharmaceutical compounds in environmental samples

Compounds	Matrix	Sample pretreatment	Extraction method	Elution solvent	LC separation		MS system	Limit of detection (LOD) (ng/l)	Reference
					Column	Mobile phase			
Multiresidue method for neutral and acidic pharmaceuticals, EDC and PCP	Surface and WW	Acidified pH 2	SPE	MeOH/MTBE	C12	Aq. formic acid/MeOH	Triple quadrupole ESI/APCI	1.0	[27]
Multiresidue method for acidic pharmaceuticals: antibiotics, lipid-regulators, antibiotics, antiphlogistics	River sediment	Not reported	Sonication followed by SPE clean up	Acetone	C18	Aq. HAc/aq. NH_4Ac/ACN	Triple quadrupole ESI/APCI	0.4–8 ng/g (LOQ)	[28]
Multiresidue method for neutral and acidic pharmaceuticals	Surface and WW	Acidified pH 3	SPE	MeOH	C18	Aq. NH_4Ac/MeOH/	Triple quadrupole ESI	10–50	[29]
Multiresidue method: analgesic/anti-inflammatory/antiphlogistic, lipid-regulators	WW	Acidified pH 2	SPE	MeOH	C18	MeOH/ACN/aq. NH_4Ac	Triple quadrupole ESI	5–20 (effluent)	[30]
Anti-inflammatory drugs	Surface, drinking and WW	No pretreatment (surface and WW); drinking water addition $Na_2S_2O_3$	SPE	MeOH/TBACl	C18	MeOH/aq. ammonium formiate	Triple quadrupole ESI	Not reported	[31]
Acidic drugs (analgesic, anti-inflammatory, lipid-regulators)	Surface and WW	Acidified pH 2–2.5	SPE	MeOH	Phenyl-hexyl	MeOH/aq. TrBA/aq.HAc (ion par)	Triple quadrupole ESI	0.15–2.5 (LOQ surface water) 0.3–6.5 (LOQ treated wastewater)	[32]
Multiresidue method: analgesic/anti-inflammatory, beta-blockers, lipid-regulators, antibiotics, anti-epilectic	Surface, drinking and ground water	Acidified pH 3	SPE	MeOH-ammonia	C18	Aq. NH_4Ac/MeOH	Triple quadrupole ESI Q-TOF ESI	5–25 (LOQ)	[33]
Multiresidue methods: antibiotics, beta-blockers, beta-sympatho-mimetics	River, drinking water and WW		SPE	MeOH	C18	Aq. NH_4Ac/ACN	Triple quadrupole	5–50	[35]
Neutral drugs (phenazone, pentoxifyline, carbamazepine)	WW	pH adjustment (pH 7.5)	SPE	MeOH	C18	Aq. NH_4Ac/ACN	Triple quadrupole ESI	0.5–1 μg/l (influent) 0.1–0.25 μg/l (effluent)	[36]
Neutral drugs (propylphenazone, phenylbutazone, diazepam, gilbenclamide, omeprazole)	River, ground and WW	pH adjustment (pH 7–7.5)	SPE	MeOH	C18	Aq. NH_4Ac/ACN	Triple quadrupole ESI	LOQ 100–250 influent 25–50 effluent 10–25 river	[37]
Lipid-regulators	Surface and WW	Acidified pH 4.5	SPE	MeOH	C18	Aq. methylamine/aq. HAc/ACN	Triple quadrupole ESI	0.1–15.4	[39]
Multiresidue method: analgesic, beta-blockers, broncholytics, secretolytics, antineoplastic, lipid-regulators	Ground water	pH adjustment (pH 7)	SPE	MeOH	C18	Aq. NH_4Ac/ACN/MeOH	Triple quadrupole ESI	7.9–44	[40]
Beta-blockers	River, drinking water and WW	Acidified pH 3.5	SPE	MeOH/aq. ammonia	C8	Aq. NH_4Ac/ACN	Triple quadrupole ESI	0.12–0.15	[41]
Atorvastatin, roxythromycin, novobiocin	River water, WW	Acidified pH 4	SPE	MeOH	C18	Aq. NH_4Ac/ACN	Triple quadrupole ESI	1 (pg) absolute 3 (pg) 2 (pg)	[38]

Multi class antibiotics: macrolides, fluoroquinolones, sulfonamides, tetracyclines	WW	Addition of Na_2EDTA (for tetracyclines)	SPE	MeOH	C18	ACN/aq. NH_4Ac/aq. formic acid	Triple quadrulple ESI	1–8	[26]
Sulphonamides, macrolides, penicillins	Tap and surface waters	Acidified pH 5 Addition of Na_2EDTA	SPE	ACN/water/TrEA	C18	Sulphonamides and microlides: aq. NH_4Ac/ACN/MeOH	Triple quadrupole ESI	3.7–21	[40]
Tetracyclines, tylosin	Ground waters	Acidified pH 4.7	SPE	Acidified MeOH	C18	Penicillins: aq. amonium formiate/MeOH aq. formic acid/ammonium formiate/ACN	Ion trap MS ESI	100	[44]
	Soil	Not reported	Vortexed with aq. citric acid/ethyl acetate					2 μg/kg	
Tetracyclines, penicillins, sulphonamides, macrolides	Surface, WW and ground waters	Acidified pH 3 addition of Na_2EDTA	SPE lyophilization	MeOH	C8	Tetracyclines: aq. oxalic acid/ACN Sulphonamides, macrolides and penicillins: aq. NH_4Ac/ACN	Triple quadrupole ESI	20–50	[42,43]
Tetracyclines	Ground waters	Acidified pH 2.5 Na_2EDTA	SPE	MeOH/TFA	C18	Aq. formic acid/ACN/MeOH	Ion trap MS ESI	200–380	[45]
Macrolides, ionophores, tiamulin	Soil	Not reported	PLE followed by SPE clean-up	ACN/aq. NH_4Ac	C18	Aq. NH_4Ac/ACN	Triple quadrupole APCI	0.2–1.6 μg/kg	[49]
Oxytetracycline, chlortetracycline, sulfadiazine, erythromycin, tylosin	Soil	Air dried to 5% water and sieved (2 mm)	PLE followed by SPE clean-up	MeOH	C18	Aq. formic acid/MeOH	Triple quadrupole ESI	1–5 μg/kg	[46]
Erythromycin, roxythromycin, tylosin	Natural and WW	Not reported	SPE addition of 5% Na_2EDTA acidified pH 5	MeOH	C18	Aq. formic acid/ACN	Ion trap ESI	30–70	[48]
Tetracyclines	Soil	Not reported	LLE	Ethyl acetate	C18	Aq. formic acid/NH_4Ac/ACN	Ion trap ESI	20 mg/kg (LOQ) 4 mg/kg (LOD)	[51]
Oxytetracyclines	Soil interstitial water	Centrifugation filtration	Not reported	Not reported	C18	Aq. formic acid/MeOH	Triple quadrupole ESI	0.1–0.25 (mg/l)	[52]
Sulphonamides	WW effluent surface waters	Acidified pH 2.5	SPE	MeOH/water	C18	Aq. formic acid/ACN	Triple quadrupole ESI	200–370 (LOD) 600–10200 (LOQ)	[53]

ACN: acetonitrile; EDC: endocrine disrupting compounds; HAc: acetic acid; LLE: liquid–liquid extraction; LOQ: limit of quantification; PLE: pressurized liquid extraction; PCP: personal care products; TrBA: tri-*n*-butylamine; TrEA: triethylamine; TBACl: tetrabutylammonium chloride; WW: wastewater.

the sample pH was adjusted to pH 2–3 in order to achieve reproducible and high recoveries. The exceptions were protocols employing the Oasis HLB cartridges that due to its chemical composition (the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone) allow working at neutral pH range.

For SPE of tetracyclines cartridge materials must not contain silanol groups, since they have been found to bind irreversibly to tetracyclines. A precaution leading to an significant improvement of extraction efficiencies is the silanisation, mostly with dimethyldichlorosilane, of all glassware getting in contact with either the water sample or the extract, or the use of other container materials, such as PTFE [10]. An additional approach to prevent chelation of metals by those kinds of compounds is adding a strong chelator to the sample, for instance Na_2EDTA, which presents optimum solubility in water and, unlike oxalic acid, does not accumulate in the capillary interface when mass spectrometric detection follows [42,43,45,48].

The presence of pharmaceutical products in soil, sediment and sludge has scarcely being investigated as compared to aquatic media [11]. Extraction of pharmaceuticals from solid matrices has normally been performed by sonication or by simple blending or stirring of the sample with polar organic solvents or mixtures of them, or with aqueous solutions [44,51]. The use of more advanced extraction techniques, such as pressurized liquid extraction (PLE), has been reported in only a limited number of occasions [46,49]. PLE presents several advantages over other extractions methods such as better reproducibility, less solvent consume and reduced time for sample pre-treatment. As in the case of water samples, tetracyclines form strong complexes with di- and tri-valent metal ions present in the soil samples. To overcome this problem different complexation agents have been tested including citric acid [44,45] in combination with the often used McIlvine buffer [46]. Following the PLE extraction, a pre-concentration and clean-up step is usually needed.

3. Chromatographic separation

Although complete separation is not necessary for the selective MS/MS detection, it generally improves detectability and reduces ion suppression effect. Both acetonitrile and methanol were tested as organic mobile phases for the LC separation. In order to obtain sufficient retention for acidic drugs and reproducible retention times the use of a buffer in the eluent or acidification of the mobile phase was recommended, although it caused the reduction of the signal intensities due to suppressing effects in the MS interface. For the analysis of acidic analgesics/anti-inflammatory drugs and antiphlogistics volatile compounds such as ammonium acetate, ammonium formiate or formic acid were preferred as mobile phase additives, at concentrations typically ranging from 2 to 20 mM (see Table 3).

In order to obtain satisfactory separation of a wide range of acidic drugs (anti-inflammatory, lipid regulators and some of their metabolites) Quintana and Reemtsma [32] applied ion-pair LC (IP–LC) using volatile ion-pairing agent tri-*n*-butylamine (TrBA). The relatively high concentration of TrBA in the eluent (10 mM) led to a very strong retention of the analytes that allowed more polar metabolites (such as salicylic acid) and adducts from selected drugs to be retained on the column and to be detected by this procedure. Chromatographic separation was carried out maintaining the column temperature at 55 °C in order to counterbalance too strong retention of some analytes. Both the elevated temperature and the high TrBA concentration resulted in a robust chromatography, as retention times turned out to be very stable.

In the analysis of antibiotics modification of the mobile phase is usually performed in attempt to improve the sensitivity of MS detection, and has been accomplished with acetate [38,42–44,49], formiate [44], oxalic acid [42,43] or formic acid [44–46,48,53].

LC separation of blood–lipid regulators and β-blockers in extract obtained from environmental waters has been carried out mainly using C18 column and the mobile phase consisting of water and methanol or acetonitrile as organic mobile phase at different pH. Miao et al. [30] and Sacher et al. [40] used a mixture of acetonitrile and methanol as organic mobile phase to lead to shorter retention times and better resolution of the analytes. The use of ammonium acetate as additive in the mobile phase is common in the reported methods to improve ESI performance in the NI mode. Acids (e.g. acetic acid, formic acid), TrBA and methylammonium acetate have been also used to improve the sensitivity of MS detection [29,32,39]. The use of methylammonium acetate as mobile phase additive was an alternative used by Miao et al. [39] obtaining highest sensitivity for the analysis of “statins” class of blood–lipid regulators.

Generally, for multiresidue methods, pH of mobile phase was adjusted to acidic or neutral conditions. An example of the separation of 13 medium-polar to polar pharmaceuticals is shown in Fig. 1. The optimised LC gradient of methanol and aqueous 2 mM ammonium acetate enables almost complete separation.

When analysing drugs with basic character such as β-blockers or β-sympathomimetics, neutral pH is preferable [35,41].

4. MS/MS detection–triple quadrupole (QqQ)

4.1. Anti-inflammatory/analgesic compounds

Precursor ions and their products ions used for quantification and confirmation purposes are summarized in Table 4. Acidic anti-inflammatory/analgesic drugs, most of them derivatives of phenyl acetic acid, were usually detected under negative ionisation conditions and deprotonated

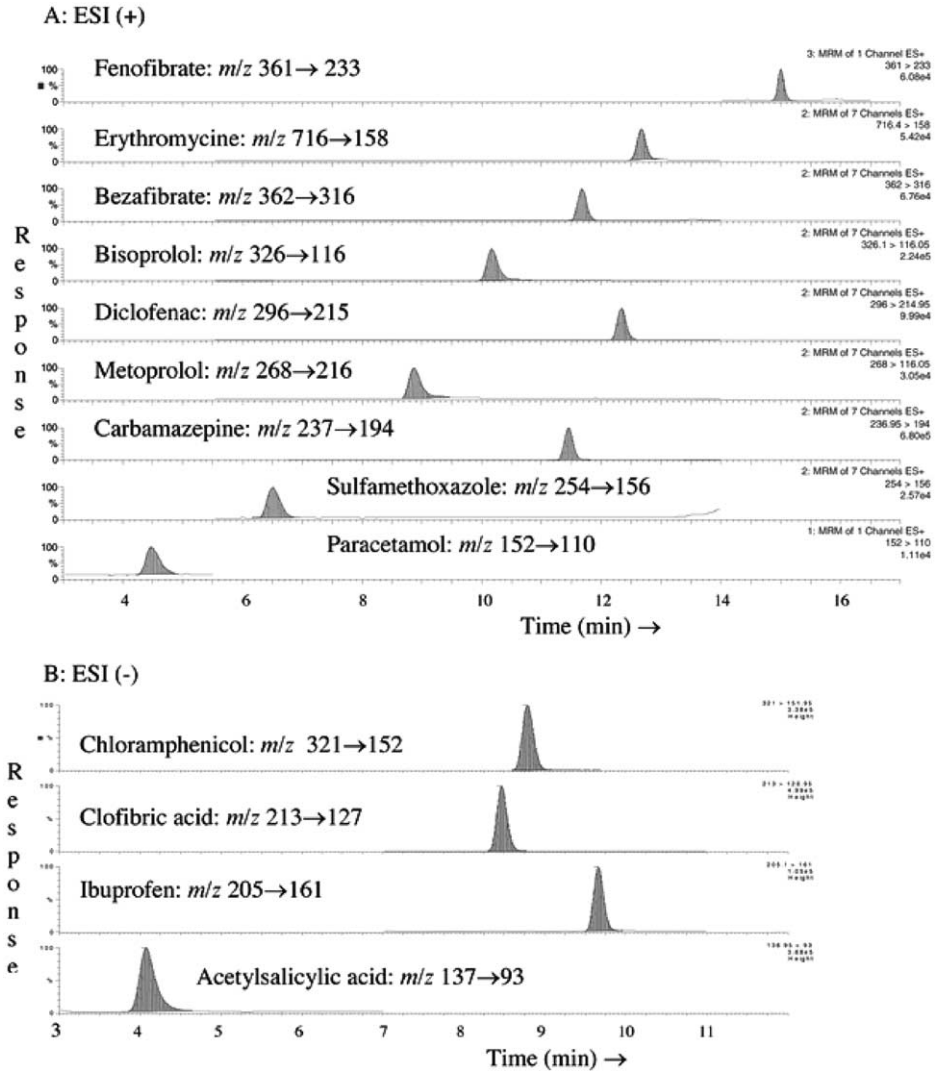

Fig. 1. (A) LC–ESI(+)-QqQ-MS screening chromatogram of blank groundwater sample fortified at 100 ng l^{-1} with the standard solution of 13 pharmaceuticals. (B) The same sample analysed in the ESI(−) mode. Reproduced with permission from [33] copyright © 2004 Springer.

molecules $[M-H]^-$ where chosen as precursor ions. Typically they showed, to a varying degrees, the characteristic tendency to lose CO_2 (*m/z* 44) in Q2 leading to a benzyl anion that is stabilised by conjugation with the aromatic ring and a limited number of other products. For example, for ibuprofen, one of the most frequently analysed human pharmaceuticals, the product ion generated by expulsion of CO_2 was the only product ion formed and hence no second MS/MS transition was available for confirmation purposes. Both, APCI and ESI interfaces were tested and for all acidic pharmaceuticals sensitivity was approximately 10-fold higher in the ESI mode than in the APCI mode [33].

For neutral anti-inflammatory/analgesic compounds (fenoprophen, acetominophen, propylphenazone and phenylbutazone) analyzed in the PI mode, all precursors ions were the result of a protonation $[M+H]^+$ of the intact, uncharged molecule.

4.2. Lipid regulating agents

The LC–MS/MS analysis for the "fibrate" and "statins" classes of blood–lipid regulators, have been carried out in most instances with ESI interface (see Table 4). With this technique, the sensitivity is approximately 10-fold higher than in the APCI mode [33]. For both "fibrate" and "statins" classes, which are acidic, NI mode of ionisation is expected to be more appropriate.

In the analysis of "statins" class, Miao et al. [39] investigated the NI and PI mode for lovastatin and simvastatin as lactone forms, and atorvastatin and pravastatin as acidic forms. They observed that PI mode was more sensitive than NI mode for both atorvastatin and lactone compounds. Although, due to the high signal intensity of $[M-H]^-$ for pravastatin, it is normally analyzed in NI mode, Miao et al. [39] proved if the use of methylammonium acetate, as a mobile phase additive, could much improve the sensitivity. They proved

Table 4
Base peaks (*m*/*z*) of precursor and product ions used for LC–MS/MS analysis of pharmaceuticals in environmental samples

Compound	Precursor ion (*m*/*z*)	Product 1 (*m*/*z*)	Product 2 (*m*/*z*)	Reference
Anti-inflammatory/analgesics/antiphlogistic				
Ibuprofen	205 $[M-H]^-$	159 $[M-H-CO_2]^-$	–	[28,30]
2-Hydroxy ibuprofen	221 $[M-H]^-$	177 $[M-H-CO_2]^-$	133	[28]
Ketoprofen	253 $[M-H]^-$	209 $[M-H-CO_2]^-$	197	[28,30]
Naproxen	229 $[M-H]^-$	185 $[M-H-CO_2]^-$	170 $[M-H-C_2H_3O_2]^-$	[28,30]
Indomethacin	356 $[M-H]^-$	312 $[M-H-CO_2]^-$	297 $[M-H-C_2H_3O_2]^-$	[28,30]
Diclofenac	294 $[M-H]^-$	250 $[M-H-CO_2]^-$	214	[28,30]
	296 $[M+H]^+$	278 $[M+H-H_2O]^+$	–	[29]
Fenoprofen	241 $[M-H]^-$	197 $[M-H-CO_2]^-$	93 $[M-H-C_9H_8O_2]^-$	[28,30]
Acetominophen	160 $[M+H]^+$	110 $[M-CH_2-CO+H]^+$	–	[27]
Hydrocodone	300 $[M+H]^+$	199	–	[27]
Propylphenazone	231 $[M+H]^+$	189 $[M-C_3H_7+H]^+$	201 $[M-2CH_3+H]^+$	[37]
Phenylbutazone	309 $[M+H]^+$	160 $[M-(C_6H_5-N)-(C_4H_9)]^+$	181 $[M-N-CO-NH_2+H]^+$	[37]
Lipid regulating agents				
Fenofibrate	361 $[M+H]^+$	233	139	[33]
Bezafibrate	362 $[M+H]^+$	276	316	[33]
	360 $[M-H]^-$	274 $[M-H-C_4H_6O_2]^-$	154 $[M-H-C_{12}H_{14}O_3]^-$	[32]
Clofibric acid	213 $[M-H]^-$	127 $[C_6H_4ClO]^-$	85 $[C_4H_5O_2]^-$	[28]
	213/215 $[M-H]^-$	127/129 $[C_6H_4ClO]^-$	85 $[C_4H_5O_2]^-$	[30,32,33]
Gemfibrocil	249 $[M-H]^-$	121 $[M-H-C_7H_{12}O_2]^-$	–	[28,30]
Simvastatin	450 $[M+CH_3NH_3]^+$	267	199	[39,40]
Atorvastatin	559 $[M+H]^+$	440	–	[38,39]
Lovastatin	436 $[M+CH_3NH_3]^+$	285 $[436-C_6H_{17}NO_2]^+$	199	[39]
Pravastatin	456 $[M+CH_3NH_3]^+$	269 $[456-C_6H_{17}NO_2]^+$	–	[39]
Mevastatin	422 $[M+CH_3NH_3]^+$	185	–	[39]
β-Blockers				
Bisoprolol	326 $[M+H]^+$	116 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	74	[35,40]
	326 $[M+H]^+$	166 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	56	[39,40]
Metoprolol	268 $[M+H]^+$	166 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	98 [(*N*-isopropyl-*N*-propenamine) + $H]^+$	[39]
Propanolol	260 $[M+H]^+$	116 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	183 $[M-H_2O-C_3H_7NH]^+$	[39]
Atenolol	267 $[M+H]^+$	190 $[M-H_2O-NH_3-$ isopropyl$^+ + 2H]^+$	145 $[190-CO-NH_3]^+$	[39–41]
Sotalol	273 $[M+H]^+$	255 $[M-H_2O+H]^+$	213	[39]
	273	213	133	[40]
Pindolol	250 $[M+H]^+$	56	72	[39,40]
Betaxolol	308 $[M+H]^+$	166 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	98 [(*N*-isopropyl-*N*-propenamine) + $H]^+$	[39]
	308	55	56	[40]
Nadolol	310 $[M+H]^+$	254 [M-tert-butyl$^+ + 2H]^+$	201	[39]
Timolol	317 $[M+H]^+$	261 [M-tert-butyl$^+ + 2H]^+$	244 [M-tert-butylamine + $H]^+$	[39]
Carazolol	299 $[M+H]^+$	116 [(*N*-isopropyl-*N*-2-hydroxypropylamine) + $H]^+$	222	[39]
Antibiotics				
Roxitromycin	838 $[M+H]^+$	158 [Desosamine + $H]^+$	680 [M – desosamine + $H]^+$	[38,43,47,48]
Erythromycin	716 $[M-H_2O+H]^+$	522 [M – desosamine – $2H_2O+H]^+$	558 [M – desosamine – $H_2O+H]^+$	[43,48]
Clarithromycin	750 $[M+H]^+$	116 [cladinose – $OCH_3+H]^+$	592 [M – desosamine + $H]^+$	[43]
Trimethoprim	293 $[M+H]^+$	123 [M – trimetoxyphenyl$]^+$	231 $[M-2CH_3O+H]^+$	[43]
Chloramphenicol	323 $[M-H]^-$	152 [Nitrobenzylalcohol carbanion$]^-$	176 $[194-H_2O]^-$	[43]
Chlortetracycline	479 $[M+H]^+$	444 $[M-H_2O-NH_3+H]^+$	462 $[M-NH_3+H]^+$	[43–45]
Doxycycline	445 $[M+H]^+$	428 $[M-NH_3+H]^+$	410 $[M-H_2O-NH_3+H]^+$	[43]
Oxytetracycline	461 $[M+H]^+$	426 $[M-H_2O-NH_3+H]^+$	443 $[M-H_2O+H]^+$	[43–45,52]
Tetracycline	445 $[M+H]^+$	410 $[M-H_2O-NH_3+H]^+$	427 $[M-H_2O+H]^+$	[43–45]

Table 4 (*Continued*)

Compound	Precursor ion (*m*/*z*)	Product 1 (*m*/*z*)	Product 2 (*m*/*z*)	Reference
Cloxacillin	453 $[M+NH_4]^+$	160 [Cleavage in β-lactam + H]$^+$	277 [Cleavage in β-lactam + H]$^+$	[43]
Dicloxacillin	487 $[M+NH_4]^+$	160 [Cleavage in β-lactam + H]$^+$	311 [Cleavage in β-lactam + H]$^+$	[43,47]
Methicillin	381 $[M+H]^+$	165 [Dimethoxybenzaldehyd]$^+$	222 [Cleavage in β-lactam + H]$^+$	[42,43]
Nafcillin	432 $[M+NH_4]^+$	171 [Ethoxynaphthyl]$^+$	199 [Ethoxynaphthylcarbonyl]$^+$	[43,47]
Oxacillin	419 $[M+NH_4]^+$	144 [Phenylisoxazolyl + H]$^+$	243 [M – methylphenylisoxazolyl]$^+$	[43,47]
Penicillin G	352 $[M+NH_4]^+$	160 [Cleavage in β-lactam + H]$^+$	176 [Cleavage in β-lactam + H]$^+$	[43,47]
Penicillin V	368 $[M+NH_4]^+$	114 $[160-CO_2+H]^+$	160 [Cleavage in β-lactam + H]$^+$	[43,47]
Sulfamethazine	279 $[M+H]^+$	186 $[M-H_2NPh]^+$	124 [Aminodimethylpyridine + H]$^+$	[43,53]
Sulfamethoxazole	254 $[M+H]^+$	156 $[H_2NPhSO_2]$	108 $[H_2NPhO]^+$	[43,47,53]
Sulfadiazine	251 $[M+H]^+$	156 $[H_2NPhSO_2]^+$	108 $[H_2NPhO]^+$	[47,53]
Ciprofloxacin	332 $[M+H]^+$	314 $[M-H_2O+H]^+$	288 $[M-H_2O-CO_2+H]^+$	[15,26]
Ofloxacin	362 $[M+H]^+$	344 $[M-H_2O+H]^+$	–	[26]
Norfloxacin	320 $[M+H]^+$	302 $[M-H_2O+H]^+$	–	[26]
Enrofloxacin	360 $[M+H]^+$	342 $[M-H_2O+H]^+$	–	[26]

the high signal intensity obtained of the methylammonium adduct ions $[M+CH_3NH_3]^+$ for lactone forms and pravastatin which were selected as the precursor ions. On the contrary, for atorvastatin, $[M+H]^+$ was the mayor ion in the full scan spectra and it was chosen as precursor ion for MS/MS experiments.

Generally, MS/MS detection of the "fibrate" class has been performed by selecting as the precursor ion, the ion $[M-H]^-$ with the mentioned exception of some of them such as fenofibrate, which analysis only has been published using ESI-PI and bezafibrate. The major product ions generated under ESI-PI MS/MS conditions applied by Stolker et al. [33] for bezafibrate were at *m*/*z* 276 and 316. In ESI-NI MS/MS conditions, the major products are at *m*/*z* 274 and 154, which correspond to losses of $C_4H_6O_2$ and $C_{12}H_{14}O_3$, respectively. From MS/MS experiments, at least two main fragment ions are obtained and they are used for determination and quantification purposes. However, the fragmentation of the precursor ion of gemfibrocil $[M-H]^-$, gives one ion product and therefore, its determination and quantification in water samples was performed using one transition. The major ion product at *m*/*z* 121 is the loss of $C_7H_{12}O_2$ from its deprotonated molecule.

Reported MS/MS spectra by Miao et al. [39] of methylammonium adduct ions $[M+CH_3NH_3]^+$ for "statins" class (lovastatin, pravastatin and simvastatin) were similar with the common fragment ion at *m*/*z* 199. The setting of SRM transition channel for monitoring these compounds was selected according to the signal intensities and structure-specificities of the product ions. Thus, for lovastatin two product ions were selected at *m*/*z* 199 and 285. This last is generated by losses of CH_3NH_2, the ester sidechain ($C_5H_{10}O_2$) and H_2O from the precursor ion $[M+CH_3NH_3]^+$.

4.3. β-Blockers

For LC–MS/MS analysis of β-blockers in environmental samples, ESI has been the ionisation technique of choice. Given its basic character, the positive ionisation mode is common among published analytical methods for these compounds (see Table 4).

The protonated molecule $[M+H]^+$ is the selected precursor ion in the MS/MS detection of β-blockers. Table 4 shows the two most intense diagnostic ions obtained under optimized MS/MS conditions. The transition $[M+H]^+ \rightarrow$ [(*N*-isopropyl-*N*-2-hydroxypropylamine) + H]$^+$ (*m*/*z* = 116) is the predominant fragmentation for the β-blockers analytes such as bisoprolol, metoprolol, propanolol and betaxolol [1,10,11]. Common transition corresponding to the second most intense MS/MS ion, $[M+H]^+ \rightarrow$ [(*N*-isopropyl-*N*-propenamine) + H]$^+$ (*m*/*z* = 98) is also obtained for metoprolol and betaxolol. Mostly, MS/MS determination of β-blockers in environmental samples has been carried out with tandem quadrupole analyzer.

4.4. Antibiotics

Antibiotics comprise a wide spectrum of substances, being the tetracycline family those studied the most. Although the most basic site in those compounds is the dimethylamino group, the dominant loss processes from the protonated tetracycline ions at low collision energies are the losses of H_2O or of NH_3 (from the tetracyclines containing a tertiary HO-group at C-6) to finally give abundant $[M-H_2O-NH_3+H]^+$ ions, as Table 4 shows. These loss processes appear to be charge site decompositions involving replaceable hydrogens rather than carbon-bound hydrogens [42–46,52].

Sulphonamides, another well-known antibiotic class, are N-substituted derivatives of the substance sulphanilamide. A typical sulphonamide's fragment loss, in positive ion mode, is detected at *m*/*z* 156, which results from the cleavage of the sulphur–nitrogen bond yielding the stable sulphanilamide moiety $[H_2NPhSO_2]^+$. This common fragment ion arising from the biologically active part of the molecules provides the best basis for the MS/MS analysis of the hole class of sulphonamides, although, the optimal collision conditions for formation of the fragment ion

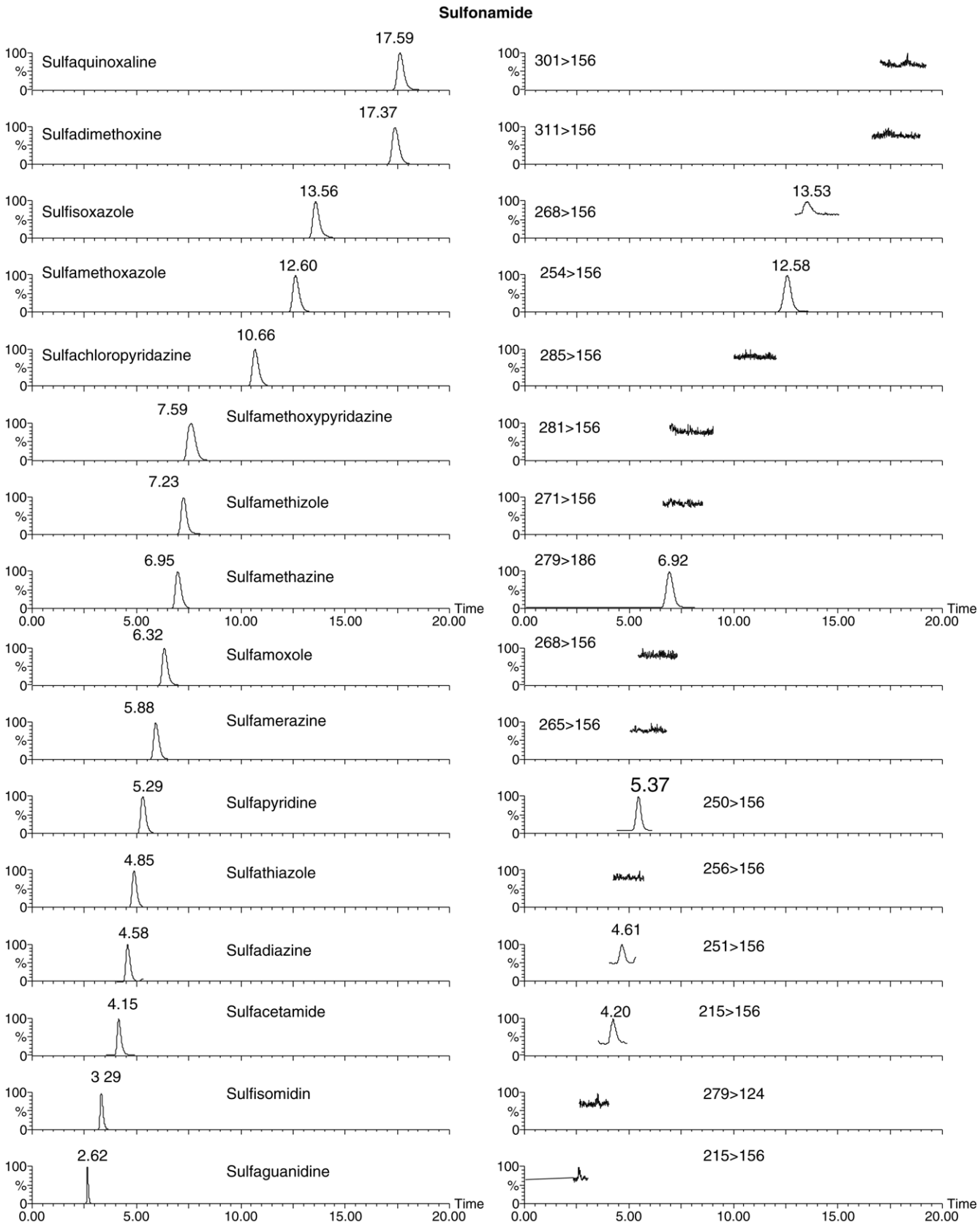

Fig. 2. Time scheduled SRM chromatograms of standards (left panels) a sample (right panels) of sulfonamides. Reproduced with permission from [26] copyright © 2004 American Chemical Society.

m/*z* 156 varied with the sulphonamide. Fig. 2. shows the time-scheduled selected reaction monitoring (SRM) chromatograms of 16 sulphonamides, using in most cases the transition $[M+H]^+ \rightarrow m/z$ 156. However, the fragmentation process yields other group-specific ions in addition to *m*/*z* 156, i.e. at *m*/*z* 108 and 92, as well as a number of compound-specific ions [12,42,43,46].

A similar behaviour can be found for penicillins, β-lactam antibiotics containing bulky side chain attached to the 6-aminopenicillanic acid nuclei. The *m*/*z* 160 ion in positive ion mode, due to opening and cleavage of the β-lactam ring, can be considered as a group-specific fragment ion, since one of the side groups in penicillins in most cases is –OH, as for instance in cloxacillin [42,47].

Macrolide antibiotics, such as erythromycin, roxythromycin and clarithromycin, are basic and lipophilic molecules constituted by a lacton ring with sugars linked via glycosidic bonds. As can be seen in Table 4, macrolides exhibited fragment ions related to the losses of their two characteristic sugars (desosamine and cladinose) and H_2O. These fragment ions can be detected working with either triple quadrupole or ion trap MS/MS detectors [43,48].

A number of LC–MS/MS methods have been reported for detection of various combinations of quinolones, fluoroquinolones and other antibacterial agents in biological matrices [13], but the use of LC–MS/MS in the analysis of environmental samples has been rarely reported. Golet et al. [14,15] used LC–MS/MS for identification purposes in combination with fluorescence detection that proved to be a specific, sensitive and quantitative methods for the determination of trace amounts of fluoroquinolones in environmental and wastewaters. The product ion mass spectra showed two major fragments of the protonated molecules corresponding to the common losses of H_2O and CO_2, both from the carboxylic group. The third transition corresponded to the loss on the piperzine substituent, which in combination with the respective retention time ensured a high specificity for fluoroquinolones and were found to be ideal for identification purposed. An example of a product ion mass spectrum of ciprofloxacin with suggested fragments is shown in Fig. 3.

Recently, Miao et al. [26] reported on the application of LC–MS/MS for the detection of 31 antimicrobials (among them six quinolones) in STP effluents. In all cases, the principal transition monitored was $[M+H]^+ \rightarrow [M+H-H_2O]^+$.

4.5. Psychiatric drugs

Carbamazepine, an important drug for the treatment of epilepsy, schizophrenia and wide range of other seizure disorders, is one of the most frequently detected pharmaceuticals in the aquatic environment. ESI-MS/MS of carbamazepine and its five main metabolites (10,11-dihydro-10,11-epoxycarbamazepine; 10,11-dihydro-10,11-dihydroxycarbamazepine, 2-hydroxycarbamazepine, 3-hydr oxycarbamazepine and 10,11-dihydro-10-hydrocarbamaze pine), was performed in PI mode [50] using ESI. Generally,

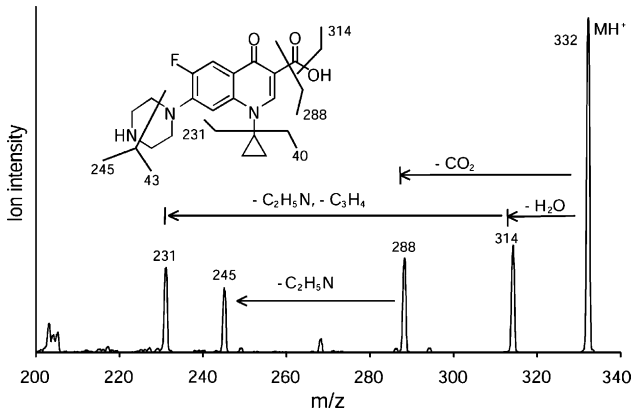

Fig. 3. Mass spectrum of ciprofloxacin obtained by LC–MS/MS. Injection volume, 20 μl, positive ion mode, spray voltage 5.1 kV, orifice skimmer potential difference 36 V and ring electron voltage 180 V. Reproduced with permission from [15] copyright © 2001 American Chemical Society.

the ESI interface was found to be more efficient for this group of drugs compared to the APCI interface resulting in a higher sensitivity. The only major ion product of carbamazepine, 2-OH-carbamazepine and 3-OH-carbamazepine corresponded to loss of the structurally characteristic carbamoyl group (HNCO, 43 Da). For other carbamazepine metabolites, rather complex product ion mass spectra were observed showing different ions corresponding to losses of H_2O, NH_3 or HNCO. Fig. 4 shows the time-scheduled SRM chromatograms of carbamazepine and its metabolites in an effluent sample. The data generated during method validation indicated that a metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine is present at higher concentrations than the parent drug in the environment.

5. MS/MS detection–time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF)

An approach for increasing the selectivity, and avoiding false positive findings is the use of time-of-flight-mass spectrometry (TOF–MS). LC–TOF–MS is rapidly becoming an important analytical tool and recently several applications have been reported for the identification of pharmaceutical compounds and their degradation products in environmental samples. Comparing the power of TOF–MS and QqQ instruments in the analysis of pharmaceutical compounds in wastewaters Benotti et al. [16] concluded that the overall sensitivity of the LC–TOF–MS operated in accurate mass mode often approached that obtained by the triple quadrupole. Main advantage of TOF/MS is the availability of full-scan mass spectra throughout each chromatogram and the accurate mass measurements that provide qualitative information that could be used to secure identification of analytes present in the samples that is not available from QqQ instruments. However, one of the most important drawbacks of using LC–TOF–MS for quantitative measurements of environmental contaminants is the effective linear dynamic range (typically two to three or-

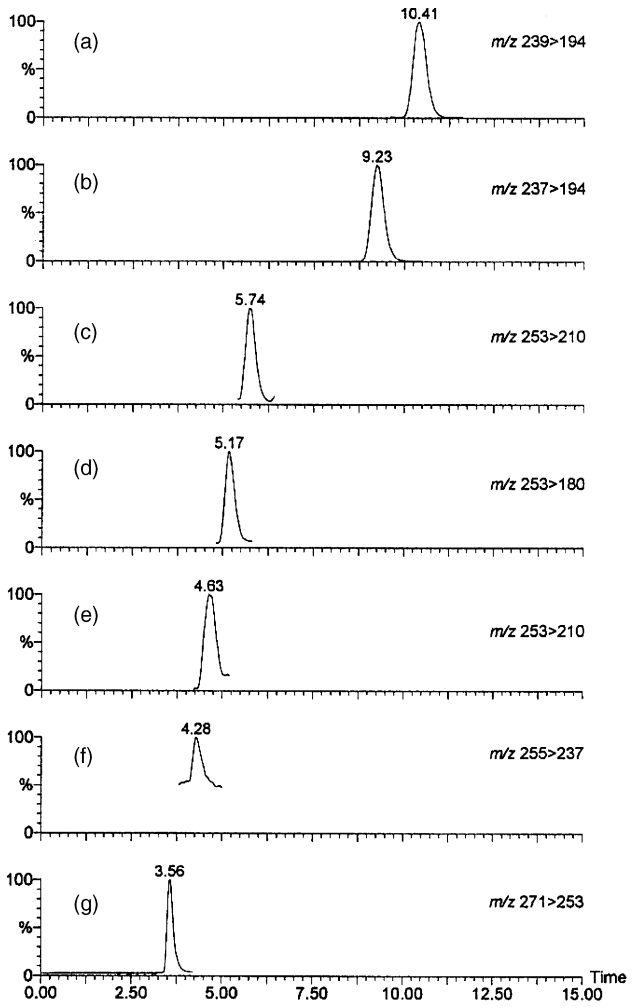

Fig. 4. Time-scheduled SRM chromatograms of carbamazepine and its metabolites in an effluent from the STP of Perborough: (a) 10,11-dihydroxycarbamazepine (CBZ-DiH), internal standard; (b) carbamazepine (CBZ); (c) 3-hydroxycarbamazepine; (d) 10,11-dihydro-10,11-epoxycarbamazepine; (e) 2-hydroxycarbamazepine; (f) 10,11-dihydro-10-hydrocarbamazepine; (g) 10,11-dihydro-10,11-dihydroxycarbamazepine. Reproduced with permission from [50] copyright © 2003 American Chemical Society.

ders of magnitude) which is significantly lower than the dynamic range observed on quadrupole instruments (typically >4 orders of magnitude).

Stolker et al. [33] used QqQ and Q-TOF–MS for screening and confirmation of pharmaceuticals in surface, drinking and ground water. The set of pharmaceuticals included selected analgesics, antibiotics, lipid regulators, β-blockers and anti-epileptics. The method permitted screening and confirmation of a large number of pharmaceuticals at low concentrations (1–100 ng/l) in one run. Comparing the performances of QqQ and Q-TOF–MS Stolker at al. [17] concluded that with both techniques fully satisfactory results were obtained, however Q-TOF–MS has the distinct advantage of the enhanced selectivity due to information obtained from the accurate mass measurements of product ions. Additional advantage is full MS/MS spectra, which are available after a single injection. Method characteristics such as linear dynamic range and repeatability were found to be the same for both techniques, but LODs of LC-QqQ MS are found to be somewhat lower.

Marchese et al. [18] compared QqQ instrument and a hybrid Q-TOF/MS for the determination of analgesics in water samples. The full TOF–MS fragment ion spectra for the $[M-H]^-$ ion of each analyte described in this study are shown in Fig. 5. The quantitation limits obtained for the TOF experiment were approximately three to five times greater than those obtained using a QqQ MS operating in SRM mode. Limits of quantification (LOQs) estimated were less than 3 ng/l for each analyte. The between-day precision and linear dynamic range results of the method with the Q-TOF analyzer were similar to those obtained using QqQ SRM analysis.

For confirmation of "positive" residues of pharmaceuticals in waters that were detected in screening analysis, Stolker et al. [33] developed confirmatory methods applying LC–MS/MS and LC–Q-TOF–MS. In this study, confirmation of pharmaceuticals such as carbamazepine or metoprolol in "positive" samples was based on the ratios of two MS/MS transition ions and the accurate masses monitored for these ions. This approach was found to be fully satisfactory for confirmatory purposes of pharmaceuticals at low concentrations. The quantification limits were in the low ng/l range for all the pharmaceuticals under investigation. Similar sensitivity was obtained for the detection of β-blockers in drinking and surface waters, using the LC–MS/MS method developed by Ternes et al. [35].

6. Matrix effects in the analysis of environmental samples

One of the limitations of LC–MS/MS is the susceptibility of API interfaces to co-extracted matrix component. The matrix effect, typically results in the suppression or, less frequently the enhancement, of the analyte signal. In general, the strategy to diminish matrix effects should take into account the variability of the matrix within the set of samples to be analysed (e.g. river water, STP influent, effluent, sediment extracts, etc.) and should be tested for each type of matrix. An appropriate internal standard (structurally similar unlabeled compound or isotopically labelled standard) may compensate, over a limited retention time window, for the signal irreproducibility that leads to erroneous results. However, the matrix effect can strongly depend upon the chromatographic retention time and more than one internal standard may be needed and finding a suitable internal standard for each analyte can be a difficult task. Another option is time-consuming and laborious standard addition method [19,20].

In the LC–ESI-MS/MS analysis of β-blockers and lipid regulating agents Hernando et al. [21] reported on the loss of signal of up to 28% in tap and river water, up to 54%

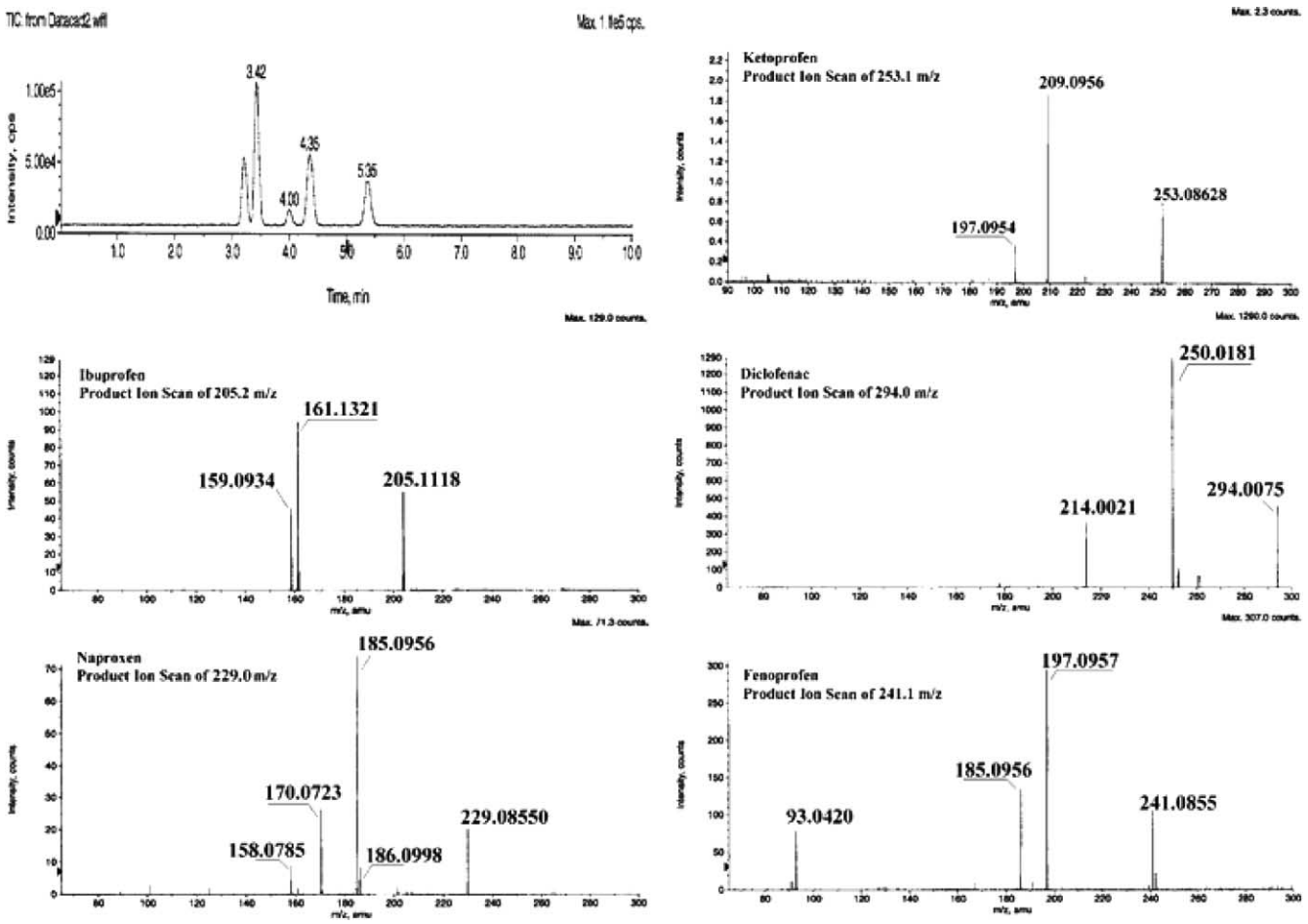

Fig. 5. TOF–MS/MS product ion spectra for $[M-H]^-$ ions of selected pharmaceuticals. Reproduced with permission from [18] copyright © 2003 Wiley.

in STP effluents and up to 60% in STP influent samples as compared to the pure standard solution. Similarly, Quintana and Reemtsma [32] observed a clear tendency of decreasing signal suppression with increasing retention time for acidic drugs, as being indicative of non-specific matrix effects of moderately polar matrix components. Signal suppression measured for early eluting compounds was almost 80% and due to the gradual decrease of the matrix effect with increasing retention time it was not possible to reliably compensate for this effect by the use of internal standards. Therefore, the authors used the standard addition method for the quantification.

7. Conclusions and future trends

The application of advanced LC–MS/MS technologies to environmental analysis has allowed the determination of a broader range of compounds and thus permitted more comprehensive assessment of environmental contaminants. The LC–MS/MS method offers an improvement over GC–MS since the derivatization step is avoided and the limits of detection (LODs) less than 1 ng/l can still be achieved. In general, LODs achieved with the LC–MS/MS methods were slightly higher than those obtained with the GC–MS, however, LC–MS/MS showed advantages in terms of versatility and less complicated sample preparation.

Currently, most efforts in environmental analysis have focused on the detection of parent compounds, while the analysis of metabolites and transformation products is still limited. Elimination of pharmaceuticals, especially polar ones, during wastewater and drinking water treatment is not satisfactory; and more research is needed to determine the breakdown pathways and to evaluate the fate of transformation products. Moreover, disinfection processes applied in water works (either chlorination or ozonation) potentially shift the assessment of the risk of human consumption of the parent compound to its degradation products. Consequently, development of generic analytical protocols that will permit simultaneous determination of parent compounds and their metabolites is required. Additionally, TOF–MS and Q-TOF instruments, with capacity to achieve accurate mass determination at sensitivities comparable to those of a QqQ instrument operating in the SRM mode, are expected to be increasingly applied for screening and identification of unknown metabolites.

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References

[1] C.G. Daughton, A.T. Ternes, Environ. Health Perspect. 107 (1999) 907.
[2] B.E. Erickson, Environ. Sci. Technol. 36 (2002) 140A.
[3] M. Petrovic, S. Gonzalez, D. Barceló, TrAC Trends Anal. Chem. 22 (2003) 685.
[4] T. Haberer, U. Dünnbier, C. Reilich, H.J. Stan, Fresenius Environ. Bull. 6 (1997) 438.
[5] K. Reddersen, T. Haberer, U. Dünnbier, Chemosphere 49 (2002) 539.
[6] T.A. Ternes, Environ. Sci. Technol. 36 (2002) 3855.
[7] T. Haberer, K. Schmidt-Bäumler, H.J. Stan, Acta Hydrochim. Hydrobiol. 26 (1998) 272.
[8] T.A. Ternes, Trends Anal. Chem. 20 (2001) 419.
[9] W. Ahrer, E. Scherwenk, W. Buchberger, J. Chromatogr. A 910 (2001) 69.
[10] M.J. López de Alda, S. Díaz-Cruz, M. Petrovic, D. Barceló, J. Chromatogr. A 1000 (2003) 503.
[11] S. Díaz-Cruz, M.J. López de Alda, D. Barceló, Trends Anal. Chem. 22 (2003) 2003.
[12] K. Klagkou, F. Pullen, M. Harrison, A. Organ, A. Firth, G.J. Langley, Rapid Commun. Mass Spectrom. 17 (2003) 2373.
[13] S.I. Kotretsou, Crit. Rev. Food Sci. Nutr. 44 (2004) 173.
[14] E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, Environ. Sci. Technol. 36 (2002) 3645.
[15] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Anal. Chem. 73 (2001) 3632.
[16] M.J. Benotti, P. Lee Ferguson, R.A. Rieger, C.R. Iden, C.E. Heine, B.J. S Brownawell, LC/MS, MS/MS and TOF/MS analysis of emerging contaminants, ACS Symposium Series 850, American Chemical Society, Washington, DC, 2003, p. 109.
[17] A.A.M. Stolker, W. Niesing, R. Fuchs, R.J. Vreeken, W.M.A. Niessen, U.A.Th. Brinkman, Anal. Bioanal. Chem. 378 (2004) 1754.
[18] S. Marchese, A. Gentilli, D. Perret, G. D'Ascenzo, F. Pastori, Rapid Commun. Mass Spectrom. 17 (2003) 879.
[19] M. Stüber, T. Reemtsma, Anal. Bioanal. Chem. 378 (2003) 910.
[20] T. Benijts, R. Dams, W. Lambert, A. De Leenheer, J. Chromatogr. A 1029 (2004) 153.
[21] M.D. Hernando, M. Petrovic, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1046 (2004) 133.
[22] R. Andreozzi, et al., Final activity report the EU project REMPHARMAWATER (EVK1-CT 2000-00048).
[23] T. Ternes, Water Res. 32 (1998) 3245.
[24] D. Ashton, M. Hilton, K.V. Thomas, Sci. Total Environ. 333 (2004) 167.
[25] C.D. Metcalfe, B.G. Kenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Environ. Toxicol. Chem. 22 (2003) 2872.
[26] X.S. Miao, F. Bishay, M. Chen, C.D. Metcalfe, Environ. Sci. Technol. 38 (2004) 3533.
[27] B.J. Vanderford, R.A. Person, D.J. Rexing, S.A. Zinder, Anal. Chem. 75 (2003) 6265.
[28] D. Löffler, T.A. Ternes, J. Chromatogr. A 1021 (2003) 133.
[29] M.J. Hilton, K.V. Thomas, J. Chromatogr. A 1015 (2003) 129.
[30] X.S. Miao, B.G. Koenig, C.D. Metcalfe, J. Chromatogr. A 952 (2002) 139.
[31] S. Marchese, D. Perret, A. Gentili, R. Curini, F. Pastori, Chromatographia 58 (2003) 263.
[32] J.B. Quintana, T. Reemtsma, Rapid Commun. Mass Spectrom. 18 (2004) 765.
[33] A.A.M. Stolker, W. Niesing, E.A. Hogendoorn, J.F.M. Versteegh, R. Fuchs, U.A.Th. Brinkman, Anal. Bioanal. Chem. 378 (2004) 955.
[34] D. Calmari, E. Zuccato, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Sci. Technol. 37 (2003) 1241.
[35] T.A. Ternes, R. Hirsch, M. Stumpf, J. Mueller, K. Haberer, Fresenius J. Anal. Chem. 362 (1998) 329.
[36] C.D. Metcalfe, B.G. Koenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Environ. Toxicol. Chem. 22 (2003) 2872.
[37] T. Ternes, M. Bonerz, T. Schmidt, J. Chromatogr. A 938 (2001) 175.
[38] X.-S. Miao, C.D. Metcalfe, J. Mass Spectrom. 38 (2003) 27.
[39] X.-S. Miao, C.D. Metcalfe, J. Chromatogr. A 998 (2003) 133.
[40] F. Sacher, F.T. Lange, H.-J. Brauch, I. Blankenhorn, J. Chromatogr. A 938 (2001) 199.
[41] K.D. Bratton, A.S. Lillquist, T.D. Williams, C.E. Lunte, Liquid chromatography/mass spectrometry MS/MS and time-of-flight MS, in: Analysis of emerging contaminants. LC/MS MS/MS and TOF/MS analysis of emerging contaminants, ACS Symposium Series 850, American Chemical society, Washington, DC, 2003, p. 188.
[42] R. Hirsch, T.A. Ternes, K. Haberer, K.L. Kratz, Sci. Total Environ. 225 (1999) 109.
[43] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K.L. Kratz, J. Chromatogr. A 815 (1998) 213.
[44] G. Hamscher, S. Sczesny, H. Höper, H. Nau, Anal. Chem. 74 (2002) 1509.
[45] J. Zhu, D.D. Snow, D.A. Cassada, S.J. Monson, R.F. Spalding, J. Chromatogr. A 928 (2001) 177.
[46] A.M. Jacobsen, B. Halling-Sorensen, F. Ingerslev, S.H. Hansen, J. Chromatogr. A 1038 (2004) 157.
[47] F. Sacher, F.T. Lange, H.J. Brauch, I. Blankenhorn, J. Chromatogr. A 938 (2001) 199.
[48] S. Yang, K.H. Carlson, J. Chromatogr. A 1038 (2004) 141.
[49] M.P. Schlüsener, M. Spiteller, K. Bester, J. Chromatogr. A 1003 (2003) 21.
[50] X.S. Miao, C.D. Metcalfe, Anal. Chem. 75 (2003) 3731.
[51] S. Sczesny, H. Nau, G. Hamscher, J. Agric. Food Chem. 51 (2003) 697.
[52] B. Halling-Sorensen, A. Lykkeberg, F. Ingerslev, P. Blackwell, J. Tjornelund, Chemosphere 50 (2003) 1331.
[53] C. Hartig, T. Storm, M. Jekel, J. Chromatogr. A 854 (1999) 163.