Analytica Chimica Acta 649 (2009) 158–179

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Review

# An overview of analytical methodologies for the determination of antibiotics in environmental waters

Marcela Seifrtová [a], Lucie Nováková [a,*], Celeste Lino [b], Angelina Pena [b], Petr Solich [a]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Group of Bromatology, Center of Pharmaceutical Studies, University of Coimbra, Coimbra, Portugal

ARTICLE INFO

*Article history:*
Received 2 March 2009
Received in revised form 10 July 2009
Accepted 14 July 2009
Available online 19 July 2009

*Keywords:*
Fluoroquinolone antibiotics
Tetracycline antibiotics
Sulfonamide antibiotics
Macrolide antibiotics
Wastewaters
Liquid chromatography–tandem mass spectrometry
SPE

ABSTRACT

The widespread occurrence of antibiotics as contaminants in the aquatic environment has increased attention in the last years. The concern over the release of antibiotics into the environment is related primarily to the potential for the development of antimicrobial resistance among microorganisms. This article presents an overview of analytical methodologies for the determination of quinolone (Qs) and fluoroquinolone (FQs), macrolide (MLs), tetracycline (TCs), sulfonamide (SAs) antibiotics and trimethoprim (TMP) in different environmental waters. The analysis of these antibiotics has usually been carried out by high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) and to a lesser extent by ultraviolet (UV) or fluorescence detection (FD). A very important step before LC analysis is sample preparation and extraction leading to elimination of interferences and prevention of matrix effect and preconcentration of target analytes.



**Contents**



*Abbreviations:* AcAc, acetic acid; ACN, acetonitrile; AmAc, ammonium acetate; APCI, atmospheric pressure chemical ionization; AZI, azithromycin; CF, concentration factor; CIN, cinoxacin; CIPRO, ciprofloxacin; CLAR, clarithromycin; CLIN, clindamycin; CTC, chlortetracycline; CZE, capillary zone electrophoresis; DAD, diode-array detector; DAN, danofloxacin; DEME, demeclocycline; DIF, difloxacin; DOXY, doxycycline; ENO, enoxacin; ENRO, enrofloxacin; ERY, erythromycin; ESI, electrospray ionization; FAc, formic acid; FD, fluorescence detection; FLE, fleroxacin; FLU, flumequine; FQs, fluoroquinolone antibiotics; GAT, gatifloxacin; HPLC, high-performance liquid chromatography; IS, internal standard; LC, liquid chromatography; LEV, levofloxacin; LLE, liquid–liquid extraction; LOD, limit of detection; LOME, lomefloxacin; LOQ, limit of quantification; MAR, marbofloxacin; MCX, mixed-cation exchange; MECLO, meclocycline; MeOH, methanol; MINO, minocycline; MLs, macrolide antibiotics; MOXI, moxifloxacin; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NAL, nalidixic acid; NOR, norfloxacin; OFLO, ofloxacin; OXO, oxolinic acid; OXY, oxytetracycline; PEFLO, pefloxacin; PIP, pipemidic acid; Qs, quinolone antibiotics; RIA, radioimmunoassay; ROXI, roxithromycin; SAD, sulfanilamide; SARA, sarafloxacin; SAs, sulfonamide antibiotics; SAX, strong-anion exchange; SCP, sulfachloropyridazine; SCT, sulfacetamide; SDM, sulfadimidine; SDT, sulfadimethoxine; SDX, sulfadoxine; SDZ, sulfadiazine; SGN, sulfaguanidine; SIM, selected ion monitoring; SM, sulfameter; SMD, sulfamethoxydiazine; SML, sulfamoxole; SMM, sulfamonomethoxine; SMO, sulfamethoxine; SMP, sulfamethoxypyrazine; SMR, sulfamerazine; SMT, sulfamethizole; SMX, sulfamethoxazole; SMZ, sulfamethazine; SNT, sulfanitran; SPAR, sparfloxacin; SPE, solid-phase extraction; SPIR, spiramycin; SPME, solid-phase microextraction; SPY, sulfapyridine; SQX, sulfaquinoxaline; SRM, selected reaction monitoring; SSM, sulfisomidin; SSX, sulfisoxazole; SSZ, sulfasalazine; STZ, sulfathiazole; TCs, tetracycline antibiotics; TET, tetracycline; TLS, tylosin; TMP, trimethoprim; TOS, tosufloxacin; UHPLC, ultra-high-performance liquid chromatography; UV, ultraviolet; VAN, vancomycin; WCX, weak-cation exchange; WWTPs, wastewater treatment plants.

* Corresponding author. Tel.: +420 495067381; fax: +420 495067164.
*E-mail address:* novakoval@faf.cuni.cz (L. Nováková).

0003-2670/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2009.07.031

## 1. Introduction

Pharmaceuticals represent a group of emerging chemicals of environmental concern widely used in human and veterinary medicine. They can enter the environment either as parent compounds or metabolites, conjugates or both. Pharmaceuticals have been found in surface waters and wastewaters at levels of up to a few $\mu g\,L^{-1}$. Antibiotics, followed by steroid compounds, analgesics/nonsteroidal and anti-inflammatory drugs, are the most widely studied classes of pharmaceuticals [1].

Antibiotics are an important group of pharmaceuticals in today's medicine and have been detected in various compartments of the aquatic environment, e.g. wastewaters, surface and ground water and in drinking water as well [2–4]. They are regarded as "pseudopersistent" contaminants due to their continual input into the ecosystem. Therefore, the occurrence of antibiotics in the environment has received considerable attention. They are generally poorly absorbed by the human body and thus excreted either unchanged or transformed, via urine and faeces [5]. There is a growing interest about their presence, persistence and fate in the environment because low levels of antibiotics can favor the proliferation of antibiotic resistant bacteria. The use of antibiotics in animal agriculture has been linked to the increased emergence of resistant strains of pathogenic bacteria that have potential to impact human health. Resistance genes and/or antibiotic resistant bacteria can be transferred from animals to humans. In addition, bacteria can develop cross-resistance between antibiotics used in veterinary medicine with those of similar structures used exclusively in human medicine [6].

Antibiotics are released to the aquatic environment in different pathways. After the administration to humans, they are excreted as metabolites but also a considerable amount is eliminated in unchanged form as parent compounds via urine and faeces into the sewage. Many researches have shown the incomplete removal of pharmaceuticals during wastewater treatment processes. Wastewater treatment plants (WWTPs) are considered to be major contributors of presence of pharmaceuticals in the environment. Pharmaceuticals along with their metabolites have been found in the effluents from WWTPs [7–9]. Therefore they can reach the surface and groundwater. There is a potential risk for the aquatic and soil organisms which is associated with the presence of trace concentrations of these bioactive compounds. Hospitals are also one of the most important contributors of the occurrence of the antibiotics into the aquatic environment [10,11].

Use of antibiotics in veterinary medicine for the treatment of bacterial infections of animals as well as prophylactic agents is another source of contamination. The animal excreta are the major source of contamination, as the most of these substances end up in manure. The manure and slurry (urine and faeces) are either stored or immediately applied to the agricultural fields as fertilizers. The unmetabolized compounds present in the manure or their biologically active metabolites may move from the manure from the field to the groundwater and eventually enter surface water, such as rivers and lakes and thus they can affect the aquatic organisms. This is depending on their mobility in the soil system. The sludge from WWTPs can be used to fertilize soils as well. In addition, antibiotics are extensively used in fish farms. They are used as feed additives or they are directly applied into the water. The result of an overfeeding is that many compounds end up in the sediments where they are slowly degraded or slowly leach out back into the surrounding waters.

Drugs may persist in solid environmental matrices for a long time. The persistence depends on their photostability, binding and adsorption capacity, degradation rate and leaching into the water. Strongly sorbing pharmaceuticals tend to accumulate in soils or sediment (TCs, FQs) and by contrast, highly mobile pharmaceuticals (SAs) have a potential to resist degradation and tend to leach into the groundwater and to be transported with the groundwater, drainage water and surface water run-off to surface waters [12]. The sorptive exchange of chemicals between a water phase and a solid phase is represented by the sorption coefficient $K_{d,solid}$, which is defined as the ratio between the concentration of the compound in the sorbent and in the water at the equilibrium [13]. Another sorption mechanism is forming complexes between antibiotic and metal ions such as $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$ or $Al^{3+}$. This is important for TCs, FQs and for MLs and their persistence in the environment.

The analysis of antibiotics in the environment represents a difficult task due both to the high complexity of the matrices analyzed and to the usually low concentrations ($ng\,L^{-1}$) at which target compounds are present in the environmental waters. This reason makes the development of very sensitive analytical methods suitable for the monitoring of these analytes in low $ng\,L^{-1}$ concentration level necessary. The typical low concentrations of antibiotics found in the environment make a preconcentration step prior to the detection imperative and essential. Off-line solid phase extraction (SPE) is the method of choice for the sample preconcentration and usually it is followed by liquid chromatography (LC) analysis. Nowadays, a new trend became the injection of water samples directly onto HPLC or ultra-high-performance liquid chromatography (UHPLC) system with MS detection. It is possible mainly due to the high sensitivity of MS detection which allows that analytes do not need to be pre-concentrated and can be quantified accurately in water samples.

Many antibiotics from different classes have been found in the aquatic environment. Therefore currently multiresidual analytical methods are preferred for the determination and monitoring of different groups of antibiotics. These methods must be sensitive, selective, not so much time-consuming and easily applicable

to analyze environmental samples. There have already been published reviews dealing with the determination of specific groups of pharmaceuticals in different scientific journals [14,15]. In this review we attempt to summarize recently published analytical methodologies for the extraction and following determination of selected antibiotics in environmental water samples. Antibiotics are characterized according to their structural and chemical properties. Members of the same group have similar structures, act by similar mechanisms, and are likely to behave similarly in the environment. For the purposes of this article following groups of antibiotics were selected: Qs and FQs, TCs, MLs, SAs and TMP. The reason was their widespread usage in the medicine and their occurrence in the aquatic environment. FQs, the second-generation of Qs are synthetic antibiotics. MLs are produced by various *Streptomyces* strains. TCs are broad-spectrum antibiotics highly effective against numerous gram-positive and gram-negative bacteria. Those groups of antibiotics are widely used in human medicine and in veterinary medicine as well. SAs are antibacterial agents, commonly used in veterinary prophylaxis of infections and also in the treatment of diseases. TMP is a dihydrofolate reductase inhibitor structurally different from SAs. It is commonly prescribed in combination with sulfamethoxazole (SMX) (as co-trimoxazole, which contains SMX:TMP in a 5:1 ratio) or it can be prescribed on its own. The β-lactam class of antimicrobials, including penicillins and cephalosporins, are used for the treatment of both humans and animals. However, due to the chemically unstable β-lactam ring, members of the lactam class of antimicrobials readily undergo hydrolysis. These compounds are not commonly detected in environmental waters that is the reason why this review does not deal with them.

## 2. Sampling, storage and stability

The whole analytical procedure typically includes five steps: sampling, sample preparation, chromatographic separation, detection and data analysis. The most important parts of the analytical process are sampling and sample preparation because they take more than 80% of the analytical time. Sampling is a selection of a small fraction of matrix enough in volume to still accurately represent the part of the environment. Sampling is so important that it can cause the main contribution to the error of the whole analytical process. The main difficulties in the sampling are representativeness and integrity. Possible errors during the sampling step can be caused by choosing improper sampling method, location and frequency of sampling and number of samples collected. Other errors could be originating from storage and handling with the sample. The sampling frequency is an important factor of the representativeness. The low sampling frequency could underestimate the occasional presence of samples with high analyte concentration. Usually, 24-h composite samples are collected in the environmental area. The composite samples are used to exclude a possibility of non-representative results, whereby samples were collected at low tide, dilution would be at a minimum and so not representative sample would be obtained [16].

The preservation of the sample is an additional problem in the sampling process. Several problems which can occur during the sampling and storage step are decomposition by means of temperature, UV irradiation, microbial activity and chemical reactions. The following approaches are applied to preserve the sample integrity. Samples should be protected from the external agents (they are collected in brown amber glass bottles) and stored at low temperature (approx. 4 °C or frozen at −20 °C) in a dark ambient until the chromatographic analysis. These precautions are very important especially for TCs which can be easily degradaded [11]. Storage at higher temperatures can enhance the bacterial growth and activity resulting in a loss of analytes. The other possible approach is an addition of preservatives which should be suitable to avoid decomposition by means of chemical reactions and microbial activity. The samples can be acidified to inhibit a bacterial growth [16]. Hydrochloric acid was used for acidification of the samples up to pH 2.0 prior to the analysis of MLs in the water samples [17]. Sulphuric acid was employed for the pH adjustment of the sample to pH 2.0 for the determination of erythromycin (ERY), SMX and TMP [18]. One method referred the addition of $Na_2S_2O_3$ as a quenching agent to consume residual chlorine contained in the samples of wastewater effluents [19].

A further important factor in the sampling process is a filtration. Generally it is performed when sample arrives to the laboratory. The filtration is usually carried out on 0.45 or 0.2 μm glass-fibre filters. The second filtration could be performed immediately before the sample preparation step or the sample could be centrifuged. The filtration step is necessary to remove particles from the water samples which can plug up the SPE cartridges and thus slow down significantly the sample preparation step. However, the filtration may lead to loss of analytes in case that they are hydrophobic and adsorb to particles in the water samples.

Considering the stability of analytes during the storage, the stability of Qs (oxolinic acid and ciprofloxacin) was determined in river water [20]. The stability has been studied both in containers and on $C_{18}$ SPE cartridges under different storage conditions: time (up to 4 months), light (sunlight *vs.* dark), and temperature (ambient, 4, and −18 °C). SPE cartridges were chosen for the study, because their easier transport and their use would considerably reduce space requirements for storage in laboratories, especially if large-volume samples must be stored at low temperatures. Results showed high influence of temperature and time of storage on the stability of studied compounds. Antibiotics were stable both in the containers and on SPE cartridges for at least 2 weeks at ambient temperature. Stability was increased substantially if samples were stored at low temperatures (4 and −18 °C) and in dark. However stability of antibiotics in SPE cartridges was significantly lower and antibiotics were degraded more quickly when stored in SPE cartridges, than in aqueous samples. After 3 months, significant degradation of analytes was observed even when cartridges were stored at −18 °C comparing to storage in containers, where the concentration of analytes was almost the same after 4 months, both at 4 and −18 °C. These results confirmed the suitability of SPE cartridges for use in the field of sampling of the antibiotics from river water and their subsequent storage, although for a shorter time.

Regarding the stability of standards, the standard solutions should be stored in amber bottles to avoid light penetration, usually below the temperature 4 °C, and warmed to room temperature before use [21]. In some papers the stability study for standards was provided. It is recommended to use the standard solutions not longer than 3 months. For example, TCs can decompose rapidly under the influence of light and atmospheric oxygen, forming degradation products. The stock standard solutions are mostly prepared in methanol (MeOH) and stored at 4 °C for 1 month.

## 3. Sample pre-treatment and extraction procedure

Sample preparation is the crucial step in environmental analysis. It is highly influenced by the physical and chemical properties of analytes studied and by matrices. The main goal is to concentrate analytes in sample, to remove interferences from matrix and to prepare analyte in suitable form for subsequent chromatographic analysis. Usually, the sample preparation step includes adjustment of solution pH, addition of chelator followed by extraction procedure, handling with the extract and final preparation for following chromatographic analysis. An overview of sample preparation procedures are given in Tables 1–4.

**Table 1**
Extraction procedures utilized for the sample preparation in multiresidue studies.

| Substances isolated | Matrix | Sample pretreatment | Sorbent type | Conditioning solvents | Washing solvent | Elution solvent | Recovery (%), concentration factor | Final analysis | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| CIPRO, DOXY, SMX, TMP, ERY (+28 pharmaceuticals) | Surface water (1 L) | pH adjustment 2.5 (by HCl), addition EDTA | Oasis MCX | 2 mL MeOH, 2 mL 2% FAc/water | 2 mL 2% FAc/water | 1 mL MeOH, 2 mL 5% ammonia/MeOH | Rec = 61.6–82.5%, CF = 2000 | UHPLC–MS/MS | [1] |
| SMX, ERY (+13 pharmaceuticals) | Surface, drinking, ground water (100 mL) | pH adjustment 3.0 (by HCl) | Oasis MCX | 5 mL acetone, 5 mL water | 6 mL water (pH 3.0) | 8 mL MeOH + ammonia (95:5) | Rec = 63–96%, CF = 200 | LC–MS/MS | [2] |
| CIPRO, NOR, ENRO, SARA, PIP, OXO, FLU, MINO, OXY, TET, DEME, CTC, DOXY, STZ, SMR, SMZ, SMT, SCP, SMX, SDT, TMP, ERY, ROXI, TLS | Drinking water (500 mL) | pH adjustment 3.0, addition EDTA | Oasis HLB | 6 mL MeOH, 3 mL MeOH + 0.1% FAc, 2× 6 mL water | 2× 6 mL water | 4× 2 mL MeOH + 0.1% FAc | Rec = 91–161%, CF = 2000 | LC–MS/MS | [4] |
| SDZ, STZ, SMZ, SPY, SMX, $N^4$-acetyl-SMX, TMP, AZI, CLAR, ERY, ROXI | WWTP: 1st effluent (50 mL), 2nd, 3rd effluent (250 mL) | Dilution with 150 mL of water, addition NaCl, pH adjustment 4.0 (by $H_2SO_4$) | Oasis HLB | 2× 1.5 mL MeOH-EtAc (1:1), 2× 1.5 mL MeOH + 1% ammonia, 2× 1.5 mL water (pH 4.0) | 1.5 mL water-MeOH (95:5) | 2× 1.5 mL MeOH-EtAc (1:1), 2× 1.5 MeOH + 1% ammonia | Rec = 30–124%, CF = 100, 500 | LC–MS/MS | [7] |
| ERY, CLAR, ROXI (+9 hormones) | WWTP influents and effluents (1 L) | | DVB-phobic speedisk cartridges, clean-up = SEC | 15 mL MeOH, 15 mL water | 15 mL water | 15 mL *tert*-butyl methyl ether, 15 mL MeOH | Rec = 81–92%, CF = 1000 | LC–MS/MS | [8] |
| CIPRO, NOR, OFLO, DOXY, SMX, TMP (+β-lactams) | Hospital wastewater (200–500 mL) | pH adjustment 3.0 (by $H_2SO_4$) | $C_2$/ENV+ | 5 mL MeOH, 5 mL MeOH–water (50:50), 5 mL water (pH 3.0) | 5 mL water (pH 3.0) | 5 mL triethylamine (5%) in MeOH | Rec = 55–87% | LC–MS/MS | [10] |
| ENRO, OXY, SMZ, SDZ, SGN, TMP (+β-lactams) | Wastewater (100 mL) | pH adjustment 4.0 | Oasis HLB | 5 mL MeOH, 5 mL water (pH 4.0) | 2 mL 2% MeOH | 2× 5 mL MeOH | Rec = 89.3–97.9%, $Rec_{SGN}$ = 11.2%, CF = 100 | LC–DAD | [12] |
| SMX, TMP, ERY-$H_2O$ (+27 pharmaceuticals) | Surface water (1 L) | pH adjustment 2.0 (by $H_2SO_4$) | Oasis HLB | 5 mL methyl *tert*-butyl ether, 5 mL water | 5 ml water | 5 mL MeOH/methyl *tert*-butyl ether (10:90), 5 mL MeOH | Rec = 71–91%, CF = 1000 | LC–MS/MS | [18] |
| CIPRO, ENRO, NOR, OFLO, SMZ, SMX, TMP | WWTP effluents (2nd, 3rd) (1 L) | Addition NaCl, pH adjustment 2.5 (by $H_3PO_4$) | Anion-exchange cartridge (on the top), Oasis HLB | 6 mL MeOH, 6 mL 4.38 mM $H_3PO_4$ | | 10 mL 95% MeOH/5% 4.38 mM $H_3PO_4$ | Rec = 37–129%, CF = 1000 | LC–MS | [19] |
| OXY, CTC, MINO, DEME, MECLO, TET, DOXY, STZ, SMX, SMR, SCP, SMZ, SDT, ERY, ROXI, TLS | River water (120 mL) | | Oasis HLB | 3 mL MeOH, 3 mL water | 9 mL water | 5 mL MeOH | Rec = 76.6–124.8%, CF = 1000 | LC–MS/MS | [21] |
| CIPRO, ENRO, NOR, OFLO, OXO, PIP, CTC, DOXY, OXY, TET, 16 SAs, CLAR, ERY, ROXI | WWTP final effluent (1 L) | pH adjustment 3.0 (by $H_2SO_4$) (Qs, TCs, SAs), addition EDTA, pH adjustment 6.0 (by $H_2SO_4$) (MLs) | Oasis HLB | 6 mL acetone, 6 mL MeOH, 6 mL 50 mM EDTA (pH 3.0), 6 mL acetone, 6 mL MeOH, 6 mL water (pH 6.0) (MLs) | | 3× 2 mL MeOH | Rec = 72–99%, CF = 1000 | LC–MS/MS | [22] |
| CIPRO, CTC, TET, SDT, SMZ, SMT, SMX, STZ, SSX, CLAR (+20 pharmaceuticals) | WWTP influent (500 mL) and effluent (1 L) | pH adjustment 6.0 (FQ, MLs), pH adjustment 3.0 (TCs, SAs) (by $H_2SO_4$) | Oasis HLB | 6 mL acetone, 6 mL MeOH, 6 mL water (pH 6) (FQs, MLs), 6 mL 50 mM EDTA (TCs, SAs) | | 3× 2 mL MeOH | Rec = 57–94%, CF = 500, 1000 | LC–MS/MS | [23] |

Table 1 (*Continued*)

| Substances isolated | Matrix | Sample pretreatment | Sorbent type | Conditioning solvents | Washing solvent | Elution solvent | Recovery (%), concentration factor | Final analysis | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| OFLO, NOR, CIPRO (+5 pharmaceuticals) | Groundwater (1 L), surface water (500 mL), WWTP influent (100 mL) and effluent (250 mL) | pH adjustment 10.0 (by NaOH) | Oasis HLB | 2 mL *n*-hexane, 2 mL acetone, 10 mL MeOH, 10 mL non-contaminated groundwater (pH 10.0) | 2 mL 5% MeOH in 2% $NH_4OH$ | 4× 1 mL MeOH | Rec = 32–97%, CF = 200, 500, 1000, 2000 | LC–MS/MS | [24] |
| CTC, DOXY, MECLO, OXY, TET, SCP, SDT, SMR, SMZ, STZ, TMP, ERY, ROXI, TLS (+pharmaceuticals) | Surface water (400 mL) | Addition EDTA, pH adjustment 8.2 (by $H_2SO_4$ or NaOH) | Oasis HLB | 5 mL MeOH, 5 mL water | 5 mL 5% MeOH | 5 mL MeOH | Rec = 65–134%, CF = 800 | LC–MS/MS | [25] |
| SMX, TMP, ERY (+13 pharmaceuticals) | Surface water, wastewater | pH adjustment 6.0 (by HCl or aq. ammonia) | Strata X | 5 mL MeOH, 5 mL water | | 5 mL acetone, 2× 5 mL MeOH | | LC–MS/MS | [26] |
| TMP, ERY (+16 pharmaceuticals) | Hospital wastewater (100 mL) | pH adjustment 7.0 (by $H_2SO_4$) | Oasis HLB | 6 mL MeOH, 5 mL water | 5 mL water | 2× 4 mL MeOH | Rec = 87.9–95.2%, CF = 100 | LC–MS/MS | [27] |
| SMX, TMP (+22 pharmaceuticals) | Surface water, ground water (1 L) | | Oasis HLB | 6 mL MeOH, 6 mL water | 1 mL 5% MeOH/water | 3 mL MeOH, 2 mL MeOH (pH 3.7) | CF = 1000 | LC–MS | [28] |
| SPY, SMX, SSX, SMZ, SDT, TMP, ERY, CLAR, ROXI | WWTP influent (100 mL) and effluent (500 mL) | | Oasis HLB | 3× 2 mL MeOH, 3× 2 mL water | | 6 mL water | CF = 100, 500 | LC–MS/MS | [29] |
| OXY, CTC, TET, DEME, DOXY, MECL, MINO STZ, SMR, SMZ, SCP, SMX, SDT | Surface waters (120 mL) | Addition EDTA, pH adjustment <3.0 (by $H_2SO_4$) | Oasis HLB | 3 mL MeOH, 3 mL HCl, 3 mL water | 3 mL water | 5 mL MeOH | Rec = 82.1–101.6%, CF = 1000 | LC–MS/MS | [32] |
| CTC, DEME, DOXY, MECLO, OXY, TET, STZ, SMZ, SCP, SMY, SDT | WWTP influent and effluent (120 mL) | Addition EDTA, citric acid, pH adjustment <3.0 (by $H_2SO_4$) | Oasis HLB | 3 mL MeOH, 3 mL HCl, 3 mL water | 3 mL water | 5 mL MeOH | $Rec_{INF}$ = 77.9–99.8%, $Rec_{EFF}$ = 83.6–103.5%, CF = 1000 | LC–MS/MS | [33] |
| Acetyl-SMX, SMX, TMP, ERY | Surface water, sewage effluent (1 L) | pH adjustment 3.0 (by HCl) | Strata X | 3× 2 mL MeOH, 3× 2 ml water, 3× 2 ml water (pH 3) | | 3× 2 mL MeOH | Rec = 56–123% | LC–MS/MS | [38,40] |
| SMX, TMP (+20 pharmaceuticals) | WWTP influent and effluent (500 mL) | pH adjustment 4.0 (by $H_2SO_4$) | Strata X | 6 mL MeOH, 6 mL water | 5 mL water | 10 mL MeOH | CF = 1000 | LC–MS/MS | [39] |
| OFLO, SMX, TMP, ERY, AZI (+27 pharmaceuticals) | Ground waters (500 mL), WWTP influents (100 mL) and effluents (200 mL) | | Oasis HLB | 5 mL MeOH, 5 mL water | 5 mL water | 2× 4 mL MeOH | Rec = 30–116%, CF = 500, 100, 200 | LC–MS/MS | [41] |
| CIPRO, OFLO, CTC, DOXY, OXY, TET, SDM, SMX, TMP, AZI, CLAR, CLIN, ERY, ROXI, SPIR, TLS, VAN | Surface water (500mL) | pH adjustment 4.0 (by $H_2SO_4$), addition EDTA | Oasis HLB (on the top), SDB-2 | 1× cart. MeOH, 3× cart. water | 2× catr. water (pH 4.0) | 4× 1 mL MeOH, 4× 1 mL MeOH-FAc | Rec = 62–106% | LC–MS/MS | [42] |
| CIPRO, ENRO, NOR, SARA, CTC, DOXY, OXY, TET, SMT, STZ, SMR, SMZ, SCP, SMX, SDT TMP, ERY-$H_2O$, ROXI, TLS | Surface water, WWTP influent and effluent (500 mL) | Addition EDTA, pH adjustment 3.0 (by $H_2SO_4$) | Oasis HLB, MCX | 2 mL water, 2 mL MeOH, 2 mL MeOH + 5% $NH_4OH$, 2 mL reagent water, 2 mL water, pH 3 ($H_2SO_4$) | 2 mL water | 6 mL MeOH, +MCX: 2 mL 5% $NH_4OH$–MeOH | Rec = 71–138%, CF = 10 000 | LC–MS | [43] |
| CIPRO, ENRO, TET, OXY, CTC, SMZ, SDT, SMX, TMP, CLIN, ERY, ROXI, TLS | Surface, ground waters, WWTP effluent (500 mL) | addition EDTA, pH adjustment 2.8–3.0 (by $H_3PO_4$) | Oasis HLB, $tC_{18}$ Sep-Pak cartridges | 6 mL ACN, 6 mL water | | 4 mL ACN | Rec = 71–117%, CF = 500 | LC–MS/MS | [44] |
| NOR, DOXY, TMP, ERY-$H_2O$ (+β-lactams) | Surface seawater (500 mL) | Addition EDTA, pH adjustment 3.0 (by FAc) | Oasis HLB | 4 mL ACN, 4 mL water | 4 mL water | 4 mL ACN | Rec = 99–116%, CF = 250 | LC–MS/MS | [45] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| OXO, SDZ, TMP | Surface water (40 mL) | pH adjustment 5.0–5.2 (by $H_3PO_4$) | Oasis HLB | 2 mL MeOH, 1 mL water | 2 mL water | 3 mL ACN | Rec = 78–84%, CF = 80 | LC–MS/MS | [46] |
| CIPRO, ENRO, CTC, DOXY, OXY, TET | River water (1 L), WWTP influent (100 mL), WWTP effluent (250 mL) | pH adjustment 2.8 (by HCl) | Oasis HLB | 5 mL MeOH, 2 mL water | | 5 mL MeOH | Rec = 88–112% | LC–MS | [47] |
| CIPRO, NOR, OFLO, DOXY, SMX, TMP, ERY-$H_2O$ (+β-lactams) | WWTP effluent (1 L) | pH adjustment 3.0 (by $H_2SO_4$) | ENV+ | 5 mL MeOH, 5 mL 50% MeOH/water, 5 mL water (pH 3.0) | 5 mL water (pH 3.0) | 2 mL MeOH, 5 mL 5% TEA/MeOH | Rec = 54–101%, CF = 1000 | LC–MS/MS | [59] |
| OFLO, SMX, TMP, AZI, ERY (+29 pharmaceuticals) | River water (500 mL), WWTP influent (100 mL) and effluent (200 mL) | | Oasis HLB | 5 mL MeOH, 5 mL water | 5 mL water | 2× 4 mL MeOH | CF = 100, 200, 500 | UHPLC–MS/MS | [61] |
| CIPRO, NOR, LOME, LEV, GAT, SPAR, MOXI, SMX, TMP (+β-lactams) | Surface water (100 mL) | pH adjustment 3.0 (by FAc) | Oasis HLB | 5 mL MeOH, 5 mL water (pH 3) | | 10 mL MeOH | Rec = 86–103%, CF = 200 | LC–MS/MS | [66] |
| ROXI (+novobicin, atorvastatin) | Surface water, WWTP effluent (500 mL) | pH adjustment 4.0 (by $H_2SO_4$) | Oasis HLB | 6 mL acetone, 6 mL MeOH, 6 mL water (pH 4.0) | 10 mL water (pH 4.0) | 3× 2 mL MeOH | $Rec_{SW}$ = 93%, $Rec_{WWTP}$ = 89% | LC–MS/MS | [67] |
| TLS (+3 veterinary antibiotics) | Surface water (30 mL) | | Oasis HLB | 6 mL MeOH, 6 mL water | 4 mL water | 5 mL MeOH + 2% FAc | Rec = 87–121% | LC–MS/MS | [68] |

The pH of sample solution significantly influences the chemical form of analytes in samples, their stability and the interaction between the analyte and SPE cartridge packing material. Therefore, for the preparation of environmental water samples the knowledge of p$K_a$ values of analytes is the most important. Antibiotics from group of FQs, TCs, SAs and MLs have acidic and/or basic functional groups and therefore their ionization is controlled by solution pH. Most antibiotics are acidic substances; thus the acidification 2 units under p$K_a$ values of target analytes in water samples in order to obtain their neutral or acidic forms is required and allows the retention of these substances in the most commonly used SPE sorbent polymeric Oasis HLB columns, whereas the negatively charged organic matter usually present in natural samples can be retained in anionic exchange materials, which improves further retention of target compounds. In majority of multiresidue studies, the sample pH was adjusted in pH range 2.5–4 by sulphuric or hydrochloric acid. Some authors used sample of pH 6.0 or higher usually when MLs were included in the study together with other antibiotics or pharmaceuticals [22–27]. In some studies the best SPE recoveries were reported with no sample pH adjustment [28]. This could be somewhat strange, because each sample has different pH and thus the charge of the analytes differs. McClure and Wong [29] used Oasis HLB cartridges for the extraction of SAs, MLs and TMP from the wastewater samples without pH adjustment and supported that Oasis HLB cartridges were effective at neutral pH for the collection of those antibiotics from environmental waters. However, pH of wastewater samples could be even higher or lower than neutral pH. Thus the recovery could be different.

The following step during the sample pre-treatment is an addition of chelating agent. Environmental matrices contain many compounds including divalent or polyvalent cations. The antibiotics from the group of TCs, FQs and MLs form complexes with those ions. Therefore special precautions have to be taken. Above mentioned antibiotics have been found to be sorbed to the residual metals on SPE cartridges and glassware, resulting in irreversible binding to the cartridge and lowering recovery. To obtain sufficient recovery from the environmental matrices some chelator should be added [30,31]. Chelating agents such as EDTA, oxalic acid and citric acid are usually applied to decrease the tendency for antibiotics to bind to cations in the matrix, to improve peak shape and to prevent interferences during the extraction of antibiotics [22]. The addition of strong chelator EDTA to the sample prior to extraction is mostly utilized to chelate metals or multivalent cations (residual metal ions) that are sufficiently soluble in water. They may be present either in solution or sorbed on the surface of the sorbent. Another way of removing metals is washing them out of the cartridge using a solution of 0.5 M HCl during the precondition step [32,33].

One study refers a salt addition step during the determination of FQs, SAs and TMP in wastewaters [19]. In this case, an addition of 0.1 M NaCl improved antibiotic extraction efficiency, particularly for SAs and TMP. Although the amount of added salt was not sufficient to salt out the antibiotics, the presence of additional electrolytes appeared to facilitated sorption of the antibiotics to Oasis HLB cartridges.

In the most instances, the preconcentration and clean-up has been performed by SPE. SPE has been the mostly preferred technique, which replaced classical liquid-liquid extraction (LLE) and become the most common sample preparation technique in the environmental area. SPE offers some advantages over LLE such as improved selectivity, specificity and reproducibility, lower organic solvent consumption, shorter sample preparation time, and easier operation and the possibility of automation. Solid-phase microextraction (SPME) has been used in some cases [29,34,35].

From the point of view concentration of analytes, the achieved concentration factor is an important parameter. The mostly referred preconcentration factor of sample was 500 or 1000 (see Table 1).

**Table 2**
Extraction procedures utilized for the sample preparation during FQs analysis.

| Substances isolated | Matrix | Sample pretreatment | Sorbent type | Conditioning solvents | Washing solvent | Elution solvent | Recovery (%), concentration factor | Final analysis | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| CIPRO, DAN, ENO, ENRO, NOR, CINO, FLU, NAL, OXO | Surface water (250 mL) | pH adjustment 4.0 | $C_{18}$ disk cartridges | 2× 1 mL MeOH, 1 mL water, 1 ml 2 mM sodium acetate buffer (pH 4.0) | 3× 500 μL acetate buffer (pH 4) + 15% ACN | 3× 1 mL 6% ammonia/MeOH | Rec = 87–101%, CF = 1000 | LC–UV | [36] |
| CIPRO, DAN, DIF, ENRO, FLU, MAR, NAL, NOR, OXO, SARA | Surface water (250–500 mL) | pH adjustment 5.5 | Oasis HLB | 5 mL MeOH, 10 mL water, 5 mL AcAc/acetate buffer (pH 5.5) | 10 mL water | 2 mL 0.01 M NaOH-ACN (75:25) | Rec = 70–99%, CF = up to 250 | LC–FD | [50] |
| CIPRO, DIF, ENRO, LOME, NOR, OFLO, PIP, SARA, TOS | WWTP effluents, Surface water (150–500 mL) | pH adjustment 3.0 (by FAc) | MCP (mixed-phase cation exchange) cartridges | 8 mL MeOH, 8 mL water (pH 3.0) | | 4 mL 5% ammonia/MeOH | Rec = 75–107%, CF = up to 250 | LC–MS, LC–FD | [51] |
| OFLO, NOR, CIPRO | WWTP effluents (250 mL) | pH adjustment 3.0 (by HCl) | Oasis WCX | 4 mL MeOH, 10 mL water (pH 3) | 100 mL water (pH 3), 5 mL MeOH | 10 mL MeOH/ACN/FAc (20/75/5) | Rec = 87–94%, CF = 250 | LC–FD, LC–MS/MS | [52] |
| OFLO, NOR, CIPRO, ENRO | Wastewater | pH adjustment 4.5 (by $H_2SO_4$) addition EDTA | SAX, Oasis HLB | 2 mL MeOH, 2 mL citric acid (pH 4.0) | 2 mL citric acid (pH 4.0), 20 ml water (pH 4.2) | 4 mL MeOH | Rec = 96–114% | LC–FD | [53] |
| NOR, CIPRO, ENRO | Surface water | pH adjustment (by $H_2SO_4$) addition EDTA | Oasis HLB | 5 mL MeOH, 4 mL water | Water (pH 4.0) | 4 mL MeOH | Rec = 76.5–97.2% | LC–FD | [54] |
| CIPRO, ENRO, FLE, FLU, LOME, MOXI, NOR, OFLO, OXO | Surface water, Municipal wastewater, WWTP effluent (500 mL) | Addition EDTA, pH adjustment 4.2 (by AcAc) | Chromabond tetracycline | EtAc, MeOH, 0.2% EDTA (pH 4.2) | 5 mL water + 0.2% EDTA (pH 4.2) | 2 mL MeOH-water (75:25), 2 mL MeOH | Rec = 81.9–104.9%, CF = 1000 | LC–FD, LC–MS | [55] |

**Table 3**
Extraction procedures utilized for the sample preparation during SAs analysis included TMP.

| Substances isolated | Matrix | Sample pretreatment | Sorbent type | Conditioning solvents | Washing solvent | Elution solvent | Recovery (%), concentration factor | Final analysis | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| SMT, SMX, SMO, SPY, SDZ, SCP, SMR, SSM, SQX, SMP, SDT | Surface water (250 mL), drinking water (1 L) | | Oasis HLB | 6 mL MeOH, 6 mL water | 1 mL 5% MeOH | 10 mL MeOH + 50 mM FAc | Rec = 87–99%, CF = 1000, 4000 | LC–MS/MS | [3] |
| SMZ, SDT, SCP, SMO | Ground water (500 mL) | | Oasis HLB | 3 mL ACN, 3 mL water | | 2× 3 mL ACN | Rec = 51–113%, CF = 500 | LC–MS/MS | [6] |
| SGN, SCT, SDZ, SPY, SMR, SMZ, SDT, SSZ | Wastewaters (500 mL) | pH adjustment 3.0 (by $H_2SO_4$) | MCX sorbent phase | 5 mL water, 5 mL MeOH, 5 mL MeOH/5% NaOH, 5 mL water (pH 3.0) | 4 mL HCl, 5 mL MeOH | 2 mL MeOH/5% ammonia | Rec = 37.3–131%, CF = 2000 | LC–MS/MS | [34] |
| SCT, SDZ, SMX, STZ, SMR, SSX, SMT, SMZ, SMM, SMP, SCP, SDX, SDT, SM | Wastewater (500 mL) | | Oasis HLB | 3 mL MeOH, 3 mL water | 3 mL water | 2× 3 mL MeOH | Rec = 22.3–87.0%, CF = 500 | LC–MS/MS | [37] |
| SDZ, SCP, SMT, SQX, SDM, SMD | Surface water (250–500 mL) | pH adjustment 3.4 (by FAc buffer) | Oasis HLB | 5 mL MeOH, 5 mL FAc buffer pH 3.5 | 10 mL water | 1 mL ACN | Rec = 73–107% | LC–FD | [57] |
| SMP, SMO, SQX, SNT, SSM, SMX, SMR, STZ, SDZ, SMT, SDM, SDT, SPY, SSX, SCP, SM, TMP | WWTP influent (250 mL) and effluent (500 mL), River water (1 L) | Addition EDTA | Double SPE, Oasis HLB, Sep-Pak silica | 6 mL dichloromethane, 6 mL MeOH, 12 mL 50 mM EDTA, 4 mL hexane | 10 mL water, 3 mL hexane, 6 ml hexane/EtAc | 6 mL dichloromethane/MeOH (2:1), 3 mL MeOH/acetone (1:1), 3 mL acetone | Rec = 62–102%, CF = 500, 1000, 2000 | LC–MS/MS | [58] |
| SAD, SDZ, STZ, SMR, SMZ, SMP, SDT, SQX | Swine wastewater (150 mL) | | Oasis HLB | 3 mL MeOH, 3 mL 0.5 M HCl, 3 mL water | 1 mL water | 5 mL ammonia/MeOH (1:19) | Rec = 31.9–106%, CF = 300 | LC–UV | [64] |

**Table 4**
Extraction procedures utilized for the sample preparation during MLs analysis.

| Substances isolated | Matrix | Sample pretreatment | Sorbent type | Conditioning solvents | Washing solvent | Elution solvent | Recovery (%), concentration factor | Final analysis | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| CLAR, ROXI, ERY-$H_2O$, SPIR, TLS | Ground water, WWTP effluents (1 L) | pH adjustment 7.0 (by $H_2SO_4$ or NaOH) | LiChrolute EN, LiChrolute RP-18 | 3× 2 mL *n*-hexane, 3× 2 mL MeOH, 6× 2 mL water (pH 3.0) | | 5× 1 mL MeOH | $Rec_{GW}$ = 59–97%, $Rec_{WWTP}$ = 66–81% | LC–MS, LC–MS/MS | [5] |
| ERY-$H_2O$, TLS | WWTP influent (100 mL) and effluent (200 mL) | Addition EDTA, citric acid (pH 6.0), pH adjustment ~5.0 (by $H_2SO_4$) | Oasis HLB | 3 mL MeOH, 3 mL HCl, 3 mL water | 5 mL water | 5 mL MeOH | Rec = 87–101%, CF = 400, 1000 | LC–MS/MS | [9] |
| AZI, CLAR, ERY, ROXI, JOS | Surface water (250 mL) | pH adjustment 6.0 (by NaOH) | Oasis HLB | 5 mL ACN, 5 mL water | 10 mL water | 1 mL 10 mM AmAc (pH 6.0) + ACN (50:50) | $Rec_{AZI}$ = 65–75%, $Rec_{MLs}$ = 84–115%, CF = 250 | LC–MS, LC–MS/MS | [17] |
| ERY-$H_2O$, ROXI, TLS | Surface waters, WWTPs (120 mL) | Addition EDTA, pH adjustment 5.0 (by $H_2SO_4$) | Oasis HLB | 3 mL MeOH, 3 mL water | 3 mL water | 5 mL MeOH | $Rec_{SW}$ = 92–94%, $Rec_{WWTP}$ = 83–86%, CF = 1000 | LC–MS/MS | [30] |

According to the sample volume used, usually 500 or 1000 mL of surface or ground water. When the wastewater sample was analyzed, lower volumes can be used for preconcentration because antibiotics occur there at higher concentration levels. For example, in the evaluation of efficiency of treatment process in WWTP, only 50 or 100 mL of influent and 250 mL of effluents from WWTPs were used [7,24,29,33]. However, nowadays, the trend is using smaller amount of sample allowing fast sample preparation step and thus shorter time of analysis or even the direct injection of water sample on LC. However, there can be a problem with matrix interferences as wastewater samples are complex matrices.

In spite of this statement, SPE procedure is excellent for the extraction of analytes from aqueous matrix, their preconcentration and clean-up. Clean-up depends on the complexity of the sample matrix. It is known that the matrix compounds present in real samples may affect the interaction of analytes with the sorbent used in SPE processes. Matrix constituents may form complexes with the target compounds, preventing their interaction with the sorbent or, more frequently, matrix components (usually present at high concentration levels) interact with the sorbent reducing the number of free sites available for the retention of the analytes [36]. Another problem encountered in the extraction of antibiotics from wastewater is the matrix interference due to the high amount of organic matter in the samples. Organic matter reduces extraction efficiency and interferes with the detection [18]. The minimal matrix effects for SAs in wastewaters have been shown in one study [33].

Processing by SPE allows simultaneous extraction of multiple samples and generally gives good recovery of target compounds. In the scientific literature there are many multiresidual analytical methods describing the simultaneous analysis of antibiotics from different groups or also with the other pharmaceuticals (Table 1). The greatest difficulty in the multiresidue analysis concerns the choice of the best SPE adsorbent and it is obvious that the optimization of SPE conditions must lead to a compromise because the compounds are characterized by different physicochemical properties. The selection of experimental conditions in some cases does not yield to obtaining the best performance and recoveries for each compound. However, there are several disadvantages associated with SPE of pharmaceuticals from the environmental samples. SPE can be laborious and time-consuming, given large samples volumes (100–1000 mL per sample) and co-extraction of unwanted matrix components which are typically present at much higher concentration than the analytes of interest in matrices such as wastewater.

The selection of the most suitable SPE sorbent has to be done with the respect to the polarity of analytes and the sample matrix. Classical SPE sorbent chemistries range from the chemically bonded silica with the $C_8$ or $C_{18}$ organic group and ion-exchange materials to the polymeric materials. Silica based sorbents have several disadvantages compared to polymeric sorbents [37]. They are unstable in a broader pH range and contain free silanol groups, which are not suitable for the extraction of TCs because of their irreversible binding to the free silanol groups.

Among various types, Oasis HLB cartridges show the most robust recovery ratio and reproducibility for both polar and non-polar compounds and they are employed in the extraction due to their chemical composition containing the lipophilic divinylbenzene units and the hydrophilic *N*-vinylpyrrolidone units allowing working in wide range of pH (from pH 1 to 14). They do not contain free silanol groups to which many amphoteric pharmaceuticals can be strongly bound and thus cannot be eluted with the conventional organic solvents. They were used in majority of studies (see Table 1) and have been selected for the extraction of TCs and MLs since they are silanol free avoiding the antibiotics binding. Only two works used Oasis MCX (mixed cation exchange) cartridges [1,2]. In four works Strata X cartridges were employed for the extraction of SAs, MLs and pharmaceuticals from environmental waters [26,38–40].

Polymeric SPE sorbents (Oasis HLB and Isolute ENV+), non-polar $C_{18}$ and mixed polymeric and strong cation sorbent (Oasis MCX) cartridges were tested in one study [41]. As the study was performed at neutral pH, only for acidic compounds good recoveries were obtained, whereas basic and neutral compounds were poorly recovered using Oasis MCX cartridge. It could be explained that Oasis MCX is a mixed reversed phase-cation exchange cartridge and can efficiently extract acidic, basic and neutral compounds at low pH values, since the cation exchanger finds the basic compounds and the reversed phase can retain both acidic and neutral ones. In order to extract efficiently basic analytes, the samples should have been adjusted at low pH values. Polymeric sorbent Isolute ENV+ was effective only for few compounds as it is recommended for very polar organic compounds that are not retained on $C_8$ or $C_{18}$ phases. However it can also retain neutral compounds at neutral pH (including MLs) through hydrophobic interactions. Non-polar $C_{18}$ sorbent provided good results for the majority of the compounds. However, comparing the polymeric sorbent Oasis HLB to the other cartridges, Oasis HLB cartridges were much more efficient, yielding high recoveries for all target compounds. This sorbent can extract acidic, neutral and basic analytes at a wide range of pHs, including neutral pH. For this reason the sorbent can be suitable for the extraction of analytes when no sample pH adjustment is done.

Another option how to improve recovery and cleaning step during SPE is using tandem of two cartridges with different properties. Two kinds of cartridges were used for the extraction of many compounds from class of MLs, FQs, TCs, and SAs [42,43]. The first mentioned study used Oasis HLB cartridge on the top of SDB cartridge, the second study used the tandem Oasis HLB and MCX cartridges. Common procedure was that cartridges were conditioned separately, then connected and sample was passed through. The elution was done separately and the eluates combined and evaporated to the dryness. The tandem Oasis HLB and strong anion-exchange cartridges (SAX) has been employed for the determination of TCs, SAs and TMP in wastewaters [18]. The pH of sample was acidified to 2.5. As a result, the neutral and cationic forms of selected antibiotics were not retained on the SAX cartridge while humic acids and highly negatively charged organic matters were retained there. This improved further retention of the target antibiotics on Oasis HLB cartridges and following LC–MS analysis, where elevated baseline and severe matrix interferences were not shown as in case when only Oasis HLB catridges were used for the extraction. The elution of SAX yielded negligible amounts of antibiotics as well, confirming that the antibiotics were not retained on these cartridges. In addition, a visual inspection of SAX cartridges after the sample percolation showed that a significant amount of organic matter had accumulated in the sorbent.

The overview of extraction procedures used in multiresidue methods is shown in Table 1. In majority studies a washing step after sample percolation was performed. However, some authors do not refer washing step. This step is important in environmental area for washing impurities before the elution step. It is due to the following elution step when impurities can be eluted together with desirable analytes. The elution of cartridges is usually done by organic solvents such as MeOH (in majority of studies), acidified MeOH [4,19] or acetonitrile (ACN) [44–46]. The elution of cation-exchange cartridges is done by the mixture of MeOH and ammonia according to the specific sorbent and guidelines from manufacturer. Then the sample extracts are evaporated to dryness under a gentle stream of nitrogen and redissolved in mobile phase or in an appropriate solvent. They are stored in amber vials or dark glass to prevent photodegradation, especially of TCs, until injection onto the chromatographic system.

Only one study refers the use of Oasis HLB cartridges for SPE repeatedly [47]. After sample percolation and elution with methanol, the cartridge was rinsed with 10 mL of ACN and was

reused again. The same cartridge was used for extraction of 10 real samples or 20 standards.

Sample preparation step can be done through the on-line SPE connection or separately in off-line SPE configuration. For the determination of FQs, comparison of off-line and on-line SPE based on the use of $C_{18}$ and SAX sorbents, respectively, for the preconcentration and clean-up steps has been performed [11]. Both SPE extractions were coupled to HPLC–UV. In both cases the degree of preconcentration and clean-up achieved was very high, allowing the use of UV detector for the identification and quantification of the studied FQs by HPLC. The limits of detection (LODs) were almost the same for off-line SPE as for on-line SPE. SPE with LC–MS through the on-line connection has been used for the determination of SAs and TCs in wastewaters [48]. Coupling of SPE procedures on-line with LC provided several advantages, such as reduction of the number of sample handling steps required, elimination the target loss by keeping in the cartridge from drying which results in recovery improvement and saving of the analytical time and minimization of the consumption of organic solvents for each analysis.

SPME can be another option for the extraction of aqueous samples [29,34,35]. SPME is an extraction technique that uses a fused-silica fibre with a solid stationary phase that collects analytes of interest. The technique is based on the partitioning of the analyte between stationary phase and matrix. Upon exposure to a sample, sorption of compounds to the solid phase occurs, resulting in simultaneous extraction, clean-up, and pre-concentration. After equilibration, adsorbed analytes are desorbed into an organic solvent, followed by chromatographic analysis. Typically, SPME method development requires the optimization of the equilibration conditions for each compound which can make the development more difficult. SPME showed some advantages over SPE such as decreased sample volume, ease and efficiency of sample processing and extraction and in some cases better elimination of matrix effects, although matrix effects are highly dependent on the type of the sample. Concerning the cost, in SPME individual fibre can be used for multiple extractions and very little organic solvent is required. In contrast, SPE cartridges are one-time use only, significantly more solvent is necessary and high volumes of samples are percolated. However, regarding to the sensitivity and precision, SPE was found more sensitive and it showed better accuracy during extraction as well [29,34] which showed the preferable use of SPE for the extraction of complex matrices.

In-tube SPME coupled with LC–MS/MS was employed for the determination of five FQs in environmental waters [35]. The in-tube SPME, a technique using an open tubular fused-silica capillary with an inner surface coating as extraction device, is simple and can easily be coupled on-line with HPLC, LC/MS and LC/MS/MS. In-tube SPME allows convenient automation of the extraction process, which not only reduces the analysis time over SPE, but also can provide better accuracy, precision, and sensitivity than off-line manual techniques. Small amount of sample (1 mL in this study) was extracted without any pretreatment and analytes were easily desorbed from the capillary by passage of the mobile phase with good recoveries. This method showed higher sensitivity than the direct injection method, because the compounds in the sample solution were preconcentrated in the capillary column during draw/eject cycles. However, this type of sample extraction is demanding special instrumentation and experiences.

There was one study that used lyophilization, because it was fast and consumption of organic solvent was very low. It was used in combination with SPE to pre-concentrate SAs [49].

### 3.1. Multiresidual methods

The overview of extraction procedures in multiresidue methods is shown in Table 1. These methods usually deal with the antibiotics from the different classes and with pharmaceuticals in some cases as well. In majority of methods the sample pH was adjusted to acidic values in order to obtain required chemical form of analytes. In some cases, pH was adjusted to high values, especially when MLs were included [22,23,25–27].

For the preconcentration and clean-up Oasis HLB columns were employed in majority of studies. It is due to their properties enabling retention of wide spectrum of different compounds achieving their good recoveries. However, other types of sorbents were utilized as well as can be seen in Table 1. In three studies the tandem of two SPE cartridges was used to achieve better clean-up step and thus high recoveries [19,42,43]. Washing step was referred in majority of studies as is suitable for extraction of analytes from the complex matrices as wastewaters.

### 3.2. Quinolone antibiotics

The structure of molecules of Qs contains carboxylic group which makes all these compounds acidic. In addition, the second-generation FQs have an amino group in the heterocyclic ring (namely piperazinyl) (Fig. 1(a)). Thus, Qs can be divided into the two groups according to the acid–base properties: acidic and piperazinyl quinolones with the heterocyclic group. Acidic quinolones have only one p$K_a$ in range between 6.0 and 6.9. In acidic conditions they are in neutral form. In contrast, piperazinyl quinolones have two dissociation constants. The reported values of p$K_1$ and p$K_2$ are in the 5.5–6.3 and 7.6–8.5 range, respectively and thus, the intermediate form is a zwitterion. At acidic conditions they are in cationic form, which is important for their retention during the extraction. At basic conditions, the anionic species of both acidic and piperazinylic quinolones are less retained in comparison to cationic, zwitterionic and neutral species on the polymeric Oasis HBL column, but they may be retained on SAX cartridge. However, the different behaviour between both groups is observed at acidic pH [11,50]. The behaviour of Qs during SPE extraction was studied [36]. At acidic pH, the acidic quinolones, present as uncharged species in solution, were less retained on $C_{18}$ cartridge. However, the piperazinyl quinolones present in cationic form at acidic pH were retained well. It is important to take this fact into account as typically sample pH is adjusted to very acidic values, far from the p$K_a$ of the molecules, in order to ensure that they will be in the desired chemical form. However, very acidic pH may not be optimum for the preconcentration purposes [36]. Another possibility is the use of cation-exchange mechanism to retain the piperazinyl quinolones over a wide range of pH values. FQs can be extracted using cation-exchange sorbents when they are in cationic form which means the pH of sample has to be below their p$K_a$ constants (2 units below the p$K_a$ constants $\leq$ 3.0) [51,52].

The overview of procedures used during the sample preparation for Qs is showed in Table 2. During the pretreatment of sample the most of authors adjust the pH of sample in the range 2.8–4.0 or 4.5 to convert the FQs into a cationic form. The best pH value to assure that FQs are in cationic form should be two units below the p$K_a$ constants. Only in one study, pH of water sample was adjusted pH 5.5 [50].

Following step in the sample preparation of Qs is an addition of chelator EDTA. FQs can be bound to divalent cations and thus could not be effectively retained on SPE cartridges and determined. However, the addition of EDTA is more important for the preparation of soil samples than water samples in the environmental analysis.

The mostly used SPE cartridges were polymeric Oasis HBL [50,53,54]. However, other types including cation-exchange [51,52], $C_{18}$ [36] and Chromabond tetracycline [55] were used. A tandem system using an anion-exchange cartridge on the top together with Oasis HLB was also described [53]. The anion-exchange column was used for pre-purification since humic acids and others

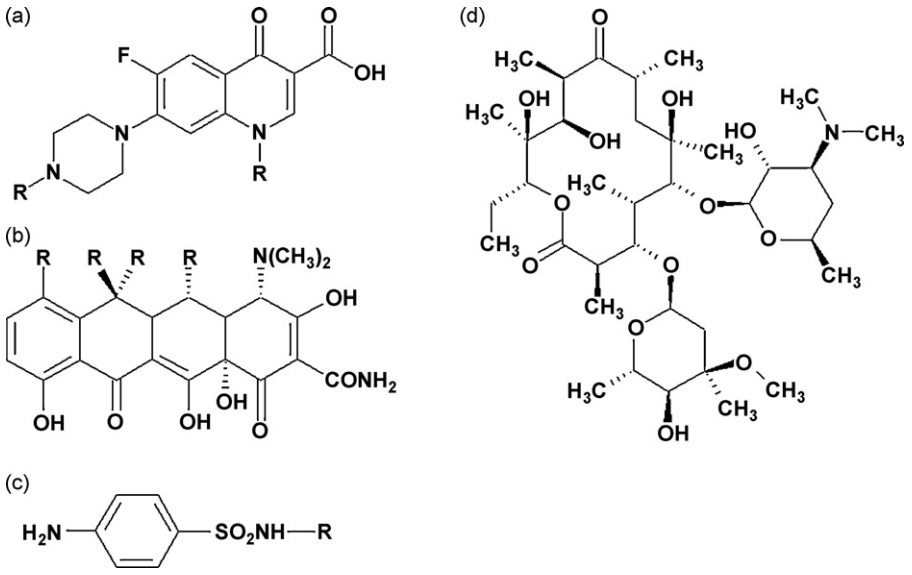


**Fig. 1.** General structures of selected groups of antibiotics included in this review: (a) fluoroquinolone, (b) tetracycline, (c) sulfonamide antibiotics, and (d) macrolide antibiotic erythromycin.

impurities were retained. Thus less impurity got onto Oasis HLB cartridge improving the clean-up procedure. As a result, a better clean-up step with less interference during the following analysis was achieved.

Different sorbents ($C_{18}$, SDB, SDP-RPS and MPC) were evaluated for the preconcentration of FQs in surface water [36]. It was concluded that the use of $C_{18}$ cartridges was preferable with the sample pH value 4. These results are in accordance with results of Ferdig et al. [56]. The best results were obtained with Chromabond Tetracycline $C_{18}$ modified silica in spite of these columns being recommended for the preconcentration of residues of TCs. The results from this cartridge were closely followed by results obtained on Oasis HLB [55,56].

Not in all studies, a concentration factor was referred. In one study following analysis was performed by LC–UV and concentration factor referred was 1000 [36]. This is sufficient preconcentration of the analytes for this kind of detection.

### 3.3. Tetracycline antibiotics

Concerning TCs none of the study dealed only with this group of antibiotics. They were involved in multiresidue studies together with other antibiotics or even pharmaceuticals. Despite of this, short chapter dealing with their properties is included. TCs contain hydronaphatacene backbone in their structure composed of four fused rings (Fig. 1(b)). Various analogues differ primarily by the different substitution. TCs show three p$K_a$ values of approximately 3, 7 and 9. Throughout the range of pH, TCs always possess a local charge, and they are zwitterionic in the approximate range of pH 3–9. Thus pH adjustment is very important step in the analysis of TCs. The pH is usually adjusted to value $\leq$3 to assume that TCs will be in cationic form which is important for their optimal extraction. The best it is to perform pH adjustment immediately before extraction because they are no longer stable in acidic media.

TCs also tend to form complexes with divalent metal ions. This complexation can prevent effective extraction and has an effect on the spectral characteristics of TCs. TCs are amphoteric and most of them have a strong tendency to be bound irreversibly to the silanol groups in silica based stationary phases resulting in peak tailing. Addition of EDTA was performed in almost all studies to prevent TCs to be bound to divalent ions and thus the recovery of extraction to be increased. Another option how to improve their recovery was using Oasis HLB cartridges. They do not contain silanol groups thus TCs cannot be bound there irreversibly and the use of Oasis HLB leads to high recoveries.

### 3.4. Sulfonamide antibiotics and trimethoprim

Table 3 is showing the recent procedures for SAs extraction from water samples in studies dealing only with the SAs determination. All of studies used Oasis HLB columns except one using cation-exchange sorbent [34]. SAs contain one basic amine group ($-NH_2$) and one acidic sulfonamide group ($-SO_2NH-$) (Fig. 1(c)). They are ampholytes with weakly basic and acidic characteristics. It is explained by the charge state of the SAs at the particular pH values because of their p$K_a$ values. The p$K_{a1}$ (2–2.5) and p$K_{a2}$ (5–8) correspond to the protonation of the aniline group and deprotonation of the sulfonylamido group, respectively. Weakly basic characteristics arise from the nitrogen of the anilinic substituent which is able to gain a proton, designated for protonation during ionization step of mass spectrometric detection, whereas the acidic characteristics arise from the N–H linkage of the sulfoamidic group which is able to release proton under specific pH conditions. Thus SAs are positively charged at acidic conditions at pH 2, neutral between pH 2 and 5, and negatively charged at alkaline conditions at pH above 5. Only two studies adjusted pH of sample to pH 3.0 or 3.4 respectively [34,57] and in the rest of studies pH adjustment was not reported. This is not usual when SPE and the interaction between the analytes and the sorbent of SPE columns are pH dependent. The interaction with the cartridge material is stronger for analytes in uncharged forms. Mostly, the sample pH was adjusted to value about 3.0, in range 2.0–4.0 in multiresidue methods as it can be seen in Table 1. This step led to good recovery rates which showed that pH adjustment of sample was very important and it was in agreement with their p$K_a$ values.

Only one method referred the addition of EDTA to water sample [58]. This step was of no importance because SAs do not form complexes with divalent and polyvalent cations. Thus, during sample preparation for SAs determination it was not necessary to add EDTA.

As was already said above, majority of studies for the determination of SAs used Oasis HLB columns for their extraction from water samples (Table 3). All methods referred washing step after sample percolation through the SPE columns which was suitable in the

environmental analysis to remove interferences. In all cases water was used, except one study where 5% MeOH was used [3]. Elution of cartridges was done by organic solvent, but 5% MeOH was so weak that it did not manage to elute desirable analytes and thus can be used during the washing step.

Concerning the concentration factors, in majority of studies MS/MS detection was used. As it is very sensitive technique high concentration factor is not necessary. However in one study UV detection was used for the SAs and concentration factor was only 300. This method was applied for the detection of SAs in swine wastewater and as the UV detection was not very sensitive one could doubt if this preconcentration was satisfactory [64].

### 3.5. Macrolide antibiotics

Macrolide antibiotics contain a basic dimethylamine [$-N(CH_3)_2$] group, which is able to gain a proton (Fig. 1(d)). Thus, according to their chemical structure, MLs are basic compounds with p$K_a$ values around 8. It was shown that their retention on reversed-phase cartridges was not pH dependent in the range 3–7 [9]. It should be noted that, in some cases, acidic pH values can promote the degradation of ERY. At pH bellow 7.0, ERY is immediately converted into its main degradation product ERY-$H_2O$ and ERY is not detected in its original form but as a degradation product (ERY-$H_2O$) with an apparent loss of one molecule of water. Thus, ERY-$H_2O$ is very often quantified in many studies [59], assuming that ERY is totally converted into ERY-$H_2O$ in SPE procedures. Additionally, since the oral administration of ERY has to pass through strongly acidic conditions in the stomach, the degraded product ERY-$H_2O$, does not exhibit the original antibiotic properties [30].

Only four studies deal with the determination of MLs alone (Table 4). Considering the recoveries of the MLs, and the sensitivity and selectivity for ERY-$H_2O$ in the acidic elution gradient, pH of sample was adjusted to higher values in range 5.0–7.0. Abuin et al. [17] showed the non-dependence of MLs in the range of pH 6–8. Addition of EDTA was performed in three studies. It is recommended as it was shown that MLs can bind divalent and polyvalent cations although they bind not so strong as TCs or FQs. The extraction was performed with polymeric Oasis HLB columns except one study using LiChrolute RP-18 sorbent [5].

An overview of extraction methods is in Table 4. The washing step was performed with water in three studies. The other study does not report the washing step although WWTPs effluents were analyzed [5]. It can be seen from recovery achieved that washing step was necessary. It can be due to the subsequent analysis by HPLC–MS because ESI source is highly susceptible to matrix interferences and thus some matrix effects could occur.

In case of MLs it can be shown that using sensitive LC–MS/MS detection, smaller amount of sample can be used for analysis of surface water samples, e.g. 250 mL [17] or 120 mL [30].

## 4. Analytical methods

There is a need for the development of analytical methods for sensitive and selective identification and quantification of antibiotics as environmental contaminations. LC–MS/MS is indicated as the technique of choice to assay relatively polar pharmaceuticals and their metabolites as it is especially suitable for the environmental analysis because of its selectivity comparing to UV [11,36] or FD [50,53,57].

In general, LC–MS can be used for quantitative purposes only when the analyte is present in simple matrices, such as tap water and bottled water, whereas LC–MS/MS is required for quantitation with simultaneous confirmation of identity of residues in complex matrices such as wastewaters [60].

### 4.1. Quantification by LC–MS/MS

In majority studies MS detection is used to identify and to quantify the substance or it can be used to confirm its molecular structure (Table 5). LC–MS/MS is often applied using triple quadrupole analyser and selected reaction monitoring (SRM) mode. This mode allows compound confirmation and providing structural information. In MS/MS, the most intensive fragment ion from precursor ion is used for quantification (transition 1). A less sensitive secondary transition is used as the second criterion for the confirmation purposes (transition 2). This mode also improves the precision and sensitivity of the analysis but does not collect full scan data. This can limit the availability of full scan data that can be used not only to the identify target analytes but also to detect additional unknown compounds. This can be good in searching of stable metabolites of antibiotics in waters that can be detected instead of parent compounds. The first step in the tandem MS detection is the selection of the precursor ion. The protonated molecular ions $[M+H]^+$ are generally considered to be the best precursor ions as can be seen in Table 5, except macrolide antibiotic ERY.

#### 4.1.1. Chromatography

Mostly, $C_{18}$ analytical columns were used for the separation of analytes (Table 5). Only one multiresidue study used $C_{12}$ analytical column [18] and in one study $C_8$ analytical column was used for the separation of FQs [43]. Typically, mixtures of ACN or MeOH with water were used as mobile phases for the LC separation. Gradient elution was used in all multiresidue studies reported. In attempt to improve the ionization of analytes and sensitivity of MS detection in the analysis of antibiotics, modification of mobile phase was usually performed and has been accomplished with volatile additives such as formic acid (FAc), acetic acid (AcAc), and ammonium acetate (AmAc) at different concentration. Babić et al. [12] used oxalic acid and ACN in mobile phase for the determination of antibiotics in wastewaters. This was possible due to UV detection and thus nonvolatile modificator could be used.

UHPLC was used for the determination of compounds from different classes of pharmaceuticals including 5 antibiotics (chromatograms are shown in Figs. 2 and 3) [1,16]. UHPLC is a modern technique, using columns packed with sub-2 μm particles, which enabled elution of sample components in much narrower, more concentrated bands, resulting in better chromatographic resolution and increased peak capacity through rapid elution from short column. The speed provided by the UHPLC system in comparison to conventional HPLC system using 5 μm particles was compared. The reduction of analysis time was substantial because of the low dead volume of the whole system allowing short equilibration times (less than 1 min between the end of the gradient and the next injection) and reducing therefore the unproductive parts of chromatogram.

#### 4.1.2. Mass spectrometry – ionization

For LC–MS and LC–MS/MS analysis of pharmaceuticals, two ionization interfaces has been the most widely used due to their sensitivity and robustness. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) satisfied the requirements. They produce protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecules. Both techniques work at atmospheric pressure which is suitable for the connection with LC system. ESI as a soft ionization technique is preferred in antibiotic residue determination due to its higher sensitivity and better reproducibility, since it is particularly suitable for both polar and non-polar analytes and for thermally labile substances. Positive electrospray ionization ($ESI^+$) was often preferred when both positive and negative ionization were possible as it can be seen in Table 5. Many antibiotic compounds are nonvolatile with high molecular weights and they respond well in $ESI^+$ which makes LC–MS or LC–MS/MS the

**Table 5**
HPLC analytical methods in multiresidue studies for the determination of different groups of antibiotics together with pharmaceuticals.

| Substances determined | Matrix sample preparation | Stationary phase, analytical column | Mobile phase | Detection | Precursor ions | Internal standard | Sensitivity LOD/LOQ | Ref. |
|---|---|---|---|---|---|---|---|---|
| CIPRO, DOXY, SMX, TMP, ERY (+28 pharmaceuticals) | Surface water, SPE | Acquity UPLC BEH $C_{18}$ (100 mm × 1 mm, 1.7 μm) (22 °C) | Gradient elution, A: water + MeOH + 0.5% AcAc, B: MeOH + 0.5% AcAc | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | Phenacetin-ethoxy-1-$^{13}C$ | LOD = 0.1–0.5 ng $L^{-1}$, LOQ = 0.5–1.5 ng $L^{-1}$ | [1] |
| SMX, ERY (+13 pharmaceuticals) | Surface, drinking, ground water, SPE | XTerra RP-18 (100 mm × 2.1 mm, 3.5 μm) (35 °C) | Gradient elution, A: 2 mM AmAc/MeOH, B: 2 mM AmAc/water | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | SDT | LOQ = 10–13 ng $L^{-1}$ | [2] |
| CIPRO, NOR, ENRO, SARA, PIP, OXO, FLU, MINO, OXY, TET, DEME, CTC, DOXY, STZ, SMR, SMZ, SMT, SCP, SMX, SDT, TMP, ERY, ROXI, TLS | Drinking water, SPE | Pursuit C-18 (150 mm × 2 mm, 3 μm) | Gradient elution, A: 0.1% FAc + water, B: ACN | $ESI^+$, MS–MS | $[M+H]^+$ | Simatone, $^{13}C_6$-SMZ | LOD = 0.5–6 ng $L^{-1}$, LOQ = 1–32 ng $L^{-1}$ | [4] |
| SDZ, STZ, SMZ, SPY, SMX, $N^4$-acetyl-SMX, TMP, AZI, CLAR, ERY, ROXI | WWTP effluent, SPE | YMC Pro $C_{18}$ (150 mm × 2.0 mm, 3 μm) (30 °C) | Gradient elution, A: water + 1% FAc (pH 2.1), B: MeOH + 1% FAc | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | SMR, TLS, JOSA | LOQ = 1–214 ng $L^{-1}$ | [7] |
| ERY, CLAR, ROXI (+hormones) | WWTP influents and effluents, SPE, SEC | Phenosphere-next $RP_{18}$ (150 mm × 2.0 mm, 3 μm) (25 °C) | Gradient elution, A: 10 mM AmAc, B: ACN | APCI, MS/MS, SRM | | (E)-9-[-O-methyloxime)]-ERY | LOQ = 2–6 ng $L^{-1}$ | [8] |
| CIPRO, NOR, OFLO, DOXY, SMX, TMP (+β-lactams) | Hospital wastewater, SPE | YMC Hydrosphere $C_{18}$ (150 mm × 4.6 mm, 5 μm) (25 °C) | Gradient elution, A: water + 0.1% FAc, B: ACN + 0.1% FAc | $ESI^+$, MS–MS, SIM | $[M+H]^+$ | ENRO, DEME, SMZ, Diaverine | LOQ = 0.01–0.68 ng inj. | [10] |
| ENRO, OXY, SMZ, SDZ, SGN, TMP (+β-lactams) | Wastewater, SPE | LiChrosphere 100CN (125 mm × 4.0 mm, 5 μm) (30 °C) | Gradient elution, A: 0.01 M oxalic acid, B: ACN | DAD, 280 nm | | | LOD = 0.1–40 μg $L^{-1}$, LOQ = 1.5–60 μg $L^{-1}$ | [12] |
| SMX, TMP, ERY-$H_2O$ (+27 pharmaceuticals) | Surface water, SPE | Synergi Max-RP C12 (250 mm × 4.6 mm, 4 μm) | Gradient elution, A: 0.1% FAc in water, B: MeOH | $ESI^+$, MS/MS | $[M+H]^+$, $[M+H\text{-}H_2O]^+$ | $[^{13}C_1]$-ERY | LOD = 1 ng $L^{-1}$ | [18] |
| CIPRO, ENRO, NOR, OFLO, SMZ, SMX, TMP | WWTP effluents ($2^{nd}$, $3^{rd}$) SPE | Zorbax SB-$C_{18}$ (150 mm × 2.1 mm, 5 μm) (30 °C) | Gradient elution, A: 1 mM AmAc + 0.007% AcAc + 10% ACN, B: ACN | $ESI^+$, MS, SIM | $[M+H]^+$ | LOME, SMR | LOD = 20–90 ng $-^1$ | [19] |
| OXY, CTC, MINO, DEME, MECLO, TET, DOXY, STZ, SMX, SMR, SCP, SMZ, SDT, ERY, ROXI, TLS | River water, SPE | Xterra MS $C_{18}$ (50 × 2.1 mm, 2.5 μm) (15 °C) (TCs, SAs) (45 °C) (MLs) | Gradient elution, A: 0.1% FAc + water (pH 2.74), B: 0.1% FAc + ACN | $ESI^+$, MS–MS, SRM | $[M+H]^+$, $[M+H\text{-}H_2O]^+$ | Simatone | LOQ = 10 ng $L^{-1}$ | [21] |
| CIPRO, ENRO, NOR, OFLO, OXO, PIP, CTC, DOXY, OXY, TET, 16 SAs, CLAR, ERY, ROXI | WWTP final effluents SPE | Genesis $C_{18}$ (150 mm × 2.1 mm, 3 μm) | Gradient elution, A: ACN, B: 20 mM AmAc (0.1% FAc, pH 4.0) (FQs, TCs, SAs), B: 20 mM AmAc (0.05% FAc, pH 4.0) (MLs) | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | | LOD = 1–8 ng $L^{-1}$ | [22] |
| CIPRO, CTC, TET, SDT, SMZ, SMT, SMX, STZ, SSX, CLAR (+20 pharmaceuticals) | WWTP influent and effluent, SPE | Lichrosphere RP-18 (150 mm × 3 mm, 5 μm) (FQs, SAs, MLs), Genesis $C_{18}$ (150 mm × 2.1 mm, 3 μm) (TCs) | Gradient elution, A: 0.1% FAc in water, B: 0.1% FAc in ACN | $ESI^+$, MS–MS | | | LOD = 0.42–8.11 ng $L^{-1}$ | [23] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| OFLO, NOR, CIPRO (+5 pharmaceuticals) | Groundwater, surface water, WWTP influent and effluent, SPE | Zorbax XDB-$C_{18}$ (50 mm × 2.1 mm, 5 μm) (30 °C) | Gradient elution, A: 1% AcAc, B: ACN | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | ENRO | LOQ = 1.6–163 ng $L^{-1}$ | [24] |
| CTC, DOXY, MECLO, OXY, TET, SCP, SDT, SMR, SMZ, STZ, TMP, ERY, ROXI, TLS (+pharmaceuticals) | Surface water, SPE | Genesis column (150 mm × 2.1 mm) (25 °C) | Gradient elution, A: 0.015% heptafluorobutyric acid + 0.5 mM AmAc + 25% MeOH, B: ACN | $ESI^+$, MS–MS, SRM | | $^{13}C_6$-sulfamethazine phenyl | LOD = 30–600 ng $L^{-1}$, LOQ = 0.1–60 ng $L^{-1}$ | [25] |
| SMX, TMP, ERY (+13 pharmaceuticals) | Surface water, wastewater, SPE | Luna $C_{18}$ (250 mm × 10 mm, 10 μm) | Gradient elution, A: water, B: MeOH, C: 10 mM AmAc, D: 0.87 M AcAc | $ESI^+$, MS–MS, SRM | | | LOD = 0.07–0.13 ng $L^{-1}$, LOQ = 0.22–0.43 ng $L^{-1}$ | [26] |
| TMP, ERY (+16 pharmaceuticals) | Hospital wastewaters, SPE | Purospher Star RP-18 (125 mm × 2.0 mm, 5 μm) | Gradient elution, A: ACN, B: 0.1% FAc | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | $^{13}$C-phenacetin, 2–4-$Dd^5$ | LOD = 3.8–9.2 ng $L^{-1}$, LOQ = 11–26 ng $L^{-1}$ | [27] |
| SMX, TMP (+22 pharmaceuticals) | Surface water, ground water, SPE | Metasil basic (150 mm × 2.0 mm, 3 μm) | Gradient elution, A: 10 mM ammonium-formate/FAc buffer (pH 3.7), B: ACN | $ESI^+$, MS, SIM | | [$^{13}$C]-caffeine, [$^{13}$C]-phenacetin | LOD = 14–23 ng $L^{-1}$ | [28] |
| SPY, SMX, SSX, SMZ, SDT, TMP, ERY, CLAR, ROXI | WWTP influent and effluent SPME, SPE | Zorbax Eclipse XDB $C_{18}$ (250 mm × 4.6 mm, 5 μm), Ultra C18 (250 mm × 4.6 mm, 5 μm) | Gradient elution, A: ACN, B: 10mM AmAc/0.1% FAc/10% ACN | $ESI^+$, MS–MS, SRM | | $^{13}C_6$-SMZ, JOSA | $LOD_{SPE}$ = 0.08–6.1 ng $L^{-1}$, $LOQ_{SPE}$ = 0.27–20 ng $L^{-1}$, $LOD_{SPME}$ = 2.8–410 ng $L^{-1}$, $LOQ_{SPME}$ = 9.2–1380 ng $L^{-1}$ | [29] |
| OXY, CTC, TET, DEME, DOXY, MECL, MINO, STZ, SMR, SMZ, SCP, SMX, SDT | Surface waters, SPE | XTerra MS $C_{18}$ (50 mm × 2.1 mm, 2.5 μm) (15 °C) | Gradient elution, A: water + 0.1% FAc, B: ACN + 0.1% FAc | UV, 360 nm (TCs), 260 nm (SAs) $ESI^+$, MS–MS, SRM | $[M+H]^+$ | Simatone | LOD = 30–50 ng $L^{-1}$ | [32] |
| CTC, DEME, DOXY, MECL, OXY, TET, STZ, SMZ, SCP, SMY, SDT | WWTP influent and effluent, SPE | XTerra MS $C_{18}$ (50 mm × 2.1 mm, 2.5 μm) (15 °C) | Gradient elution, A: water + 0.1% FAc, B: ACN + 0.1% FAc | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | Simatone | $LOD_{INF}$ = 40–70 ng $L^{-1}$, $LOD_{EFF}$ = 30–50 ng $L^{-1}$ | [33] |
| SMX, acetyl-SMX, TMP, ERY (+13 pharm) | Surface water, sewage effluents, SPE | $C_{18}$ Luna (250 mm × 2 mm, 5 μm) | Gradient elution, A: 40 mM AmAc (pH 5.5 by FAc), B: MeOH, C: water | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | [$^{13}$C]-phenacetin | LOD = 10–50 ng $L^{-1}$ | [38,40] |
| SMX, TMP (+20 pharmaceuticals) | WWTP influent and effluent, SPE | Sunfire $C_{18}$ (150 mm × 4.6 mm, 3.5 μm), Sunfire $C_{18}$ narrow bore (50 mm × 2.1 mm, 3.55 μm) | Gradient elution, A: 0.1% AmAc in water (pH 6.2), B: 0.1% AmAc in ACN, A: 20:80 ACN:water + 0.1% AmAc, B: 80:20 ACN:water + 0.1% AmAc | UV, 270 nm, $ESI^-{}_{SMX}$, $ESI^+{}_{TMP}$, MS–MS | $[M-H]^-$, $[M+H]^+$ | | $LOD_{influent}$ = 72–171 ng $L^{-1}$, $LOQ_{influent}$ = 241–570 ng $L^{-1}$, $LOD_{effluent}$ = 20–166 ng $L^{-1}$, $LOQ_{effluent}$ = 67–553 ng $L^{-1}$ | [39] |
| OFLO, SMX, TMP, ERY, AZI (+27 pharmaceuticals) | Ground waters, WWTP influents and effluents, SPE | Purospher Star RP-18 (125 mm × 2.0 mm, 5 μm) | Gradient elution, A: ACN + MeOH (2:1), B: 5 mM AmAc/AcAc buffer, pH 4.7 | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | $^{13}$C-phenacetin | LOD = 1–43 ng $L^{-1}$, LOQ = 3–120 ng $L^{-1}$ | [41] |
| CIPRO, OFLO, CTC, DOXY, OXY, TET, SDM, SMX, TMP, AZI, CLIN, CLAR, ERY, ROXI, SPIR, TLS, VAN (+β-lactams) | Surface water, SPE | Phenomenex SYNERGI Hydro-RP C18, 4 μm | Gradient elution not specified | $ESI^+$, MS–MS | $[M+H]^+$ | | LOQ = 0.5–30 ng $L^{-1}$ | [42] |

Table 5 (*Continued*)

| Substances determined | Matrix sample preparation | Stationary phase, analytical column | Mobile phase | Detection | Precursor ions | Internal standard | Sensitivity LOD/LOQ | Ref. |
|---|---|---|---|---|---|---|---|---|
| CIPRO, ENRO, NOR, SARA CTC, DOXY, OXY, TET, SMT, STZ, SMR, SMZ, SCP, SMX, SDT, TMP, ERY-$H_2O$, ROXI, TLS | Ground water, WWTP influent and effluent, SPE | Luna $C_8$ (150 mm × 3.0 mm, 3 μm) (FQs), MS Xterra $C_{18}$ (150 mm × 3.0 mm, 3.5 μm) (TCs), Luna phenylhexyl (150 mm × 3.0 mm, 3.5 μm) (SAs, MLs) | Gradient elution, A: 20 mM ammonium-formate + 0.3% FAc + MeOH, B: MeOH + 20 mM ammonium-formate + 0.5% FAc, Gradient elution, A: 20 mM AmAc (pH 6.5) + ACN, B: 20/80 A/ACN (SAs, MLs) | $ESI^+$, MS | $[M+H]^+$ | $^{13}C_6$-SMZ, [$^{13}$C]-ERY-$H_2O$, MECLO, terbuthylazine | $LOQ_{SW}$ = 10–50 ng $L^{-1}$, $LOQ_{WWTP}$ = 50–100 ng $L^{-1}$ | [43] |
| CIPRO, ENRO, TET, OXY, CTC, SMZ, SDT, SMX, TMP, CLIN, ERY, ROXI, TLS | Surface, ground water, WWTP effluent, SPE | Beta-Basic-18 $C_{18}$ (100 mm × 2.1 mm, 3 μm) (30 °C) | Gradient elution, A: ACN, B: MeOH, C: water + 0.3% FAc | $ESI^+$, MS-MS, SRM | $[M+H]^+$ | $^{13}C_6$-SMZ | LOD = 27–190 ng $L^{(1}$, LOQ = 100–650 ng $L^{(1}$ | [44] |
| NOR, TET, TMP, ERY-$H_2O$ (+β-lactams) | Surface water, SPE | XBridge $C_{18}$ (50 mm × 2.1 mm, 5 μm) | Gradient elution, A: 0.01% FAc in water, B: MeOH | $ESI^+$, MS–MS | | | LOD = 2–13 ng $L^{-1}$ | [45] |
| OXO, SDZ, TMP | Surface water, SPE | Luna $C_{18}$ (150 mm × 4.6 mm, 5 μm) (25 °C) | Gradient elution, A: 10% (v/v) MeOH + 0.1% FAc, B: 90% (v/v) MeOH + 0.1% FAc | $APCI^+$, MS–MS, SRM | $[M+H]^+$ | | LOD = 1–2 ng $L^{-1}$ | [46] |
| CIPRO, ENRO, TET, OXY, DOXY, CTC | River water, WWTP influent and effluent, SPE | Kromasil 100 $C_{18}$ (250 mm × 4.6 mm, 5 μm) (35 °C) | Gradient elution, A: water + 1% AcAc (pH 2.8), B: ACN | $ESI^+$, MS, SIM | $[M+H]^+$ | | LOD = 4–6 ng $L^{-1}$ | [47] |
| OXY, MINO, DOXY, DEME, MECLO, CTC, TET, SDT, SMX, STZ, SCP, SMR, SMZ, SMM | Surface water, wastewater, on-line SPE | Atlantis d$C_{18}$ (50 mm × 2.1 mm, 3 μm) | Gradient elution, A: 0.1% FAc in water, B: 0.1% FAc in ACN | DAD, $API^+$, MS | $[M+H]^+$ | Simatone | LOD = 30–110 ng $L^{-1}$ | [48] |
| CIPRO, NOR, OFLO, DOXY, SMX, TMP, ERY-$H_2O$ (+β-lactams) | WWTP effluent, SPE | YMC Hydrosphere $C_{18}$ (150 mm × 4.6 mm, 5 μm) (25 °C) | Gradient elution, A: water + 0.1% FAc, B: ACN + 0.1% FAc | $ESI^+$, MS–MS, SRM | $[M+H]^+$, $[M+H-H_2O]^+$ | ENRO, DEME, SMZ, Diaverine | LOQ = 6–160 ng $L^{-1}$ | [59] |
| OFLO, SMX, TMP, AZI, ERY (+29 pharmaceuticals) | River water, WWTP influent and effluent, SPE | Acquity UPLC BEH $C_{18}$ (50 mm × 2.1 mm, 1.7 μm) | Gradient elution, A: 5 mM AmAc/AcAc (pH 4.8) B: ACN + MeOH (2:1) | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | $^{13}$C-phenacetin | $LOD_{influent}$ = 10–500 ng $L^{-1}$ | [61] |
| CIPRO, NOR, LOME, LEV, GAT, SPAR, MOXI, SMX, TMP (β-lactams) | Surface water, SPE | Inertsil ODS-3V $C_{18}$ (250 mm × 4.6 mm, 5 μm) | Gradient elution, A: 0.1% TFA, B: ACN | PDA, 280 nm, $ESI^+$, MS, SIM | $[M+H]^+$ | | LOD = 0.6–8.1 μg $L^{-1}$, LOQ = 2.7–24 μg $L^{-1}$ | [66] |
| ROXI (+novobicin, atorvastatin) | Surface water, WWTP effluent, SPE | Microbore column YMC ODS-AQ (100 mm × 1.0 mm, 3 μm) | Gradient elution, A: ACN, B: 10 mM AmAc | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | | IDL = 3 pg | [67] |
| TLS (3 veterinary antibiotics) | Surface water, SPE | Thermo Hypersil Gold (50 mm × 2.1 mm, 5 μm) | Gradient elution, A: ACN + 20 mM heptafluorobutyric acid, B: water + 20 mM heptafluorobutyric acid | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | | IQL = 10 g $L^{-1}$, LOQ = 35 ng $L^{-1}$ | [68] |
| OXO, NAL, FLU, MAR, OFLO, ENRO, PEFL, CIPRO, PIP, NOR (+β-lactams) | Groud and surface water, on-line SPE | Kromasil C-18 (100 mm × 2.1 mm, 5 μm) | Gradient elution, A: water + 0.1% FAc, B: MeOH + 0.1% FAc | $ESI^+$, MS–MS, SRM | $[M+H]^+$ | | | [69] |

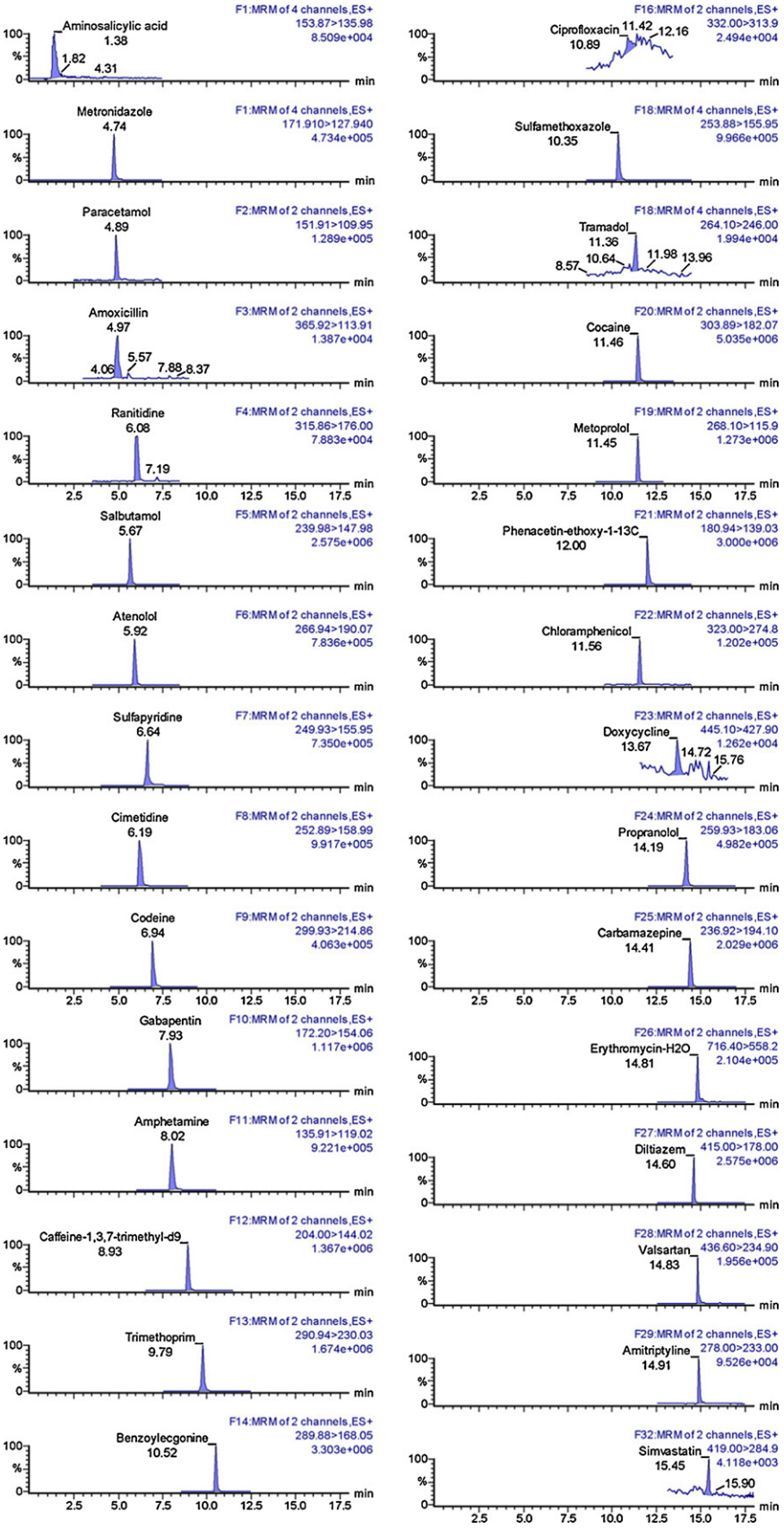


**Fig. 2.** UHPLC–MS/MS separations for chosen pharmaceuticals spiked into water and extracted by SPE (concentration of pharmaceuticals, 100 ng $L^{-1}$; IS, 200 ng $L^{-1}$) (antibiotics included – CIPRO, SMX, DOXY, ERY-$H_2O$, TMP). Reprinted from [1] - copyright Elsevier.

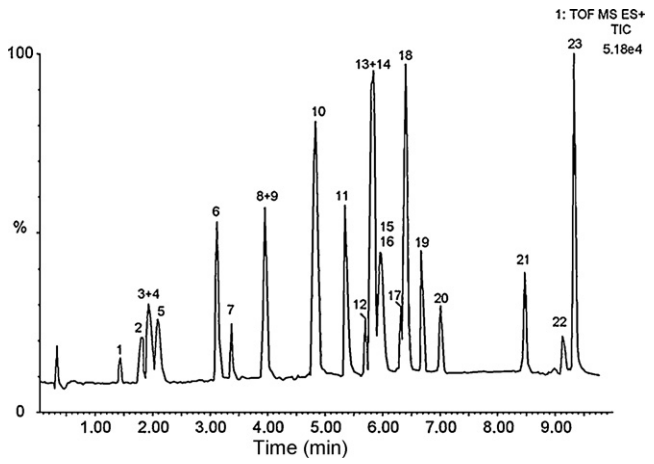


**Fig. 3.** UPLC–TOF total ion chromatogram showing the separation of 23 pharmaceutical compounds (analgesics, beta-blockers, lipid regulators, antiulcer agents, antihistaminicsm, psychiatric drugs and antibiotics ERY, AZI, SMX, TMP, OFLO) analyzed in positive ion mode (100 ng mL$^{-1}$ standard solution). Reprinted from [61] - copyright Elsevier.

technique of choice for their separation and analysis. Only in two multiresidue studies APCI ionization technique is referred in comparison to the rest of studies using ESI for ionization of analytes [8,46].

The performance of APCI and ESI in the analysis of diverse drugs was compared. ESI was found to provide the best LODs of MLs in influents and effluents of WWTPs [8]. However, due to the matrix effects in LC–MS/MS it was concluded that APCI mode should be preferred than ESI, even though less sensitivity is obtained in standard solutions. Also, the addition of AmAc to the mobile phase for the separation of MLs increased the ionization performance. Stolker et al. [2] have referred 10-fold higher sensitivity for the most of pharmaceuticals in ESI mode than in APCI mode in his study which was according to results of Schlüsener and Bester [8]. Both, positive and negative ionization modes were evaluated for the analysis of different groups of antibiotics (TCs, MLs, SAs, TMP) [25]. TCs and MLs, in general, can be easily protonated and analyzed in positive ion mode. SAs can be readily detected in both negative and positive ESI modes.

#### 4.1.3. Matrix effects

In LC–MS analysis, especially in the environmental analysis, the signal intensity of antibiotics may be considerably suppressed in wastewater matrices. Matrix effects occur very often in ESI MS analysis. It is the main disadvantage of ESI MS, because ESI source is highly susceptible to other components present in the matrix. As a result, signal suppression leads to erroneous results. The decrease of method sensitivity can be caused by several factors. Firstly, the antibiotics may sorb to organic matter in the samples which causes that sample preparation step is not effective in the point of view concentration of antibiotics and those are more difficult to detect. Secondly, contaminants in the sample matrix may interfere with the analyte peaks by raising the chromatogram baseline. Thirdly, contaminants may reduce the ionization efficiency of the analytes by taking up some of the limited number of excess charged sites on the surfaces of electrospray droplets [1,18,41]. Previously cited work studied the signal suppression [18]. The observation suggested that comparison of signal suppression among the antibiotics indicated that antibiotics within the same class generally exhibited a similar degree of signal suppression (FQs *vs.* SAs *vs.* TMP were compared). It was concluded that FQs were more susceptible to signal suppression than SAs and TMP. Signal suppression may be minimized by improving selective extraction, effective sample clean-up procedures after the extraction, improvement of the chromatographic separation and quantification by internal standards or standard addition method.

Another complication encountered in the LC–MS analysis is that retention times of antibiotics might drift significantly. One study refers observation of drift up to 2 min in some wastewater samples for FQs and TMP [18]. However, the molecular and confirming ions provided enough evidence to identify the antibiotics in these cases. This was solved by adjusting the eluent buffer concentration to minimize the drift in retention time and improve the peak shape. On the other hand, increasing the buffer concentration can reduce signal intensity.

#### 4.1.4. Internal standard

For the environmental analysis it is suitable to use internal standard (IS) to improve accuracy of quantitation. It is necessary to choose the compound which does not occur in the environment to avoid false positive results. IS could be used as surrogate standards—they are added prior to the enrichment to assess possible losses during the analytical procedure or like instrumental standards that are added to the final extracts prior to measurement. While the surrogate standards are used for the quantification, the instrumental standards are used to check the instrument performance during measurement [7].

In many multiresidue studies, simatone was chosen for MS detection as IS because it is eluted within the same chromatographic time frame as analytes, responded well in ESI$^+$ mode and had no noticeable matrix effects [4,21,32,33,48]. $^{13}$C-phenacetin was used very often in studies where antibiotics were analyzed together with other pharmaceuticals [1,24,27,38,40,41,61]. Although simatone or phenacetin are good ISs, it is desirable that both IS and target compounds should have the structural similarities in order to reflect the properties of the target compound during the entire analytical procedure. This indicates that it would be more desirable to have an IS (e.g., an isotopically labelled compounds, a structurally similar compound) for each individual antibiotic or at least for each class of antibiotics. In an attempt to represent the analytes most effectively, carbon-13- and deuterium-labelled surrogate were used as ISs (when commercially available, because only a limited number of isotopically labelled antibiotic substances are currently commercially available). This will certainly play an important role in further improvement of ease and accuracy of the determination of antibiotics in the environmental samples. It should be noted that a labelled standard will only be effective if it perfectly matches the target analyte(s) in terms of chromatographic retention time. Therefore a structurally identical isotopically labelled IS should ideally be added for every analyte to be determined [10]. Some compounds from the same class of antibiotics which are not used in human therapy can be employed as IS because they are not expected at significant concentration in municipal wastewaters.

### 4.2. Screening methods

Screening analytical methods can be based on the strategy of screening and confirmation. Screening methods are methods that are used to detect the presence of an analyte or class of analytes at the level of interest. Confirmatory methods are methods that provide full or complementary information enabling the analyte to be identified unequivocally at the level of interest [2].

Radioassay has also been reported as a screening method for the detection of antibiotics; however its low selectivity allows only semi-quantitative results [31]. Immunoassay is a next method of analysis that relies on specific interactions between antibodies and antigens to measure a variety of substances. Qualitative, semi-quantitative or quantitative immunoassay techniques are based on

the fundamental concept that antibodies prepared in animals can recognize and bind with high specificity to the antigen that stimulated their production. Immunoassays are an appropriate method of analysis in cases where real-time data are critical, field assays are needed and most samples are expected to be negative. Therefore, Immunoassays are well-suited for large-scale surveys and monitoring programs where sophisticated laboratory capability is not available [31]. Radioimmunoassays (RIAs) have been used as both qualitative or semi-quantitative tool and a screening method to reduce sample load for direct analyses. RIAs generally employ radiolabelled antigens and specific antibodies for the quantitative detection of antigens such as drugs and hormones. Yang and Carlson [31] assessed the applicability of SPE/RIA method for the routine monitoring of antibiotics in water and wastewater sources. The efficiency between SPE/RIA and SPE/LC–MS was compared for determination of TCs and SAs in water samples.

### 4.3. Multiresidue methods

Table 5 refers the recently published methods dealing with the determination of antibiotics from different classes even together with some pharmaceuticals. LC–MS or LC–MS/MS was used in majority of studies. One study referred UV detection of antibiotics in wastewaters [12]. However, in some studies, UV detection was used during the development step; MS detection was employed for further quantification and confirmation of analytes. $ESI^+$ was chosen for the ionization in all studies, except two studies using APCI [8,46].

For separation, $C_{18}$ analytical columns were mostly used. However, in some studies, $C_8$ [43], $C_{12}$ [18] and phenylhexyl [43] analytical columns were employed. The gradient elution mode was utilized in all studies.

Two works used UHPLC in tandem with MS [1,61] using $C_{18}$ analytical columns with 1.7 μm particles.

### 4.4. Fluoroquinolone antibiotics

FQs are widely used in human medicine. The methods deal mostly with norfloxacin (NOR), ciprofloxacin (CIPRO) and ofloxacin (OFLO) which are intended for humans use. Concerning the veterinary compounds, the most studied was enrofloxacin (ENRO) and sarafloxacin (SARA). An overview of methods dealing with the determination of Qs and FQs in environmental waters is shown Table 6. LC coupled to tandem MS method is nowadays a technique of choice for the determination of very low concentrations of FQs in environmental waters because of its selectivity and specificity. However, LC with FD was used in six works [50–55]. FQs are naturally highly fluorescent compounds and RP-LC–FD can be used for their determination. They were monitored at excitation wavelength 278 nm and emission wavelength 450 nm. Nevertheless, some studies used different wavelengths for each compound [50,55]. UV detection was also reported for the determination of FQs [36] but it was not as specific as FD. The detection was performed at 275 nm [36] or at 255 nm [36] depending on group of the compounds studied.

LC–MS/MS methods employed $ESI^+$ in all studies. For the quantitation and confirmation, SRM experiments were used [35,52,55]. In some studies single MS was utilized together with $ESI^+$ and SIM (selected ion monitoring) experiment [51,55,66]. Protonated molecule peak $[M+H]^+$ was used as a precursor ion for the quantitation.

Separation was performed on $C_{18}$ stationary phase [36,55]. However, RP-amide $C_{16}$ stationary phase [51] and $C_8$ analytical column were utilized [35,50,52]. A monolithic column was used for LC separation in two studies [53,54]. In four studies the separation was performed using gradient elution [36,50,51,55] and in four studies by isocratic elution [35,52–54]. Mobile phases contained MeOH or ACN or both together with volatile additive such as FAc or ammonium formate for the improvement of the ionization during MS analysis. Nonvolatile oxalic acid [50] and phosphoric acid [53,54] were added to mobile phases when FD was used.

Only one study used isotopically labelled CIPRO as IS [52]. Other studies did not refer using IS. In some multiresidue studies ENRO [10,24,59] or lomefloxacin [18] were used as ISs. These ISs were suitable because of similarity with the analyzed compounds, but there was a risk about their possible occurrence in the environment. ENRO can be used in veterinary medicine and thus can enter the environment leading to false results. For this reason it is better to use isotopically labelled ISs.

Capillary electrophoresis is another possible analytical technique that has scarcely been applied by environmental researches. This technique is less robust and less suitable for the routine monitoring antibiotics in environmental waters than HPLC. Capillary zone electrophoresis (CZE) with FD was employed for separation of 9 FQs in surface water samples [56]. CZE with UV detection was used for simultaneous determination of 5 FQs [62].

### 4.5. Tetracycline antibiotics

As it was already mentioned in the chapter extraction any of studies dealed only with the determination of TCs in environmental waters. TCs were determined only in multiresidue studies together with other antibiotics or even with other pharmaceuticals (Table 5). However, LC–MS/MS methods are nowadays technique of choice for the analysis of TCs in environmental waters. Some authors employed HPLC with UV detection. The detection was performed at 360 nm [32,48] or at 280 nm [11]. 280 nm was used for the detection in multiresidue study in which other veterinary antibiotics were determined, although UV detection was not as sensitive and specific as MS detection. FD can be used for detection of TCs if they are derivatized before.

Concerning MS detection for quantitation of TCs the protonated molecule $[M+H]^+$ can be chosen as a precursor ion. TCs exhibit characteristic fragment ions such as a neutral loss of 17, $[M+H\text{-}NH_3]^+$, and 35 amu, $[M+H\text{-}NH_3\text{-}H_2O]^+$ and 18 amu loss, $[M+H\text{-}H_2O]^+$ of tetracycline (TET) and oxytetracycline (OXY).

It is important that TCs strongly bind to silanol groups and consequently peak tailing could be observed. Using $C_{18}$ end-capped column and an addition of 1% AcAc to aqueous solvent of mobile phase was employed to avoid tailing peaks during determination of TCs [47].

As it was already mentioned, the use of isotope labelled IS is the best approach for the quantification in environmental analysis. However using isotope labelled IS was not referred in any study. Some compounds from the class of TCs were also utilized as IS in multiresidue studies: demeclocycline [10,59] or meclocycline [3].

### 4.6. Sulfonamide antibiotics and trimethoprim

SAs belong to the most studied group of antibiotics. Among them, mostly SMX and TMP are studied due to their wide use in human medicine for the treatment of bacterial infections. From veterinary compounds, sulfathiazole (STZ), sulfamethazine (SMZ), sulfadiazine (SDZ) and sulfachloropyridazine (SCP) have been included in many studies dealing with their determination in environmental water samples. In most cases, SAs were not studied alone but their determination was made simultaneously with other antibiotics. They have been determined in different kinds of environmental waters mainly in surface waters and wastewater samples. SPE was preferred technique of sample preparation in most cases. The overview of methods dealing only with SAs determination is shown in Table 7.

**Table 6**
HPLC analytical methods for the determination of FQs.

| Substances determined | Matrix sample preparation | Stationary phase, analytical column | Mobile phase | Detection | Precursor ions | Internal standard | Sensitivity LOD/LOQ | Ref. |
|---|---|---|---|---|---|---|---|---|
| ENO, OFLO, CIPRO, NOR, LOME | Surface water, wastewater SPME | CAPCELL PAK $C_8$ (100 mm × 2.1 mm, 5 μm) (40 °C) | 5 mM ammonium-formate (pH 3) + ACN (85/15) | $ESI^+$ MS/MS SRM | $[M+H]^+$ | | LOD = 7–29 ng $L^{-1}$ | [35] |
| CIPRO, DAN, ENO, ENRO, NOR, CINO, FLU, NAL, OXO | Surface water, SPE | Polarity $dC_{18}$ (150 mm × 3 mm, 3 μm) | Gradient elution, A: FAc (pH 2.5), B: ACN | DAD, 275 or 255 nm | | | LOD = 8–20 ng $L^{-1}$ | [36] |
| CIPRO, DAN, DIF, ENRO, FLU, MAR, NAL, NOR, OXO, SARA | Surface water, SPE | Inertsil $C_8$ (250 mm × 4.6 mm, 5 μm) | Gradient elution, A: 10 mM oxalic acid buffer (pH 4) + ACN (89:11), B: ACN | FD, 248–297 nm, 361–507 nm | | | LOD = 0.05–1 μg $L^{-1}$ | [50] |
| CIPRO, DIF, ENRO, LOME, NOR, OFLO, PIP, SARA, TOS | WWTP effluents, surface water, SPE | YMC ODS-AQ S-3 (50 mm × 4.0 mm) (23 °C), Discovery RP-Amide C16 (50 mm × 4.0 mm) | Gradient elution, A: water (pH 3.0) + ACN (98:2), B: ACN | $ESI^+$, MS, SIM, FD, 278 nm, 445–500 nm | $[M+H]^+$ | | $LOD_{MS}$ = 8.6–49 ng $L^{-1}$ | [51] |
| OFLO, NOR, CIPRO | WWTP efluents, SPE | Zorbax SB-$C_8$ (150 mm × 2.1 mm, 3.5 μm) | ACN/MeOH/FAc/water (6/12/0.5/81.5) | FD (278, 450 nm), $ESI^+$, MS, SIM, $ESI^+$, MS/MS, SRM | $[M+H]^+$ | CIPRO-$^{13}C_3$ $^{15}N$ | LOQ = 2–10 ng $L^{-1}$ | [52] |
| OFLO, NOR, CIPRO, ENRO | Wastewater, SPE | Chromolith Performance RP-18e (100 mm × 4.6 mm) | 0.025 M $H_3PO_4$ (pH 3.0 by TBA) + MeOH + ACN (920/70/10) | FD, 278 nm, 450 nm | | | LOD = 8.5–85 ng $L^{-1}$, LOQ = 25–250 ng $L^{-1}$ | [53] |
| NOR, CIPRO, ENRO | Surface water, SPE | Chromolith Performance RP-18e (100 mm × 4.6 mm) | 0.025 M $H_3PO_4$ (pH 3.0 by TBA)–MeOH (960/40) | FD, 278 nm, 450 nm | | | LOQ = 25 ng $L^{-1}$ | [54] |
| CIPRO, ENRO, FLE, FLU, LOME, MOXI, NOR, OFLO, OXO | Surface water, municipal WW, WWTP effluent, SPE | ID YMC-Pack Pro $C_{18}$ (250 mm × 4.6 mm, 3 μm) | Gradient elution, A: 50 mM FAc, B: MeOH | FD, 278–320 nm, 365–500 nm, MS, SIM, SRM | $[M+H]^+$ | | $LOQ_{FD}$ = 11–60 ng $L^{-1}$, $LOQ_{MS}$ = 0.3–7.0 ng $L^{-1}$ | [55] |

**Table 7**
HPLC analytical methods for the determination of SAs and TMP.

| Substances determined | Matrix Sample preparation | Stationary phase, analytical column | Mobile phase | Detection | Precursor ions | Internal standard | Sensitivity LOD/LOQ | Ref. |
|---|---|---|---|---|---|---|---|---|
| SMT, SMX, SMO, SPY, SDZ, SCP, SMR, SSM, SQX, SMP, SDT | Surface, drinking water, SPE | Alltima RP $C_{18}$ (150 mm × 2.1 mm, 5 μm) | Gradient elution A: ACN + 1mM FAc, B: water + 1mM FAc | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | | LOQ = 5–21 ng $L^{-1}$ | [3] |
| SMZ, SDT, SCP, SMO | Ground water, SPE | BetaBasic-18 $C_{18}$ (100 mm × 2.1 mm, 3 μm) (30 °C) | Gradient elution, A: ACN, B: water + 0.3% FAc | $ESI^+$, MS/MS | $[M+H]^+$ | $^{13}C_6$-SMZ | LOD = 20–70 ng $L^{-1}$, LOQ = 70–240 ng $L^{-1}$ | [6] |
| SGN, SCT, SDZ, SPY, SMR, SMZ, SDT, SSZ | Wastewaters, SPE, SPME | SPE: XTerra MS $C_{18}$ (250 mm × 2.1 mm, 5 μm) (35 °C), SPME: Ulracarb ODS $C_{18}$ (150 mm × 4.6 mm, 5 μm) (35 °C) | Gradient elution, A: 20 mM AmAc (0.1% FAc, pH 3.0), B: 20 mM AmAc (in 2:1 ACN/MeOH) | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | $^{13}C_6$-SMZ, $^{13}C_3$-caffeine | $LOD_{SPE}$ = 2.88–9.00 ng $L^{-1}$, $LOD_{SPME}$ = 9.04–55.3 μg $L^{-1}$ | [34] |
| SCT, SDZ, SMX, STZ, SMR, SSX, SMT, SMZ, SMM, SMP, SCP, SDX, SDT, SM | Wastewater, SPE | Symmetry $C_{18}$ (150 mm × 2.1 mm, 3.5 μm) (20 °C) | Gradient elution, A: 0.2% FAc in MeOH, B: 0.2% FAc in water | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | | LOQ = 1–3 ng $L^{-1}$ | [37] |
| SDZ, SCP, SMT, SQX, SDM, SMD | Surface water, SPE | LiChrospher 100 RP-$C_{18}$ (250 mm × 4 mm, 5 μm) | Gradient elution, A: 10 mM AcAc buffer pH 3.4, B: ACN | FD, 405, 485 nm (pre-column derivatization) | | | LOD = 1–8 ng $L^{-1}$ | [57] |
| SMP, SMO, SQX, SNT, SSM, SMX, SMR, STZ, SDZ, SMT, SDM, SDT, SPY, SSX, SCP, SM, TMP | WWTP influent and effluent, river water, SPE | Acquity UPLC BEH $C_{18}$ (100 mm × 2.1 mm, 1.7 μm) (40 °C) | Gradient elution, A: MeOH, B: water + 0.1% FAc | $ESI^+$, MS/MS, SRM | | $^{13}C_6$-SMZ | $LOD_{influent}$ = 20–200g $L^{-1}$ $L^{-1}$, $LOD_{effluent}$ = 16–120g $L^{-1}$ $L^{-1}$, $LOD_{river\ water}$ = 8–60g $L^{-1}$ $L^{-1}$ | [58] |
| SDZ, STZ, SPY, SMZ, SMX | Surface water, wastewaters, mixed hemimicelles SPE | Diamonsil-$C_{18}$ (250 mm × 4.6 mm, 5 μm) | Gradient elution, A: water (pH 3.4 adjusted by $H_3PO_4$), B: ACN–water (75:25) | UV, 260 nm | | | LOD = 0.15–0.35 μg $L^{-1}$ | [63] |
| SAD, SDZ, STZ, SMR, SMZ, SMP, SDT, SQX | Swine wastewater, SPE | Supelcosil $C_{18}$ (250 mm × 4.6 mm, 5 μm) (35 °C) | Gradient elution, A: 0.5% AcAc in water, B: ACN | UV, 272 nm | | | LOQ = 5–7.5 ng $L^{-1}$ | [64] |
| SDZ, STZ, SMZ, SMX, SDT and their acetylforms | Surface water, online SPE | Nucleodur $C_{18}$ Gravity (125 mm × 2 mm, 5 μm) | Gradient elution, A: 20 mM FAc | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | $D_4$-SDZ, $D_4$-STZ, $^{13}C_6$-SMZ, $D_4$-SMX, $D_4$-SDT | LOD = 1–3 ng $L^{-1}$ | [65] |

SAs themselves are not fluorescent, but they can be easily derivatized with fluorescamine to form highly fluorescent derivatives. FD with pre-column derivatization was used at $\lambda_{exc}$ 405 nm and $\lambda_{em}$ 485 nm, respectively [57]. HPLC–UV detection at 260 nm [63] or at 272 nm was used [64]. In some studies UV detection was only used for the optimization of chromatographic separation development and then for the determination of SAs in real samples MS detection was applied. Although, HPLC–UV or HPLC–FD have been used for the detection of SAs, these methods did not achieve good sensitivity comparing to MS detection. The sensitivity of method also depends on the purpose of the method and its application on real samples. Babić et al. [12] achieved LOD and LOQ suitable for the detection of analytes in wastewaters, where the concentrations were higher than in surface waters.

LC analyses were performed of $C_{18}$ analytical columns for the separation in all referred methods. In all cases gradient elution was employed. Mobile phases containing MeOH or ACN were used as organic modificators. For MS detection, FAc or AmAc were added. In case of UV detection, pH of water was adjusted by phosphoric acid which can be used in UV detection.

Concerning MS/MS detection, $ESI^+$ mode was chosen for the ionization of SAs because of its high sensitivity. The protonated molecule $[M+H]^+$ was chosen as precursor ion for quantitation in all developed methods. The identification of individual antibiotics was mostly based on chromatographic retention time and two the most intensive transitions for each compound. SAs exhibit characteristic fragmentation in the $ESI^+$ mode. Protonated molecule $[M+H]^+$ is usually base peak of spectra in SIM mode. The specific fragment ions are $m/z$ 92 $[M\text{-}RNH_2\text{-}SO_2]^+$, 108 $[M\text{-}RNH_2\text{-}SO]^+$ and 156 $[M\text{-}RNH_2]^+$. The common fragment ion, $m/z$ 156, representing the sulfanilyl ring is used for the quantification of the majority of SAs.

In case of SAs $^{13}C_6$-sulfamethazine was used the most often as IS [6,34,58,65]. Other SAs isotope labelled compounds were employed as well, e.g. $D_4$-sulfadiazine, $D_4$-sulfathiazole, $D_4$-sulfamethoxazole, and $D_4$-sulfadimethoxine [65].

Only one work used UHPLC in tandem with MS. $C_{18}$ column with 1.7 μm particles was employed [58].

### 4.7. Macrolide antibiotics

Only four methods dealed with the determination of MLs. Analytical methods for the determination of MLs employed HPLC with tandem MS (as is shown in Table 8). MLs do not have any suitable chromophore group and this causes that MLs show poor UV absorbance, indicating that specific, selective and sensitive UV detection of these compounds in environmental waters is difficult [30]. One study refers the monitoring ERY, roxithromycin (ROXI) and tylosin (TLS) by UV, but the tandem MS was used for the accurate quantitation [30]. FD of MLs is possible with their derivatization. Thus, it is clear that MS detection is the most suitable technique for this group of antibiotics from the point of view of sensitivity and specificity.

The methods for the determination of MLs employed MS/MS using specific SRM conditions with $ESI^+$. The precursor ion chosen for the quantitation was $[M+H]^+$ in almost all studies, except of ERY-$H_2O$, for which $[M+H\text{-}H_2O]^+$ was used as a precursor ion [5,9,17,30].

MLs were separated on the $C_{18}$ analytical column. For the separation, gradient elution was utilized in all cases. ACN was used as a part of mobile phase, together with AmAc buffer of FAc to enhance ionization. MeOH was used in one study as a third part of mobile phase [9].

Isotope labelled ERY [8,18,43] was used as IS in multiresidue studies [3]. Structurally related compounds e.g., kitasamycin [17], spiramycin [9], josamycin [5,7,24] or oleandomycin [5] were also employed.

**Table 8**
HPLC analytical methods for the determination of MLs.

| Substances determined | Matrix sample preparation | Stationary phase, analytical column | Mobile phase | Detection | Precursor ions | Internal standard | Sensitivity LOD/LOQ | Ref. |
|---|---|---|---|---|---|---|---|---|
| CLAR, ROXI, ERY-$H_2O$, SPIR, TLS | Ground water, WWTP effluent, SPE | Nucleosil 100-5 $C_{18}$ HD (125 mm × 5 mm) (30 °C) | Gradient elution, A: 10 mM AmAc buffer (pH 6.0) + ACN (90:10), B: ACN | $ESI^+$, MS/MS, SIM | $[M+H]^+$ | JOSA, Oleandomycin | LOQ = 2–35 ng $L^{-1}$, LOQ = 5–70 ng $L^{-1}$ | [5] |
| ERY-$H_2O$, TLS | WWTP influent and effluent, SPE | Xterra MS $C_{18}$ (50 mm × 2.1 mm, 2.5 μm) (45 °C) | Gradient elution, A: 0.1% FAc in water, B: 0.1% FAc in ACN, C: MeOH | $ESI^+$, MS/MS, SRM | $[M+H]^+$ | SPIR | LOD = 10–60 ng $L^{-1}$ | [9] |
| AZI, CLAR, ERY, ROXI, JOS | Surface water, SPE | Hypurity $C_{18}$ (250 mm × 2.1 mm, 5 μm) | Gradient elution, A: ACN + 10 mM AmAc (pH 6.0) (10:90), B: ACN | $ESI^+$, MS, SIM, MS/MS, SRM | $[M+H]^+$ | Kitasamycin | $LOD_{MS}$ = 0.14–1.9 μg $L^{-1}$, $LOQ_{MS}$ = 0.47–6.3 μg $L^{-1}$, $LOD_{MS/MS}$ = 13–140 ng $L^{-1}$, $LOQ_{MS/MS}$ = 43–470 ng $L^{-1}$ | [17] |
| ERY-$H_2O$, ROXI, TLS | Surface waters, WWTP, SPE | XTerra MS $C_{18}$ (50 mm × 2.1 mm, 2.5 μm) (45 °C) | Gradient elution, A: water + 0.1% FAc, B: ACN + 0.1% FAc | UV 215,205, 287 (resp.), $ESI^+$, MS/MS, SRM | $[M+H\text{-}H_2O]^+$, $[M+H]^+$, $[M+H]^+$ | Simatone | LOD = 30–70 ng $L^{-1}$ | [30] |

## 5. Conclusions and future trends

This review summarizes recently developed analytical methodologies for the determination of different groups of antibiotics in environmental waters. A challenge is presented in the simultaneous extraction and analysis of multiple classes of compounds due to the wide range of polarities, solubilities, p$K_{as}$ and other properties under the acidic and basic conditions.

To prevent emergence of resistant bacteria, it is necessary to monitor the concentration, fate and removal of antibiotics in environmental samples. Many studies showed the incomplete elimination during wastewater treatment processes, thus the next task is to improve the treatment process.

Detailed knowledge of the behaviour of antimicrobials in wastewater treatment and the aquatic environment will help to achieve a reliable basis of environmental risk assessment (e.g., by providing measured environmental concentrations, MECs). MECs can be used in environmental risk assessment studies since they provide accurate indications of actual concentration present in environmental systems. Investigations of the occurrence and fate of antimicrobial agents in various wastewater treatment steps can be exploited in order to evaluate wastewater treatment technologies with the respect to elimination of specific contaminants. Reducing the release of residual antibiotics into the aquatic environment would presumably decrease any potential environmental risks. During monitoring receiving surface waters as well as wastewater treatment plants, location of particular concern can be identified and mitigated specifically.

## Acknowledgements

The authors gratefully acknowledge the financial support from the Grant Agency of the Academy of Sciences of the Czech Republic (KJB 601100901) and the financial support of Grant Agency of the Ministry of Education of the Czech Republic (MSM 0021620822).

## References

[1] B. Kasprzyk-Horden, R.M. Dinsdale, A.J. Guwy, J. Chromatogr. A 1161 (2007) 132.
[2] A.A.M. Stolker, W. Niesing, E.A. Hogendoorn, J.F.M. Versteegh, R. Fuchs, U.A.T. Brinkman, Anal. Bioanal. Chem. 374 (2004) 955.
[3] D. Perret, A. Gentili, S. Marchese, A. Greco, R. Curini, Chromatographia 63 (2006) 225.
[4] Z. Ye, H.S. Weinberg, M.T. Meyer, Anal. Chem. 79 (2007) 1135.
[5] C.S. McArdell, E. Molnar, M.J.-F. Suter, W. Giger, Environ. Sci. Technol. 37 (2003) 5479.
[6] A.L. Batt, D.D. Snow, D.S. Aga, Chemosphere 64 (2006) 1963.
[7] A. Göbel, C.S. McArdell, M.J.-F. Suter, W. Giger, Anal. Chem. 76 (2004) 4756.
[8] M.P. Schlüsener, K. Bester, Rapid Commun. Mass Spectrom. 19 (2005) 3269.
[9] S. Yang, J. Cha, K. Carlson, Anal. Bioanal. Chem. 385 (2006) 623.
[10] R. Lindberg, P.-A. Jarnheimer, B. Olsen, M. Johansson, M. Tysklind, Chemosphere 57 (2004) 1479.
[11] E. Turiel, G. Bordin, A.R. Rodríguez, J. Sep. Sci. 28 (2005) 257.
[12] S. Babić, D. Ašperger, D. Mutavdžić, A.J.M. Horvat, M. Kaštelan-Macan, Talanta 70 (2006) 732.
[13] M.S. Díaz-Cruz, M.J. López de Alda, D. Barceló, Trends Anal. Chem. 22 (2003) 340.
[14] F. Hernández, J.V. Sancho, M. Ibáñez, C. Guerrero, Trends Anal. Chem. 26 (2007) 466.
[15] M. Farré, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1203.
[16] Y. Madrid, Z.P. Zayas, Trends Anal. Chem. 26 (2007) 293.
[17] S. Abuin, R. Codony, R. Compañó, M. Granados, M.D. Prat, J. Chromatogr. A 1114 (2006) 73.
[18] B.J. Vanderford, R.S. Pearson, D.J. Rexing, S.A. Snyder, Anal. Chem. 75 (2003) 6265.
[19] J.E. Renew, C.H. Huang, J. Chromatogr. A 1042 (2004) 113.
[20] E. Turiel, A. Martín-Esteban, G. Bordin, A.R. Rodríguez, Anal. Bioanal. Chem. 380 (2004) 123.
[21] S.C. Kim, K. Carlson, Anal. Bioanal. Chem. 387 (2007) 1301.
[22] X.S. Miao, F. Bishay, M. Chen, C.D. Metcalfe, Environ. Sci. Technol. 38 (2004) 3533.
[23] A.L. Spondberg, J.D. Witter, Sci. Total Environ. 397 (2008) 148.
[24] N.M. Vieno, T. Tuhkanen, L. Kronberg, J. Chromatogr. A 1134 (2006) 101.
[25] C. Hao, L. Lissemore, B. Nguyen, S. Kleywegt, P. Yang, K. Solomon, Anal. Bioanal. Chem. 384 (2006) 505.
[26] C. Nebot, S.W. Gibb, K.G. Boyd, Anal. Chim. Acta 598 (2007) 87.
[27] M.J. Gómez, M. Petrović, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1114 (2006) 224.
[28] J.D. Cahill, E.T. Furlong, M.R. Burkhardt, D. Kolpin, L.G. Anderson, J. Chromatogr. A 1041 (2004) 171.
[29] E.L. McClure, C.S. Wong, J. Chromatogr. A 1169 (2007) 53.
[30] S. Yang, K.H. Carlson, J. Chromatogr. A 1038 (2004) 141.
[31] S. Yang, K. Carlson, Water Res. 38 (2004) 3155.
[32] S. Yang, J. Cha, K. Carlson, Rapid Commun. Mass Spectrom. 18 (2004) 2131.
[33] S. Yang, J. Cha, K. Carlson, J. Chromatogr. A 1097 (2005) 40.
[34] V.K. Balakrishnan, K.A. Terry, J. Toito, J. Chromatogr. A 1131 (2006) 1.
[35] K. Mitani, H. Kataoka, Anal. Chim. Acta 562 (2006) 16.
[36] E. Turiel, G. Bordin, A.R. Rodríguez, J. Chromatogr. A 1008 (2003) 145.
[37] S. Ye, Z. Yao, G. Na, J. Wang, D. Ma, J. Sep. Sci. 30 (2007) 2360.
[38] M.J. Hilton, K.V. Thomas, J. Chromatogr. A 1015 (2003) 129.
[39] C. Lacey, G. McMahon, J. Bones, L. Barron, A. Morrissey, J.M. Tobin, Talanta 75 (2008) 1089.
[40] P.H. Roberts, K.V. Thomas, Sci. Total Environ. 356 (2006) 143.
[41] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678.
[42] T. Christian, R.J. Schneider, H.A. Färber, D. Skutlarek, M.T. Meyer, H.E. Goldbach, Acta Hydrochim. Hydrobiol. 31 (2003) 36.
[43] K.G. Karthikeyan, M.T. Meyer, Sci. Total Environ. 361 (2006) 106.
[44] A.L. Batt, D.S. Aga, Anal. Chem. 77 (2005) 2940.
[45] A. Gulkowska, Y. He, M.K. So, L.W.Y. Yeung, H.W. Leung, J.P. Giesy, P.K.S. Lam, M. Martin, B.J. Richardson, Marine Poll. Bull. 54 (2007) 1287.
[46] L.K. Sørensen, T.H. Elbæk, Chromatographia 60 (2004) 287.
[47] S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Chromatogr. A 1010 (2003) 225.
[48] K.J. Choi, S.G. Kim, C.W. Kim, S.H. Kim, Chemosphere 66 (2007) 977.
[49] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K.L. Kratz, J. Chromatogr. A 815 (1998) 213.
[50] M.D. Prat, J. Benito, R. Compañó, J.A. Hernández-Arteseros, M. Granados, J. Chromatogr. A 1041 (2004) 27.
[51] H. Nakata, K. Kannan, P.D. Jones, J.P. Giesy, Chemosphere 58 (2005) 759.
[52] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr. A 1139 (2007) 45.
[53] M. Seifrtová, A. Pena, C.M. Lino, P. Solich, Anal. Bioanal. Chem. 391 (2008) 799.
[54] A. Pena, D. Chmielova, C.M. Lino, P. Solich, J. Sep. Sci. 30 (2007) 2924.
[55] M. Ferdig, A. Kaleta, W. Buchberger, J. Sep. Sci. 28 (2005) 1448.
[56] M. Ferdig, A. Kaleta, T.D. Thanh Vo, W. Buchberger, J. Chromatogr. A 1047 (2004) 305.
[57] J. Raich-Montiu, J. Folch, R. Compañó, M. Granados, M.D. Prat, J. Chromatogr. A 1172 (2007) 186.
[58] H. Chang, J. Hu, M. Asami, S. Kunikane, J. Chromatogr. A 1190 (2008) 390.
[59] R.H. Lindberg, P. Wennberg, M.I. Johansson, M. Tysklind, B.A.V. Andersson, Environ. Sci. Technol 39 (2005) 3421.
[60] M.S. Díaz-Cruz, D. Barceló, Anal. Bioanal. Chem. 386 (2006) 973.
[61] M. Petrovic, M. Gross, D. Barceló, J. Chromatogr. A 1124 (2006) 68.
[62] A.F. Faria, M.V.N. Souza, M.V. Almeida, M.A.L. Oliveria, Anal. Chim. Acta 579 (2006) 185.
[63] J.D. Li, Y.Q. Cai, Y.L. Shi, S.F. Mou, G.B. Jiang, J. Chromatogr. A 1139 (2007) 178.
[64] N.T. Malintan, M.A. Mohd, J. Chromatogr. A 1127 (2006) 154.
[65] K. Stoob, H.P. Singer, C.W. Goetz, M. Ruff, S.R. Mueller, J. Chromatogr. A 1097 (2005) 137.
[66] R. Nageswara Rao, N. Venkateswarlu, R. Narsimha, J. Chromatogr. A 1187 (2008) 151.
[67] X.S. Miao, C.D. Metcalfe, J. Mass Spectrom. 38 (2003) 27.
[68] W. Song, M. Huang, W. Rumbeiha, H. Li, Rapid Commun. Mass Spectrom. 21 (2007) 1944.
[69] O.J. Pozo, C. Guerrero, J.V. Sancho, M. Ibánez, E. Pitarch, E. Hogendoorn, F. Hernández, J. Chromatogr. A 1103 (2006) 83.