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REVIEW

An overview of sample preparation procedures for LC-MS multiclass antibiotic determination in environmental and food samples

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Abstract Antibiotics are a class of pharmaceuticals that are of great interest due to the large volumes of these substances that are consumed in both human and veterinary medicine, and due to their status as the agents responsible for bacterial resistance. They can be present in foodstuffs and in environmental samples as multicomponent chemical mixtures that exhibit a wide range of mechanisms of action. Moreover, they can be transformed into different metabolites by the action of microorganisms, as well as by other physical or chemical means, resulting in mixtures with higher ecotoxicities and risks to human health than those of the individual compounds. Therefore, there is growing interest in the availability of multiclass methods for the analysis of antimicrobial mixtures in environmental and food samples at very low concentrations. Liquid chromatography (LC) has become the technique of choice for multiclass analysis, especially when coupled to mass spectrometry (LC-MS) and tandem MS (LC-MS^2). However, due to the complexity of the matrix, in most cases an extraction step for sample clean-up and preconcentration is required before analysis in order to achieve the required sensitivities. This paper reviews the most recent developments and applications of multiclass antimicrobial determination in environmental and food matrices, emphasizing the practical aspects of sample preparation for the simultaneous extraction of antimicrobials from the selected samples. Future trends in the application of LC-MS-based techniques to multiclass antibiotic analysis are also presented.

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Introduction

Antibiotics are defined as drugs of natural, semisynthetic or synthetic origin with antibacterial, antifungal or antiparasitic activity [1]. These drugs are used as chemotherapeutic agents in the treatment of infectious diseases in humans and animals. They can also be used as growth promoters in livestock farming (although EU legislation has forbidden this practice since January 1, 2006 [2]), as well as in agriculture to control bacterial diseases when growing fruit or vegetables and in bee-keeping [1].

Antibiotics have attracted a great deal of attention during the last decade, especially in relation to food safety and their presence, behavior and fate in the environment. Antibiotic use is the major contributor to the selection and propagation of resistant bacterial strains that represent a serious health risk to humans and animals. Resistance to some antibiotics can emerge after controlled treatment, after the prolonged use and/or following the application of concentrations that are too low to cure but are high enough to promote the emergence of resistant bacterial strains.

The main antibiotics used in both human and veterinary medicine fall into the following classes : β-lactams (β-LCs), tetracyclines (TCs), macrolides (MCs), aminoglycosides (AGs), amphenicols (AMPs), quinolones (Qs)/fluoroquinolones (FQs), sulfonamides (SAs), lincosamides (LCs), glycopeptides (GPs) and polyether ionophores (IPhs) (Fig. 1) [1]. They can be classified according to their chemical structure or mechanism of action. Antibiotics are used extensively in human and veterinary medicine, as

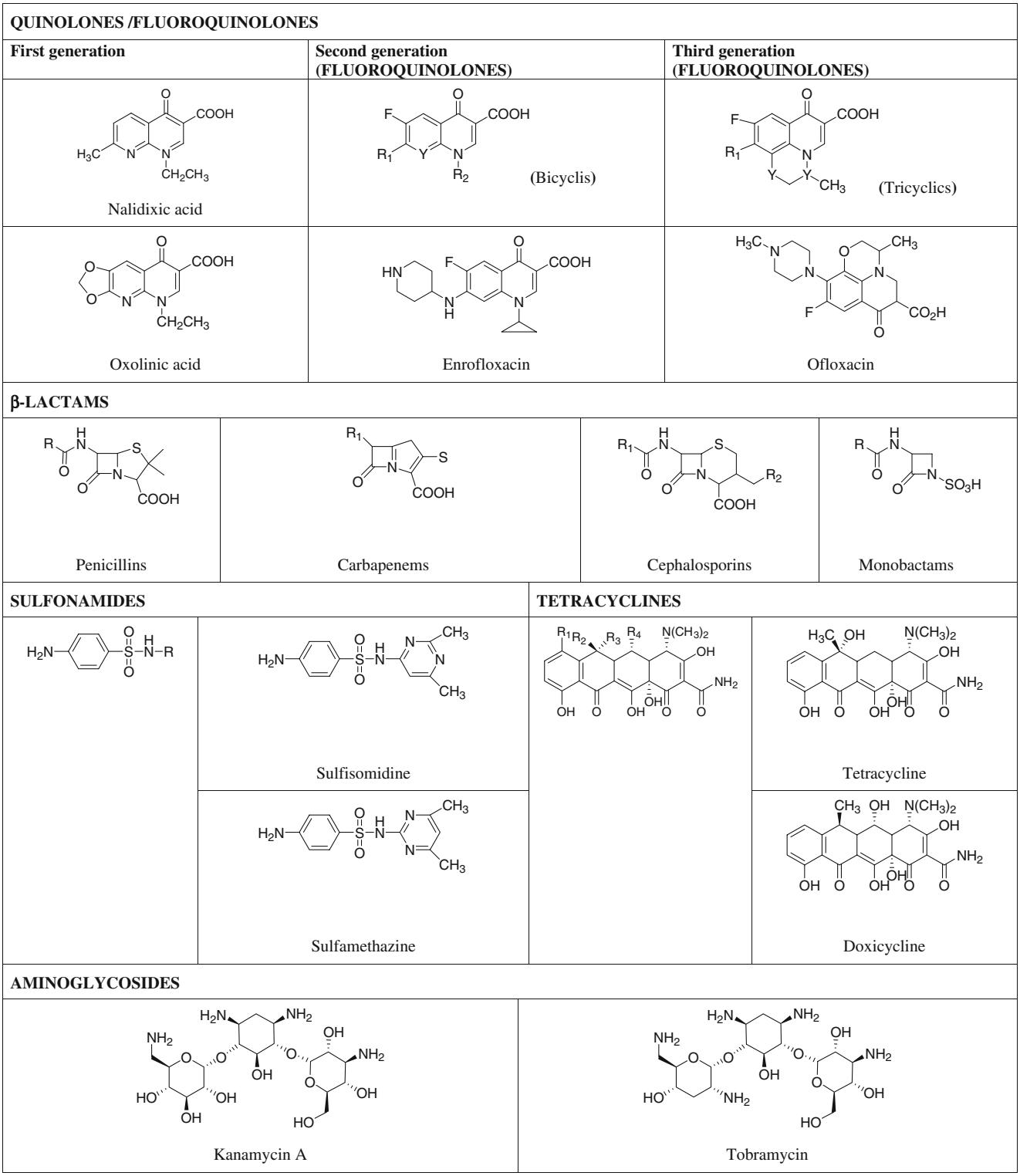

Fig. 1 Chemical structures of the main classes of antibiotics

well as in aquaculture, in order to prevent (prophylaxis) or treat microbial infections.

These pharmaceuticals can be administered by injection (intravenously, intramuscularly, or subcutaneously), orally, topically on the skin, or by intramammary and intrauterine infusions in veterinary medicine. All of these routes can lead to the appearance of residues in foods of animal origin, especially when the drugs are used in the wrong or an abusive way (e.g., with withdrawal periods that are too short, incorrect doses, self-medication, etc.).

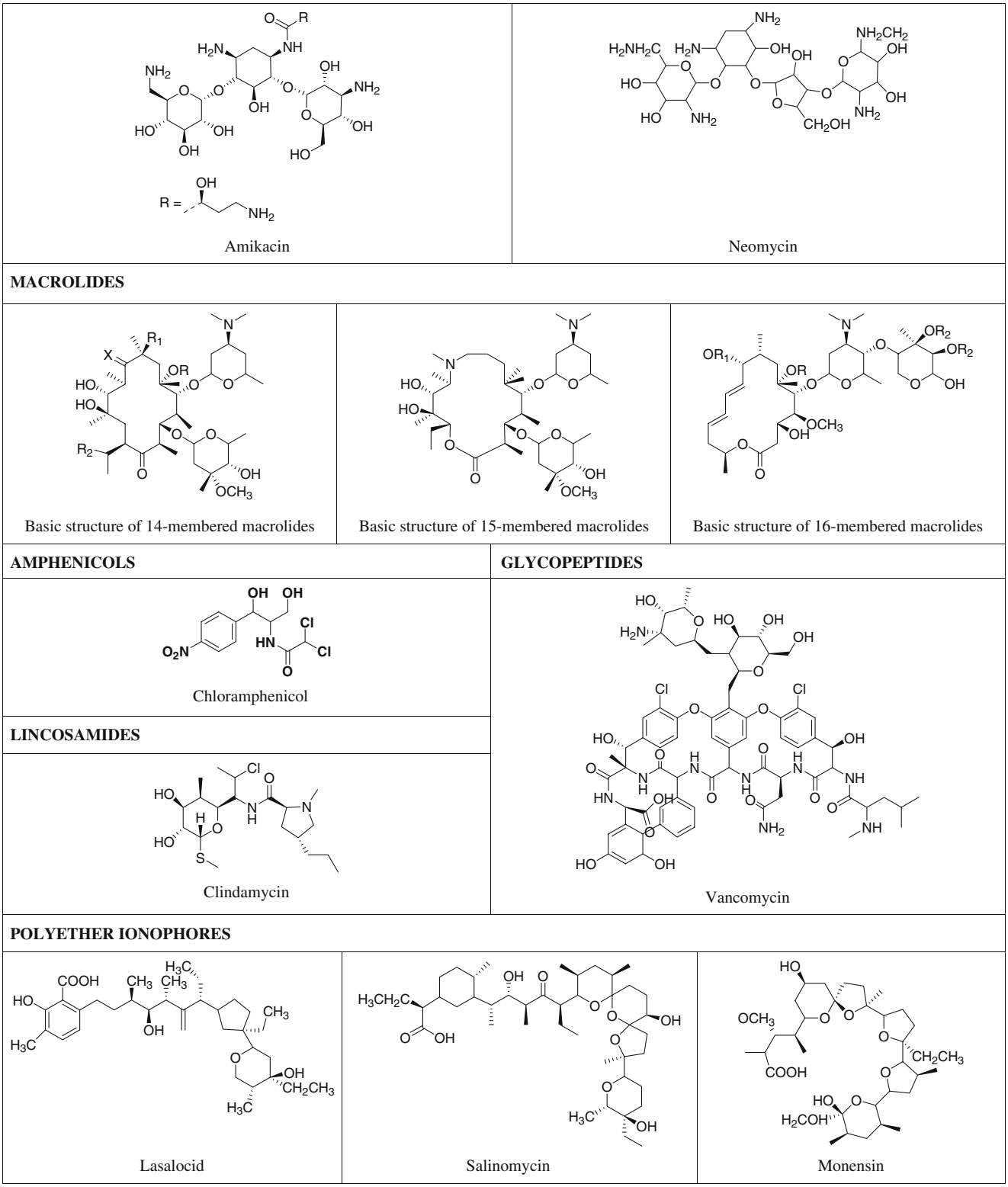

Fig. 1 (continued)

Data on antibiotic consumption at international level are scarce and heterogeneous. The total antibiotic consumption around the world was estimated at between 100,000 and 200,000 tons per year [3]. According to a survey carried out in 2000 by the European Federation of Animal Health (FEDESA), 13,216 tons of antibiotics were used in the EU and Switzerland in 1999, 65% of which were applied in human medicine, 29% in veterinary medicine, and 6% as

growth promoters. Between 1997 and 1999, the total antibiotic consumption increased by about 10%, although their use as growth promoters decreased by 51% during this period. According to a recent report in the United States, approximately 11,200 metric tons of antibiotics were used as growth promoters for cattle, hogs and poultry [4].

The European Surveillance of Antimicrobial Consumption (ESAC) webpage provides information about the specific consumption of antibiotics for human medicine in Europe, expressed as "defined daily doses" (DDD, World Health Organisation definition) [5]. There is great variation in the use patterns as well as in the classes of antibiotics used in different countries. Figure 2 summarizes data on the application of antimicrobials for systemic use in DDD/100 inhabitants/day during 2006 in 24 European countries [5]. The group of β-LC antibiotics—especially penicillins (PCs), cephalosporins (CPs) and, to a minor extent, carbapenems (CPNs)—accounts for approximately 50–70% of ambulatory antibiotic consumption in these selected countries [5, 6]. The second most important group are the MCs, followed by the TCs, FQs and SAs.

There are significant differences among the different classes of antimicrobials in terms of their importance as environmental contaminants. β-LCs, the most widely used antibiotics, are easily hydrolyzed and are not usually thought to be a serious threat to the environment, but SAs, MCs and FQs are much more stable [7] and have been detected in different environmental compartments [1].

After consumption, antibiotics are often metabolized only partially and are excreted via urine or feces (Fig. 3). The degree of metabolization varies across chemical classes and within a chemical class and depends mainly on the animal species and mode of application. For instance, the degrees of metabolism of TCs, MCs and amoxicillin (a β-LC) are lower than 20%, whereas it is higher than 80% for SAs [8, 9]. Antibiotic metabolites can be more toxic to humans than the parent compounds, although not much information is available on this area [10].

Disposal of unused therapeutic drugs or residues from the plants that produce them can also represent a source of environmental contamination [11]. Once they are released into the environment, the nonmetabolized active drug, along with its metabolites, may be degraded, transported and distributed between different environmental compartments. The parent compounds and metabolites are only partially eliminated at sewage treatment plants (STPs). Effluents from the STPs are discharged into receiving surface waters, and trace amounts of these pharmaceuticals have been detected in surface and ground waters and—more rarely—in drinking water [12].

They can also be present in sediments, especially beneath fish farms [1], where antimicrobials are fed directly into the water to treat infections in farmed fish. The application of sewage sludge or manure to soils can also be a potential route for antibiotics to enter the terrestrial environment. The manure and slurry are typically collected and stored before being applied to the land as fertilizer. During storage, some antibiotics can degrade (particularly SAs, β-LCs, MCs and AGs), whereas others (such as Qs and TCs) are more persistent [13].

Once the drugs reach the soil, they can be partially adsorbed onto the soil particles transported to surface waters via overland flows or drain flows, leached into groundwater, and/or degraded. The adsorption of antibiotics

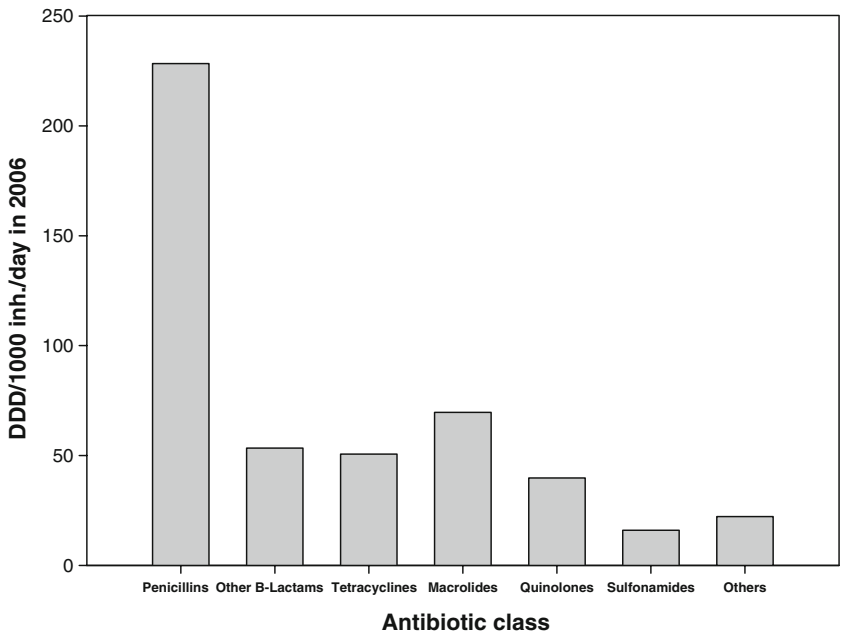

Fig. 2 Data on the total use of antimicrobials for systemic use, expressed in DDD/1000 inhabitants/day in 2006, corresponding to Austria, Belgium, Bulgaria, Croatia, Cyprus, Denmark, Finland, France, Greece, Hungary, Iceland, Ireland, Israel, Italy, Lithuania, Luxembourg, Netherlands, Norway, Portugal, Russia, Slovakia, Slovenia, Spain and Sweden [5]

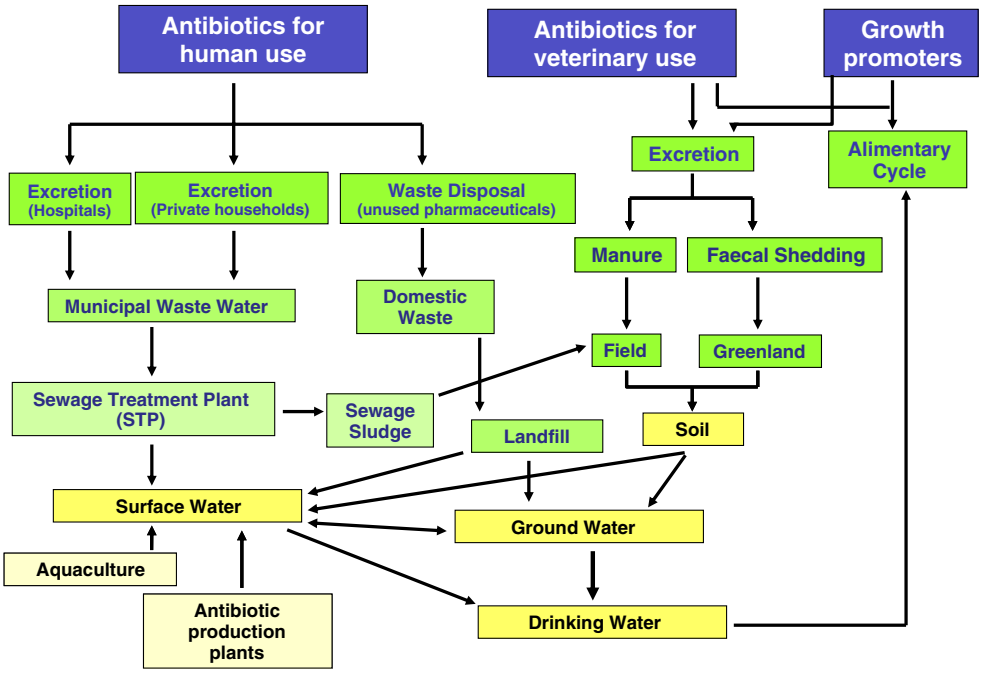

Fig. 3 Sources and pathways for antibiotic residues in the environment (adapted from [6])

at the organic and mineral exchange sites in soils is mostly attributed to charge transfer and ion interactions and not to hydrophobic partitioning [14]. Therefore, sorption will be strongly influenced by the pH of the medium, which will affect antibiotic mobility and transport. Antibiotic degradation in the environment can occur through the action of microorganisms, photodegradation processes and/or hydrolysis [13]. It has been shown that the binding of antibiotics to soil particles or to sediments hampers biodegradation, increasing their persistence in the environment [15]. For example, Qs are strongly adsorbed onto sewage sludge, soils and sediments, and some studies have shown that are not biodegraded in sediments [4]. However, they are rapidly photodegraded in waters, as are TCs, ivermectin and furazolidone, although this process is also less effective when the drugs are bound to the soil surface [15].

The significance of the presence of these compounds at low levels in the environment is currently unclear, but according to EU directives 2001/83/EC [16] and 81/852/EC [17] and their amendments, all new pharmaceuticals must undergo an environmental risk assessment before they are introduced into the market. Guidelines on the assessment of the environmental impact of veterinary and human pharmaceuticals have been issued by the European Medicines Evaluation Agency (EMEA) and by the Food and Drug Administration (FDA) in the USA [18, 19]. On the other hand, the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) has developed more specific guidelines for veterinary products in an effort to ensure a harmonized approach for Japan, the USA, and the EU [20, 21].

Concentration limits or tolerance levels of antibiotics in the environment are not regulated, but they vary between the higher μg/L range in hospital effluents, the lower μg/L range in municipal wastewater, and the higher and lower μg/L ranges in different surface waters, groundwater and seawater [1].

Regarding foodstuffs, the FDA in the USA and the EU have set maximum residue limits (MRLs) for veterinary drug residues in foods of animal origin to ensure consumer safety [22–24]. However, legislation can differ considerably between different countries, especially in developing regions, where, in many cases, national food regulations are still being created and updated [25]. Moreover, MRLs are not established for all of the antibiotics found in each food commodity and food-producing species. Therefore, veterinary drug-residue control represents an important issue in ensuring consumer protection.

As mentioned before, antibiotic residues do not occur as isolated parent drugs in the environment. They can be transformed into different metabolites by the action of microorganisms, as well as by other physical or chemical means. On the other hand, a broad range of pharmaceuticals are applied for human and veterinary uses, so they will be present in the environment as multicomponent chemical mixtures with a wide range of different mechanisms of action. This "cocktail" usually has a synergic effect, showing a higher ecotoxicity than the individual compounds, as some authors have reported [26, 27]. According to the current guidelines for medical product authorization issued by EMEA, ecotoxicological assessment is not necessary if the predicted environmental concentrations (PEC) of human pharmaceuticals in the aquatic environ-

ment are below 0.01 μg L^{-1} or below 10 μg kg^{-1} and 0.1 μg L^{-1} in soil and groundwater, respectively, in the case of veterinary medicines. Obviously, these cut-off values are not sufficient for mixtures of substances with similar or even dissimilar activities [28]. Therefore, there is growing interest in the availability of multiclass methods for the analysis of pharmaceutical mixtures in the various environmental compartments that allow realistic evaluation of the ecotoxicological effects of these drugs for different organisms. On the other hand, they are also requested by regulatory agencies as part of their requirement to monitor the food supply for regulated veterinary residues and contaminants. Moreover, the application of multiresidue analysis methods also allows the time and cost of analysis to be reduced.

Antibiotics, and in general pharmaceutical compounds, in contrast to chemical industrial pollutants, possess special characteristics that make multiclass analysis difficult. They are comparatively large and chemically complex molecules, with several functionalities and multiple ionization sites within the same molecule. They can therefore be cationic, anionic or zwitterionic, and the solution pH can affect their physicochemical properties, sorption behaviors, photostabilities, as well as their antimicrobial activities and toxicities [29]. The diversity of the chemical properties of different antibiotics, and the low concentration levels at which they can be present in samples, increase the difficulty involved in finding generic analytical procedures for multiclass analysis, especially for complex matrices such as food or sludge.

The simultaneous analysis of different antimicrobials requires chromatographic separation using gas (GC) or liquid chromatography (LC). The first has found limited applicability in antibiotic analysis. Due to the polar nature, low volatility and thermal stability of these drugs, they require derivatization before analysis. Therefore, LC has become the technique of choice for multiclass analysis, especially when coupled to mass spectrometry (MS) and tandem MS (LC-MS^2), the latter allowing increased sensitivity and selectivity in complex matrices [30, 31]. Despite these characteristics, due to the low concentration levels of antibiotics found in most environmental [32] and food [33] samples, the majority of applications require a extraction step, for both sample clean-up and preconcentration, before analysis.

In this review we present an overview of recent developments in the analysis of multiclass antibiotic residues in environmental and food samples. Most of the references included herein correspond to methods published in the last five years for the analysis of more than two antimicrobial classes. We discuss the main sample treatment methods, as well as the analytical procedures reported for multiresidue determination in the selected matrices, emphasizing their analytical characteristics and future prospects.

Analytical methodology

Sample preparation

The aim of sample preparation procedures for single-class residue analysis is to obtain the maximum recoveries of the analytes in a given matrix. In contrast, during the development of multiclass methods, recovery optimization remains an important but secondary issue, because the main goal is the simultaneous extraction of as many analytes from multiple classes as possible. Obviously, the more variation there is in their physicochemical properties (e.g., pK_a, polarity, solubility, stability, etc.), the greater the difficulty in finding a generic extraction procedure for all analytes with acceptable recoveries of them. In addition, the complexity of some matrices, especially when considering food samples, requires clean-up steps or dilution of the extracts prior to LC-MS determination in order to avoid matrix effects [30]. Thus, up to now, multiclass methods have been relatively scarce compared to multimatrix or single-class residue methods. However, the situation is changing rapidly, and the availability of straightforward multiclass extraction procedures is increasing in the literature due to the interest in applying them to routine monitoring programs that would drastically reduce the time and effort devoted to sample preparation. Whenever possible, sample preparation procedures for multiclass analysis should be as simple as possible in order to achieve high sample throughput.

Environmental matrices

Multiclass antibiotic analysis has mainly been carried out in environmental water samples and, to a lesser extent, in sludge and soil, which are much more complex matrices. Sample treatment methods for liquid samples (e.g., surface water, groundwater or drinking water) are usually based on solid-phase extraction (SPE) using polymeric sorbents, although other techniques such as lyophilization or liquid–liquid extraction (LLE) have also been reported [34, 35]. Prior to SPE, the samples are filtered to remove particles in suspension. The relatively low antibiotic concentrations predicted in environmental waters require preconcentration factors that are typically of the order of 1000, and sample volumes are usually in the range 100–1000 mL [30]. Tables 1 and 2 summarize the sample preparation procedures as well as the analytical methods available for the determination of multiclass antibiotics in environmental samples.

Table 1 Summary of methods used for the extraction of antibiotics in environmental samples

Compounds	Matrix	Pretreatment	Treatment/SPE	Recoveries (%)	References
7 SAs, 3 MCs, 7 FQs, 6 TCs, TMP	CDW	Addition of ascorbic acid, 25 mg/L Filtration (0.45 μg nylon filters) Addition 4 mL 2.5 g/L Na_2EDTA per litre of sample	Sample pH 3.0. Offline SPE: Oasis HLB Sample volume 500 mL	86 - 125% for MCs, FQs. TMP and most TCs	[12]
3 SAs, TMP, OTC, ENRO, PEN G	WW	Filtration (Whatman filter paper + 0.45 μm nylon membrane filter)	Sample pH 4.0. Offline SPE: Oasis HBL. Sample volume: 100 mL. Preconcentration: ×100	11.2–97.9%; RSD≤10.1%	[36]
4 MCs, 6 SAs + NAc-SMX, TMP	EFFL	Filtration (0.45 μm cellulose nitrate filters). Addition of 1 g NaCl	Sample pH 4.0 (H_2SO_4). Offline SPE: Oasis HLB. Sample volume: 50 mL (1° EFFL); 250 mL (2° and 3° EFFL). Concentrations: ×25 and ×500, respectively	78–124% except TMP (30–47%); RSD<15%	[37]
5 SAs, 4 MCs, 3 FQs, TMP	WW, RW	Filtration (2.7 μm glass fiber filter GF/D)	Sample pH 3.0 (formic acid). Offline SPE: Oasis HLB. Sample volume: Raw WW, 100 mL; WW effluent, 200 mL and RW, 500 mL	RW: 72–116%. Secondary effluent: 49–119%. Primary effluent: 53–112%; RSD≤11%	[38]
3 SAs, 2 FQs, 3 TCs, 1 LCs, 3 MCs, TMP	SW, GW, WW	Filtration (1.2 μm filter + 0.2 μm filter). Addition of 2 mL 5% EDTA	Sample pH 3.0 (40% orthophosphoric acid). Offline SPE: Oasis HLB. Sample volume: 500 mL. Concentration: ×500	SW: 74–129%; RSD≤13%. GW: 51–120%; RSD≤11%. WW: 75–126%; RSD≤23%	[39]
CAP, OFLO, 2 SAs	WW, GW, RW, TW	Centrifuge 1500 rpm, 15 min filtration (0.7 μm, 47 mm i.d. glass microfiber filter)	Sample pH 3.0 (HCl). Offline SPE: Oasis HLB. Sample volume: 100/250 mL (spiked samples/WW)	64–99%; RSD≤9%	[42]
3 SAs, TMP, 3 FQs, CAP	Urban waters	Addition of sodium azide (0.5 g L^{-1}). Filtration (glass fiber filter)	Addition of sodium chloride (2.93 g/L). Sample pH 4.2 (HCl). Offline SPE: HLB cartridge. Sample volume: 350–1000 mL	WWE: 63–126%; RSD≤12%. WWI: 65–112%; RSD≤30%	[43]
ORN, TMP, 2 SAs, 13 FQs	RW	Filtration (0.47 μm glass fiber filter)	Sample pH 7.0 (orthophosphoric acid 25%). Offline SPE: Oasis HBL. Sample volume: 100 mL. Preconcentration: ×200	70–120%; RSD<15%	[44]
3 MCs, 6 FQs + 2 QdNOs, 16 SAs, 4 TCs	WW	Filtration (0.1 μm glass microfiber filters)	Offline SPE: Oasis HLB cartridges. Sample volume: 1000 mL. Concentration: ×1000. Method a (macrolides): sample pH 6.0 (3.0 M H_2SO_4). Method b (all others): sample pH 3.0 (3.0 M H_2SO_4) + 0.5 g EDTA	72–99%; RSD≤10%	[46]
1 PC, 2 FQs, 6 MCs, 1 LCs, 1 SA, 1 TC (+ oPs)	STPs	Filtration (glass microfiber filter GF/D, 2.7 μm)	Offline SPE: a) sample pH 2 (37% HCl) + EDTA + OASIS MCX; b) sample pH 7 (30% NH_4OH) + LiChrolut EN. Sample volume: 500 mL. Preconcentration: ×5000	31–131%; RSD≤7.6%	[47]
ERY, CAP, SMX (+ oPs)	SW, GW, DW	Filtration	Sample pH 3.0 (HCl). Offline SPE: Oasis MCX. Sample volume: 100 mL. Preconcentration: ×200	63–96%; RSD≤16%	[48]
SMX, CAP (+ oPs)	SW	Filtration (0.7 μm glass fiber filter GF/F)	Sample pH 2.0 (31% HCl). Offline SPE: Oasis MCX. Sample volume: 1 L	SMX: 27.2%; RSD 4.5%. CAP: 37.0%; RSD 1.4%	[49]
TMP, SMX, AMOX, CAP, ERY, MET (+ oPs)	SW, WW	Filtration: SW, 0.7 μm glass fiber filter GF/F WW, GF/D 2.7 μm glass fiber filter + 0.7 μm glass fiber filter GF/F	Sample pH 2.0 (31% HCl). Offline SPE: Oasis MCX. Sample volume: SW, 1000 mL; WW, 250 mL. Concentrations: ×2000 and ×500, respectively	Ultrapure water: 35–104%. SW: 27–84%. WWE: 7–76%. WWI: 4–69%; RSD≤12%	[50]
3 TCs, 4 SAs, TMP, 2 LCs, 4 MCs, 2 PCs, 4 FQs (+ oPs)	SW	Filtration (0.7 μm TCLP glass fiber filter): 250 mL + 0.2 g Na_2EDTA + H_2SO_4 or NH_4OH (pH 5.0)	Offline SPE: Strata–X. Sample volume: 250 mL	51–109%; RSD≤24%	[51]
SMX, NAc-SMX, TMP, ERY (+ oPs)	WWE SW	Filtration (0.45 μm glass fiber filter GFC)	Sample pH 3 (HCl). Offline SPE: Phenomenex Strata X	56–123%; RSD≤20%	[52]
10 FQs, 6 PCs	GW, SW	Centrifuge 3500 rpm, 5 min	Sample pH 2.5 (automated acidification, 700 μL 10%	GW: 57–123%; RSD≤11%.	[53]

Table 1 (continued)

Compounds	Matrix	Pretreatment	Treatment/SPE	Recoveries (%)	References
		(if suspended particulate matter is visible), 200 μL NH_4COOH 500 mM/12 mL sample	HCOOH): a) on-line SPE: C18, sample volume: 9.8 mL; b) offline SPE	SW: 34–117%; RSD≤24%	
3 FQs, 1 CE, 2 PCs, 1 SA, 1 NTI, 1 TC, TMP	Sewage water	Filtration (0.45 μm MF membrane filter)	Sample pH 3 (H_2SO_4). Offline SPE: layered C2/ENV + sample volume 200–500 mL. Concentration: ×200–500)	48–90%; RSD≤12%	[54]
4 FQs, 3 SAs, TMP	WW	Addition of 2 mg/L $Na_2S_2O_3$. Filtration (0.5 μm glass fiber filter). Addition of 0.1 M NaCl	Sample pH 2.5 (H_3PO_4). Offline SPE: anion-exchange Isolute + Oasis HLB. Sample volume: 1000 mL. Concentration: ×1000	Deionized water: 84–102%. Secondary effluent: 37–124%. Final effluent: 64–114%; RSD 10–56%	[55]
3 MCs, 2 SAs, 4 TCs, 7 PCs, TMP, CAP	Water	Filtration with 0.45 μm glass fiber filter	Method a: lyophilization: 1 mg/mL EDTA; method b: offline SPE (excep tetracyclines), sample pH 3.0 (H_2SO_4), Lichrolute EN + Lichrolute C18. Sample volume: 1000 mL. Concentration: ×1000)	Lyophilization: 54–108%. SPE: 15–120%; RSD≤15%	[56]
4 TCs, 8 MCs, 3 SAs, 6 FQs, TMP, CAP, MET, NIF (+ oPs)	Environmental waters	Filtration (1 μm glass fiber filters + 0.45 μm nylon membrane filters) Sample: 500 mL (SW); 200 mL (WWE); 100 mL (influent wastewater, WWI) Addition of 5% EDTA (0.1% in the sample) 10 mL SW 4 mL WWE 2 mL WWI	Offline SPE: Oasis HLB	SW 54–135% WWE 31–121% WWI 21–151% RSD ≤25%	[58]
TMP, MET, ERY (+oPs)	Hospital WWEs	Filtration (0.7 μm glass fiber filter)	Sample pH 7.0 (2 M H_2SO_4). Offline SPE: Oasis HBL. Sample volume: 100 mL. Preconcentration: ×100	TMP: 87.9%; RSD 1.4%. MET: 83.8%; RSD 4.9%. ERY: 95.2%; RSD 2.4%	[59]
2 MCs, SMX, TMP, OFLO (+oPs)	SW, WWI, WWE	Filtration (1 μm glass fiber filter + 0.45 μm nylon membrane filter)	No sample pH adjustment. Offline SPE: Oasis HBL. Sample volume: 500 mL (SW); 200 mL (effluent); 100 mL (influent). Preconcentrations: ×500, ×200, ×100, respectively	SW: 30–108%; RSD≤9%. WWE: 30–116%; RSD≤15%. WWI: 40–111%; RSD≤9%	[60]
ERY, AZI, SMX, TMP, OFLO (+oPs)	GW, RW, WWI, WWE	Filtration (1 μm glass fiber filter + 0.45 μm nylon membrane filter)	Offline SPE: Oasis HLB. Sample volume: 500 mL ground and RW; 200 mL effluent; 100 mL influent. Preconcentrations: ×500, ×200 and ×100, respectively	Not clearly reported	[61]
13 FQs, 32 SAs, 12 PCs, 19 MCs, 5 TCs (+ other veterinary drugs)	Urine	Sample dilution 1:10 with 5% acetonitrile	–	4–377%	[62]
3 MCs, 3 SAs, TMP (+ oPs)	River sediment	Autoclave. USE 50 g sample 2 x 45 mL MeOH + 45 mL acetone + 45 mL ethyl acetate Rotary-evaporation at 40 °C, 150-200 mbar	Offline SPE: Lichrolute EN + Lichrolute C18	Absolute recovery: 41–82% RSD ≤16% Relative recovery: 71–121% RSD ≤23%	[63]
6 TCs, 5 SAs	WWI, WWE	Centrifugation at 3000 rpm × 40 min, 4 °C (influent). Filtration with 0.4 μm glass fiber filter. Addition of 1 mL 5% EDTA + 30 mL 0.1 M citric acid	Sample pH <3.0 (40% H_2SO_4). Offline SPE: Oasis HLB. Sample volume: 120 mL. Concentration: ×1000	Deionized water: 94.6–101%; RSD≤10.4%. WWI: 77.9–99.8%; RSD ≤ 13.9%. WWE: 83.6–103.5%; RSD ≤ 13.4%	[64]

5 SAs, TMP, 3 MCs, OME, RAN	Sludge	Lyophilization of sample	PLE: 5 g sample + aluminium oxide; water (pH 3)–MeOH (1:1, v/v), 1500 psi, 80 °C, 5 min preheating, 60% flush volume, 120s nitrogen purge and 1 extraction cycle (5 min)	54–95%; RSD≤15%	[66]
3 MCs, 4 SAs, TMP	Activate sludge Digested sludge	1. Lyophilisation 2. Grind in a mortar 3. PLE 200 mg sample + quartz sand; MeOH/H_2O (1:1, v/v), 100 bar (1450 psi), 100 °C, 5 min preheating time and 5 min static time, 120% flush volume and 3 extraction cycles (5 min per cycle), 60 s nitrogen purge 4. Diluted sludge extract. (Sample pH 4 or 7)	Offline SPE: Oasis HLB	Absolute recovery: 41–95% RSD ≤6%	[69]
9 SAs, 2 PCs	Sludge	Lyophilization of sample PLE: 5 g sample + 1 g Na_2EDTA + Hydromatrix; acetone–MeOH (1:1, v/v), 1500 psi, 75 °C, 60% flush volume and 3 extraction cycles	Evaporation of PLE extracts under nitrogen stream. Reconstitution into MeOH–H_2O (1:19, v/v). Offline SPE: Oasis HLB	1–85%; RSD<54%	[70]
TMP, SMX, TRI, (+oPs)	Soils, digested sludge	Lyophilization of sample PLE: 2.7 g sample + analytical grade sea sand; MeOH–H_2O (1:1, v/v), 1500 psi, 60 °C, 5 min heat time, 100% flush volume and 2 extraction cycles (5 min per cycle), 60 s nitrogen purge	Sample pH 5.5 (formic acid or ammonia solutions). Offline SPE: Waters Oasis HLB	85–130%; RSD<13%	[71]
2 TCs, 1 SA, 2 MCs	Agricultural soils	Air dried. PLE: 10 g sample + 10 g Ottawa sand; MeOH/citric acid 0.2 M, pH 4.7 (1:1, v/v); 1500 psi; RT; 10 min preheating; 1 extraction cycle	Offline SPE: SAX and HLB in tandem	Sandy soil 45.4–134.4% RSD ≤67% Loamy sand soil 31.3–126.6% RSD ≤32%	[72]
4 TCs, 2 FQs	Well water, RW, STP	Filtration: STP samples: Rundfilter MN 615 filter paper + 0.45 µm membrane filter. River and well samples: 0.45 µm membrane filter	Sample pH 2.8 (HCl). Offline SPE: Oasis HLB. Sample volume: WWI, 100 mL; WWE, 250 mL; RW and well water, 1000 mL. Concentrations: ×100, ×250 and ×1000, respectively	RW: 88–112%. Well water: 41–87%. WWE: 68–89%. WWI: 73–103%; RSD<15%	[73]
5 TCs, 6 SAs	GW, SW	Filtration (0.7 µm glass fiber filter). Addition of 75 µL 40% H_2SO_4 + 1 mL scoop of EDTA	Sample pH 2.5. Offline SPE: HLB Waters. Sample volume: 123 mL	83.8–130% (except minocycline); RSD<20%	[74]
ERY, SMX, TMP, TRI (+oPs, endocrine disruptors and PCPs)	Water		Sample pH 2.0 (H_2SO_4). Offline SPE: HLB Waters. Sample volume: 1000 mL. Concentration: ×1000	71–91%; RSD≤17%	[75]
5 SAs, 5 TCs, 7 MCs, TMP, CAP, CAR, (+oPs)	Water	Addition of 2 g Na_2EDTA	Sample pH 8.2 (H_2SO_4 or NaOH solution). Offline SPE: HBL Waters. Sample volume: 400 mL. Concentration: ×800	48–150%; RSD≤15%	[76]
TMP, 1 SA, FLOR, OXO	SW	Centrifugation for 5 min at 3000×g	Sample pH 5.0–5.2 (H_3PO_4). Offline SPE: Oasis HLB. Sample volume: 40 mL. Concentration: ×80	78–96%; RSD<11%	[77]
AMOX, TMP, SMX (+oPs)	SW, GW	Filtration (0.7 µm GF/F glass fiber filter)	Offline SPE: Oasis HLB. Sample volume: 1000 mL. Concentration: ×1000	8–123%; RSD<21%	[78]
6 TCs, 6 SAs, 3 MCs	RW	Water samples: Filtration (0.2-µm glass fiber filter)	Offline SPE: Oasis HLB.	RW: TCs: 100–127% SAs: 76–124%	[79]

Table 1 (continued)

Compounds	Matrix	Pretreatment	Treatment/SPE	Recoveries (%)	References
		pH sample 2.0–2.5 (TCs and SAs), 5.0 (MLs) Sediment: Air-dried in the dark. Passed through 2 mm and 75 μm sieves	Sample volume: 120 mL Concentration (x1000)	MLs: 89–114% Sediment: TCs: 40–114% SAs: 62–11% MLs: 53–128%	
3 TCs, 3 SAs, 2 FQs, 3 MCs, TMP	Urban WW	500 mg Na_2EDTA + 5 mL 10% Na_2SO_3 (per 1000 mL sample) Filtration (0.45 μL acetate cellulose filter)	Sample pH 2.8-3.0. Online SPE: Oasis HLB. Sample volume 50 mL	Deionized water: 94–120% RSD ≤25% WW (effluent): 40–92% RSD ≤17%	[80]
13 FQs, 2 SAs, ORN, TMP	MW, SW	Filtration (0.45 μm glass fiber filter)	Sample pH 7. Offline SPE: Oasis HLB	70–130% RSD ≤20%	[81]
4 SAs, 4 FQs, 4 TCs, CAP	GW, LW, WWI, WWE	Filtration (1 mm filter + 0.45 μm glass fiber filter) Addition of 1% formaldehyde Addition of 5 mL 5% (w/v) EDTA	Sample pH 4.0. Offline SPE: Oasis HLB Sample volume: GW: 500 mL; LW: 250 mL; piggery wastewater: 50 mL	GW: 76.9–115.1% LW: 68.6–92.6% WWE: 62.5–93.1% WWI: 58.4–98.7% RSD ≤10%	[82]

AMOX, amoxicillin; *AS*, agricultural soils; *AZI*, azithromycin; *CAP*, chloramphenicol; *CAR*, carbadox; *CDW*, chlorinated drinking water; *CEs*, cephalosporines; *DW*, drinking water; *EFFL*, effluents of municipal wastewater treatment plants; *ENRO*, enrofloxacin; *ESI-MS*^{2}, electrospray ionization–tandem mass spectrometry; *ERY*, erythromycin; *FLOR*, florfenicol; *FQs*, fluoroquinolones; *GW*, groundwater; *HLB*, hydrophilic–lipophilic balance; *LCs*, lincosamides; *LW*, lake water; *MCs*, macrolides; *MET*, metronidazole; *MW*, mineral water; *NAc-SMX*, N-acetyl sulfamethoxazole; *NIF*, nifuroxazide; *NMZs*, nitroimidazoles; *NTIs*, nitroimidazoles; *OFLO*, ofloxacin; *OME*, omeprazole; *oPss*, other pharmaceuticals; *ORN*, ornidazole; *OTC*, oxytetracycline; *OXO*, oxolinic acid; *PCs*, penicillins; *PEN G*, penicillin G; *PCPs*, personal care products; *PLE*, pressurized liquid extraction; *QdNOs*, quinoxaline dioxides; *Qs*, quinolones; *RAN*, ranitidine; *RW*, river water; *SAs*, sulfonamides; *SMX*, sulfamethoxazole; *SPE*, solid-phase extraction; *STPs*, sewage treatment plants; *SW*, surface water; *TCs*, tetracyclines; *TFA*, trifluoroacetic acid; *TMP*, trimethoprim; *TRI*, triclosan; *TW*, tap water; *USE*, ultrasound-assisted solvent extraction; *WW*, wastewater; *WWE*, effluent WW; *WWI*, influent WW

a) Waste water, surface water and groundwater

Babic et al. [36] have described the preconcentration of three SAs, a sulfonamide synergist, trimethoprim (TMP), a TC, a FQ and a β-LC from wastewater of a pharmaceutical company using Oasis HLB cartridges. Recoveries of between 89.3% and 97.9% were obtained, except for penicillin G/procaine (68.3%) and sulfaguanidine (11.2%). Reported intraday and interday reproducibilities were lower than 8.4% and 10.1%, respectively.

Göbel et al. [37] applied HLB sorbents for the extraction of four MC antibiotics, six SAs, the human metabolite of sulfamethoxazole, N^4-acetylsulfametoxazole, and TMP in primary effluents (1° EFFL, after mechanical treatment), secondary effluents (2° EFFL, after biological treatment), and tertiary effluents (3° EFFL, after sand filtration). The most influential parameter for sample extraction was pH, which had a significant impact on the retention of the compounds, especially on SAs; due to their amino groups, SAs showed their highest recoveries at pH 4, whereas the recoveries of MCs and TMP showed almost no pH dependence. Under these conditions, the metabolite N^4-acetylsulfametoxazole was stable during sample treatment, but erythromycin was unstable, transforming into erythromycin-H_2O. Recoveries of the antibiotics in the different sample matrices were above 80% (RSD≤18%), except for TMP (30–47%), due to the use of a nonideal surrogate sulfamethazine-phenyl-$^{13}C_6$ ($^{13}C_6$SMZ) for its determination.

Senta et al. [38] compared the performances of three popular extraction cartridges: Envi C_{18}, a classical lipophilic sorbent, and two polymeric SPE phases, Oasis HLB and Strata X, for the extraction of three classes of antimicrobials, including six SAs, TMP, three FQs and five MCs. Higher recoveries were achieved with the polymeric cartridges (>66%), illustrating the importance of the hydrophilic groups in the retention mechanism. In comparison to the protocol described by Göbel et al. [37], the optimized retention pH for all of the antibiotic classes was 3.0, and on this occasion it was also crucial to the retention of FQs. The use of basic conditions (1% NH_3 in methanol) during the elution step allowed higher extraction recoveries, especially for MC antibiotics.

Batt et al. [39] developed an SPE-LC-MS^2 method for the analysis of 13 antibiotics and caffeine in surface waters and wastewaters. The selected compounds were representative of different classes used in both human and veterinary medicine, including TCs, FQs, SAs, LCs, and MC antibiotics, which are expected to have different environmental fates and effects. Caffeine monitoring was also included, as it behaves as an anthropogenic marker of untreated domestic wastewater contamination, and its presence in surface waters is indicative of untreated sewage overflows. The antibiotics were preconcentrated using Oasis HLB cartridges, as in the previous papers, but the sample pH was adjusted to between 2.8 and 3.0 to increase the retention of the TCs and FQs, which decreases at neutral pH. However, below pH 7.0 erythromycin is immediately degraded to erythromycin-H_2O, which is quantified in the extracts instead of the parent molecule. The recoveries of MC antibiotics were increased by adding a chelating agent, EDTA, that binds to the soluble metals, increasing the antibiotic extraction efficiency. The eluting solvent was ACN instead of MeOH, as it allowed improved recoveries of erythromycin-H_2O and roxithromycin. The optimized SPE-LC-MS^2 procedure was applied to the extraction of these compounds from three different matrices (groundwater, surface water and wastewater), and the analysis reported the presence of clindamycin (1.1 μg L^{-1}) in surface water from the Niagara River (US) and multiple antibiotics (0.10–1.3 μg L^{-1}) in wastewater effluents from two different STPs.

Ye et al. [40] reported a multirun analytical method for the trace determination of 24 antibiotics, including seven SAs, three MCs, seven Qs/FQs, six TCs, and TMP, in chlorinated drinking water using SPE followed by LC-MS^2 analysis. Some antibiotics, sulfamethoxazole, ciprofloxacin, enrofloxacin and erythromycin, can react with free chlorine and are expected to undergo substantial transformations under extended exposure to residual chlorine in finished drinking water [41]. Ascorbic acid was added to the samples to increase the storage time, as it quenches residual chlorine without affecting the analysis and stability of the antimicrobials. Preconcentration was carried out in the HLB cartridges with sample loading at pH 3.0, and elution in acidified methanol (0.1% formic acid) to increase the recovery of the piperazinylic quinolones. All antibiotics were successfully extracted from the drinking water samples with recoveries ranging from 90 to 106%. However, TC and SA losses were observed after solvent evaporation before the chromatographic analysis. The TC losses were attributed to antibiotic sorption on the glass surfaces, but for the SAs, the lower recoveries were explained by their possible association with matrix components that precipitate during solvent reduction and are removed by filtration before analysis.

HLB cartridges have also been applied in several studies to evaluate the occurrence and fate of multiclass antibiotics in environmental water samples in several countries. For example, the occurrence of SAs, FQs, chloramphenicol (CAP) [42] and TMP [43] in urban river water and wastewater in Guangzhou, China, was evaluated by SPE followed by LC-DAD/FLD, indicating that activated sludge treatment is effective at and necessary for removing antimicrobial substances from municipal sewage.

These cartridges have also been applied to the analysis of 17 antibiotics, belonging to four groups, Qs, SAs, NMZs

Table 2 Summary of multiclass LC-MS methods used for antibiotic residue analysis in environmental samples

Compounds	Matrix	LC		MS		Sensitivity		References
		Column	Mobile phase	Type	Ionization	LOD	LOQ	
7 SAs, 3 MCs, 7 FQs, 6 TCs, TMP	CDW	Pursuit C-18 (150×2 mm, 3 μm)	Formic acid and ACN (gradient)	QqQ	ESI (+)	0.5–6 ng L^{-1}	1-36 ng L^{-1}	[12]
3 SAs, TMP, OTC, ENRO, PEN G	WW	Lichrosphere 100 CN (125×4.0 mm, 5 μm)	10 mM oxalic acid and ACN (gradient)			0.1–40 μgL^{-1}	1.5–100 μg L^{-1}	[36]
4 MCs, 6 SAs + NAc-SMX, TMP	EFFL	YMC Pro C18 (150×2 mm, 3 μm)	1% formic acid and MeOH (gradient)	QqQ	ESI (+)		3–214 ng L^{-1}	[37]
5 SAs, 4 MCs, 3 FQs, TMP	WW, RW	YMC ProC18 (150×2.1 mm, 3 μm)	0.1% formic acid and MeOH (gradient)	QqQ	ESI (+)	1–13.2 ng L^{-1}		[38]
3 SAs, 2 FQs, 3 TCs, 1 LCs, 3 MCs, TMP	SW, GW, WW	BetaBasic-18 C18 (100×2.1 mm, 3 μm)	0.3% formic acid, MeOH and ACN (gradient)	IT	ESI (+)	27–190 ng L^{-1}	100–650 ng L^{-1}	[39]
CAP, OFLO, 2 SAs	WW, GW, RW, TW	(a) Zorbax SB-C18 (100×2.1 mm, 3.5 μm); (b) Zorbax Eclipse XDB-C18 (150×3.0 mm, 3.5 μm)	(a) 0.1% AcH and ACN (gradient); (b) ACN and 50 mM NH_4Ac/HAc (gradient)				20–500 ng L^{-1}	[42]
3 SAs, TMP, 3 FQs, CAP	Urban waters	Zorbax Eclipse XDB-c18 (150×3.0 mm, 3.5 μm)	0.05% formic acid and ACN (gradient)				35–100 ng L^{-1}	[43]
ORN, TMP, 2 SAs, 13 FQs	RW	Acquity UPLC BEH C18 (100×1 mm, 1.7 μm)	0.01% formic acid and ACN (gradient)	QqQ	ESI (+)		10 ng L^{-1}	[44]
3 MCs, 6 FQs + 2 QdNOs, 16 SAs, 4 TCs	WW	(a) and (d): Genesis C18 (50×2.1 mm, 3 μm); (b) and (c): Genesis C18 (150×2.1 mm, 3 μm)	(a) ACN, 20 mM NH_4Ac, 0.05% formic acid (gradient); (b) and (c) ACN, 20 mM NH_4Ac, 0.1% formic acid (gradient); (d) ACN, 20 mM NH_4Ac, 0.1% formic acid and 4 mM oxalic acid (gradient)	QqQ	ESI (+)	1–8 ng L^{-1}		[46]
1 PC, 2 FQs, 6 MCs, 1 LCs, 1 SA, 1 TC (+oPs)	STPs	Luna C8 (50×2 mm, 3 μm)	(a) 0.1% formic acid and ACN; (b) 0.05% TEA and ACN (gradient)	QqQ	ESI (+), ESI (–)		0.15–2.08 ng L^{-1}	[47]
ERY, CAP, SMX (+oPs)	SW, GW, DW	RP-18 Waters XTerra (100×2.1 mm, 3.5 μm)	MeOH and 2 mM NH_4Ac (gradient)	(a) QqQ; (b) QTOF	ESI (+), ESI (–)		5–10 ng L^{-1}	[48]
SMX, CAP (+oPs)	SW	Acquity UPLC BEH C18 (100 mm x 1 mm, 1.7 μm)	MeOH, 0.5% AcH and 10 mM TrBA (gradient)	QqQ	ESI (–)	0.05 ng L^{-1}	20 ng L^{-1}	[49]
TMP, SMX, AMOX, CAP, ERY, MET (+oPs)	SW, WW	Acquity UPLC BEH C18 (100×1 mm, 1.7 μm)	MeOH and AcH (gradient)	QqQ	ESI (+), ESI (–)	0.1–2.5 ng L^{-1}	0.2–87 ng L^{-1}	[50]
3 TCs, 4 SAs, TMP, 2 LCs, 4 MCs, 2 PCs, 4 FQs (+oPs)	SW	Supelco Discovery HS C19 (150×4.6 mm, 3 μm)	0.1% formic acid and ACN (gradient)	QqQ	ESI (+), ESI (–)		0.5–98 ng L^{-1}	[51]
SMX, NAc-SMX, TMP, ERY (+oPs)	WWE, SW	C18 Luna (250×2 mm, 5 μm)	40 mM NH_4Ac, formic acid and MeOH (gradient)	IT	ESI (+)	10–5 ng L^{-1}		[52]
10 FQs, 6 PCs	GW, SW	Kromasil C18 (100×2.1 mm, 5 μm)	MeOH, 0.1% formic acid (gradient)	(a) QqQ; (b) QTOF	ESI (+)	0.4–4.3 ng L^{-1}		[53]
3 FQs, 1 CE, 2 PCs, 1 SA, 1 NTI, 1 TC, TMP	Sewage Water	YMC Hydrosphere C18 (150×4.6 mm, 5 μm)	0.1% formic acid and ACN (gradient)	IT	ESI (+)		0.01–0.68 ng L^{-1}	[54]
4 FQs, 3 SAs, TMP	WW	Zorbax SB-C18 (150×2.1 mm, 5 μm)	1 mM NH_4Ac, 0.007% AcH and ACN (gradient)	Q	ESI (+)	2–90 ng L^{-1}		[55]
3 MCs, 2 SAs, 4 TCs, 7 PCs, TMP, CAP	Water	(a) Macherey and Nagel LiChrospher 100 RP-8 end-capped (125×3 mm, 5 μm) ; (b) and (c) Merck LiChrospher 100 RP-18 end-capped (125×3 mm, 5 μm)	(a) 10 mM oxalic acid and ACN (gradient); (b) and (c) 10 mM NH_4Ac and ACN (gradient)	QqQ	ESI (+), ESI (–)		20–50 ng L^{-1}	[56]
4 TCs, 8 MCs, 3 SAs, 6 FQs, TMP, CAP, MET, NIF (+oPs)	Water	Purospher Star RP-18 endcapped (125×2.0 mm, 5 μm)	(a) Water, ACN and MeOH (gradient); (b) 0.1% formic acid and ACN (gradient)	QqLIT	ESI (+), ESI (–)	0.02–24 ng L^{-1}	0.1–74 ng L^{-1}	[58]

TMP, MET, ERY (+oPs)	Hospital WWEs	Purospher Star RP-18 endcapped (125×2.0 mm, 5μm)	ACN and 0.1% formic acid (gradient)	QqQ	ESI (+)	3.8–40 ng L^{-1}	11–112 ng L^{-1}	[59]
2 MCs, SMX, TMP, OFLO (+oPs)	SW, WWI, WWE	Purospher Star RP-18 endcapped (125×2.0 mm, 5μm)	ACN, MeOH and 5 mM NH_4Ac/AcH (gradient)	QqQ	ESI (+)	1–43 ng L^{-1}	3–120 ng L^{-1}	[60]
ERY, AZI, SMX, TMP, OFLO (+oPs)	GW, RW, WWI, WWE	Waters Acquity C18 (50×2.1 mm, 1.7μm)	5 mM NH_4Ac/AcH, ACN and MeOH (gradient)	QTOF	ESI (+)	10–500 ng L^{-1}		[61]
13 FQs, 32 SAs, 12 PCs, 19 MCs, 5 TCs (+ other veterinary drugs)	Urine	Waters Acquity C18 MS (50×2.1 mm, 1.7μm)	Formic acid and ACN (gradient)	TOF	ESI (+)		0.2–45 μg L^{-1}	[62]
3 MCs, 3 SAs, TMP (+oPs)	River sediment	LiChrospher RP-18 (125×3 mm, 5μm)	20 mM NH_4Ac and ACN (gradient)	QqQ	ESI (+)		3–20 μg kg^{-1}	[63]
6 TCs, 5 SAs	WWI, WWE	Xterra MS C18 (50×2.1 mm, 2.5μm)	0.1% formic acid and ACN (gradient)	IT	ESI (+)	0.03–0.07 ng L^{-1}		[64]
5 SAs, TMP, 3 MCs, OME, RAN	Sludge	Kromasil 100 C18 (250×4.6 mm, 5μm)	AcH and ACN (gradient)	Q	ESI (+)	2–8 μg kg^{-1}	20–100 μg kg^{-1}	[66]
4 MCs, 5 SAs, TMP	Sludge	Chromolith Performance RP-18e (100 x 4.6 mm)	10 mM NH_4Ac and ACN (gradient)	QqQ	ESI (+)		3–41 μg kg^{-1}	[69]
6 SAs, 2 PCs	Sludge	Atlantis C18 LC (150×2.1 mm, 3μm)	1% formic acid and ACN (gradient)	QqQ	ESI (+)	1–270 ng kg^{-1}	5–590 ng kg^{-1}	[70]
TMP, SMX, TRI (+ oPs)	Soils, digested sludge	Waters Sunfire (150×2.1 mm, 3.5μm)	10 mM NH_4Ac and ACN (gradient)	IT	ESI (+)	3–150 ng L^{-1}		[71]
2 TCs, 1 SA, 2 MCs	AS	Xterra MS-C18 (100×2.1 mm, 3.5μm)	80 mM formic acid and MeOH (gradient)	QqQ	ESI (+)	0.4–5.6 μg kg^{-1}	1.1–12.8 μg kg^{-1}	[72]
4 TCs, 2 FQs	Well water, RW, STP	Kromasil 100 C18 (250×4.6 mm, 5μm)	AcH and ACN (gradient)	Q	ESI (+)	4–6 ng L^{-1}		[73]
5 TCs, 6 SAs	GW, SW	Luna C8 (100×4.6 mm, 3μm)	10 mM ammonium formate, 0.3–0.5% formic acid and MeOH (gradient)	Q	ESI (+)		100 ng L^{-1}	[74]
ERY, SMX, TMP, TRI (+ oPs, endocrine disruptors and PCPs)	Water	Synergi Max RP (250×4.6 mm, 4μm)	0.1% formic acid and MeOH (gradient)	QqQ	ESI (+), ESI (–)			[75]
5 SAs, 5 TCs, 7 MCs, TMP, CAP, CAR (+ oPs)	Water	(a) Genesis (150×2.1 mm); (b) Apex (150×2.1 mm)	(a) 0.015% HFBA, 0.5 mM NH_4Ac, MeOH and ACN (gradient); (b) NH_4Ac and ACN (gradient)	QqQ	ESI (+), ESI (–)		0.03–1.4 μg L^{-1}	[76]
TMP, 1 SA, FLOR, OXO	SW	Luna C18 (150×4.6, 5μm)	0.1% formic acid and MeOH (gradient)	QqQ	APCI (+)	0.5 1.0 ng L^{-1}	0.7–1.8 ng L^{-1}	[77]
AMOX, TMP, SMX (+ oPs)	SW, GW	Metasil Basic C18 (150×2.0 mm, 3μm)	10 mM ammonium formate/formic acid and ACN (gradient)	Q	ESI (+)	14–30 ng L^{-1}		[78]
6 TCs, 6 SAs, 3 MCs	RW, river sediment	Xterra MS C18 (50×2.1 mm, 2.5μm)	0.1% formic acid and ACN (gradient)	IT	ESI (+)	0.03–0.07 ng L^{-1}		[79]
2 TCs, 3 SAs, 2 FQs, 4 MCs, TMP	Urban WW	BetaBasic C18 endcapped (150×2 mm, 3μm)	0.1% formic acid, 0.1% TFA and ACN (gradient)	IT	ESI (+)	1–46 ng L^{-1}	4–152 ng L^{-1}	[80]
13 FQs, 2 SAs, 1 NTI, TMP	MW, SW	Acquity UPLC BEH C18 (100×2.1 mm, 1.7μm)	0.01% formic acid and ACN (gradient)	QqQ	ESI (+)	0.2–7.7 ng L^{-1}	0.8–25.5 ng L^{-1}	[81]
4 SAs, 4 FQs, 4 TCs, CAP	GW, LW, WWI, WWE	Dionex Acclaim C18 (150×2.1 mm, 4.6μm)	(a) 0.1% formic acid and ACN (gradient); (b) water and ACN (gradient)	QqQ	ESI (+), ESI (–)		0.8–104.4 ng L^{-1}	[82]

AcH, acetic acid; *AMOX*, amoxicillin; *APCI*, atmospheric pressure chemical ionisation; *AS*, agricultural soils; *AZI*, azithromycin; *CAP*, chloramphenicol; *CAR*, carbadox; *CDW*, chlorinated drinking water; *CEs*, cephalosporines; *DW*, drinking water; *EFFL*, effluents of municipal wastewater treatment plants; *ENRO*, enrofloxacin; *ESI-MS*^{2}, electrospray ionization–tandem mass spectrometry; *ERY*, erythromycin; *FLOR*, florfenicol; *FQs*, fluoroquinolones; *GW*, groundwater; *HFBA*, heptafluorobutyric acid; *IT*, ion trap; *LCs*, lincosamides; *LW*, lake water; *MCs*, macrolides; *MET*, metronidazole; *MW*, mineral water; *NAc-SMX*, N-acetyl sulfamethoxazole; *NH_{Ac4}*, ammonium acetate; *NIF*, nifuroxazide; *NMZs*, nitroimidazoles; *NTIs*, nitroimidazoles; *OFLO*, ofloxacin; *OME*, omeprazole; *ORN*, ornidazole; *OTC*, oxytetracycline; *OXO*, oxolinic acid; *PCs*, penicillins; *PEN G*, penicillin G; *QdNOs*, quinoxaline dioxides; *QqQ*, triple quadrupole; *QqLIT*, hybrid triple quadrupole–linear ion trap mass spectrometer; *QTOF*, hybrid quadrupole time of flight; *Qs*, quinolones; *RAN*, ranitidine; *RW*, river water; *SAs*, sulfonamides; *SMX*, sulfamethoxazole; *STPs*, sewage treatment plants; *SW*, surface water; *TCs*, tetracyclines; *TEA*, triethylamine; *TFA*, trifluoroacetic acid; *TMP*, trimethoprim; *TOF*, time of flight; *TrBA*, tributylamine; *TRI*, triclosan; *UPLC*, ultraperformance liquid chromatography; *TW*, tap water; *WW*, wastewater; *WWE*, effluent WW; *WWI*, influent WW

and diaminopyrimidines, in the Seine River inner estuary using ultraperformance liquid chromatography (UPLC) coupled to MS^2 [44]. In a different work, Oasis HLB coupled to LC-MS^2 was used to evaluate the occurrence and distribution of nine selected antibiotics from five different antibacterial families—FQs, SAs, diaminopyrimidines, TCs and MCs—in a small Mediterranean stream (Arc River, Southern France) [45], and thirty-one antimicrobials—MCs, Qs, quinoxaline dioxide, SAs, and TCs—in final (treated) effluents from eight wastewater treatment plants in five Canadian cities [46].

Other cartridges that have also been tested for the enrichment of antibiotics from aqueous samples include Oasis MCX [47–50], Strata X [51, 52], LiChrolut EN [47], C_{18} [53], and mixed-phase C_2/ENV^+ [54].

Oasis MCX is a strong cation-exchange mixed-mode polymeric sorbent that is capable of both cation-exchange and reversed-phase interactions. MCX sorbent is based on HLB copolymer, but the additional presence of sulfonic groups allows for cation-exchange interactions [50]. It can extract acidic, basic and neutral compounds at low pH values. Strata X is styrene-divinylbenzene (SDVB) polymer that has been surface-modified with *N*-methyl-2-piperidone moieties that possess both hydrophobic and hydrophilic properties [51].

The LiChrolut EN cartridge is an ethylvinylbenzene-divinylbenzene copolymer that can extract polar organic compounds. At pH 7, this polymeric sorbent can also retain neutral drugs through hydrophobic interactions. Castiglioni et al. [47] developed a multiresidue analytical method to measure 30 pharmaceuticals belonging to different therapeutic families, including three MCs, four LCs, two FQs, one SA, one TC and one β-LC, in urban wastewaters. Due to the different physicochemical properties of these compounds, SPE was carried out using two different cartridges: a) LiChrolut EN at pH 7.0 for the extraction of seven pharmaceuticals, including three LCs (clarithromycin, erythromycin, spiramycin) and one MC (tylosin); b) Oasis MCX for the rest. Extraction was carried out at pH 1.5–2 with the second cartridge, in the presence of EDTA to prevent the TCs complexing with Ca^{2+} and Mg^{2+} ions and residual metals during the SPE. Recoveries were higher than 70% in the Oasis MCX cartridges, except for amoxicillin, ciprofloxacin and oxofloxacin (36%, 32%, 31%, respectively), and they were slightly lower when using the LiChrolut EN (47–64%). Sample analysis revealed the presence of nineteen pharmaceuticals in the sewage treatment plant at concentrations ranging from 0.5 to 2000 ng L^{-1}, including FQs and SAs.

Pozo et al. [53] have described the application of reversed-phase C18 cartridges, a nonpolar sorbent, for the online SPE of 16 antibiotics (ten FQs and six β-LCs) in water. A notable breakthrough was observed for the FQs that could be reduced by acidifying the samples at pH 2.5 with formic acid. This low pH favored penicillin degradation, and the acid had to be added immediately before loading the sample into the SPE-LC system.

Isolute C_2/ENV^+ solid-phase extraction sorbents are based on highly crosslinked polystyrene polymers that are specially derivatized to obtain a wettable surface. They are specially suited to the extraction of analytes that are highly soluble in water and have been applied [54] to the analysis of FQs, β-LCs (penicillins and cephalosphorins), doxycycline, sulfamethoxazole, TMP and metronidazole in hospital sewage water. This sorbent does not show ion exchange properties, but the authors found that the use of triethylamine in MeOH (instead of MeOH alone) as an eluting solvent significantly improved recoveries (48–90%), probably due to a reduction in the secondary interactions between the analytes and the residual silanol groups on the particle surface. The authors reported the presence of antibiotic concentrations on the order of 100 μg L^{-1} for some antibiotics in the hospital waste waters, and found large temporal variations in the analyte concentrations.

Most of the methods described previously were based on offline SPE procedures, but online procedures have also been reported for multiclass environmental analysis. Feitosa-Felizzola et al. [45] compared an online SPE coupled to LC-MS^2 with the offline mode for the analysis of several classes of antibiotics (TCs, SAs, FQs and MCs) in urban wastewaters. The application of the online mode facilitates method automation, minimizes sample manipulation, and reduces analysis time and organic solvent consumption. However, the sample volume is limited by the system and the enrichment factors can be lower than those obtained with offline SPE.

In some applications, two or more SPE cartridges have been used either in tandem [55–57] or in parallel to preconcentrate different groups of analytes [47]. Nevertheless, in most cases, the use of one cartridge is preferred in an effort to simplify the sample preparation procedure as much as possible for the simultaneous preconcentration of a wide range of pharmaceuticals from different therapeutic groups, rather than using more complex procedures that yield higher recovery rates. In that regard, Gros et al. [58] have evaluated the application of MCX and HLB cartridges in tandem versus the use of the individual cartridges separately for the simultaneous preconcentration of 73 pharmaceutical residues, including 25 antibiotics, mainly from the MC, TC, FQ and SA families, in surface and wastewater. The use of two cartridges did not significantly improve the recovery rates of the target compounds, and Oasis HLB cartridges—with EDTA addition prior to extraction—were selected for sample preconcentration since they provided the best overall recoveries (from 50% up to 100%, with some exceptions). The addition of the

chelating agent had more of an effect than adjusting the sample pH to acidic values (2.5–3) in terms of enhancing the retention of FQs, TCs and MCs in the polymeric sorbent, so samples were loaded at their natural pH in the presence of 0.1% EDTA. The use of an analysis method based on LC-MS^2 and using a hybrid quadrupole–linear ion trap mass analyzer (QqLIT) allowed high sensitivity, selectivity and reliability of results.

In conclusion, the optimization of multiclass antibiotic SPE-based preconcentration methods for the analysis of environmental water samples requires careful selection of both the nature of the cartridge and the extraction conditions. Sample pH is an important consideration for maximizing the retention of the antibiotics, especially those with a strong pH dependence, and avoiding antibiotic degradation [37, 53]. The coextraction of matrix components such as humic and fulvic acids, which complicate the analysis of the more polar antibiotics that elute at the beginning of the chromatogram in particular, also depends on the pH of the extracted sample and has been found to decrease at neutral pH values versus extraction in acidic media [59–62]. Nevertheless, it has been shown that the presence of humic acids can improve the extraction of MC antibiotics from spiked groundwater [63].

Chelating agents (such as EDTA) can be added to the sample prior to analysis to remove residual metals from the matrix or glassware, or those sorbed onto the surface of the cartridge sorbent, which can cause low extraction recoveries of MCs, FQs, and especially TCs [39, 47, 58, 64]. Salt addition (e.g., sodium chloride) can also be applied in order to improve antibiotic extraction efficiency in hydrophilic–lipophilic balance polymers, particularly for SAs and TMP [57, 65].

b) Sewage sludge and sediments

The presence of antibiotics, and pharmaceuticals in general, in solid environmental samples such as sewage sludge or sediments has been studied to a lesser extent than in water samples, probably due to their greater complexity. However, the analysis of antibiotics in these matrices is important, as it allows the eliminating power of sewage treatment plants (SPTs) to be controlled and the safety of the sewage sludge used as manure to be evaluated, and finally the incorporation of antibiotics into the food chain to be avoided (Fig. 3) [66].

The main antibiotic classes that have been measured in solid matrices include SAs, MCs, TCs and FQs [14]. The concentrations of antibiotics observed in solid waste samples seemed to be significantly higher than those in aqueous media, suggesting some form of preconcentration onto the solid samples. These drugs differ considerably in terms of their sorption and fixation in soils due to their different physicochemical properties, such as their molecular structures, sizes, shapes, solubilities and hydrophobicities, and also because the cation exchange properties, cation bridging, surface complexation and hydrogen bonding can change from sample to sample [67]. Their antibacterial power usually decreases after sorption and fixation, but in most cases they still produce some antimicrobial effects, which can influence the selection of antibiotic-resistant bacteria in the terrestrial environment. For example, FQ antibacterials such as ciprofloxacin and norfloxacin have been shown to be sorbed specifically to sewage sludge in waste water treatment plants, and concentrations in the mg kg^{-1} range have been found in digested sludges [68]. TCs have also been shown to bond strongly to soil organic matter due to their ability to form complexes with doubly-charged cations, such as calcium, that occur at high concentrations in soil [14].

Antibiotics may occur together with other potentially interfering compounds that will require further separation before the analysis. Preparative techniques for soils and sludge generally include multistep procedures that first involve the extraction of the antibiotics from the solid samples into the liquid phase. This step can be based on mechanical shaking or ultrasound-assisted solvent extraction (USE) [63, 69], using high volumes of different organic solvents in most cases. Alternatively, pressurized liquid extraction (PLE) has also been applied, and it provides high recoveries for most analytes in a shorter time and with decreased organic solvent consumption [66]. These techniques are further combined with SPE for sample clean-up and preconcentration of the target analytes in order to avoid matrix effects during chromatographic analysis [69, 71].

One of the major challenges in the analysis of solid environmental samples is the preparation of synthetic standards for method characterization and validation. To do this, the analytes must be incubated for a defined time prior to extraction in order to attain sorption–desorption equilibrium. On the other hand, the microbial and enzymatic activities in the sediments may transform the analytes and/or influence their binding to the solid matrix, affecting the extraction yields [32]. In order to minimize the influences of any biotransformation processes, the samples are usually lyophilized before analysis [66, 70]. Other authors have proposed sterilizing the sediments prior to extraction using γ radiation or autoclaving for example [63].

Díaz-Cruz et al. [70] have described the simultaneous determination of eleven antibiotics, nine SAs and two PCs in sludge from infiltration basins. The extraction of antibiotics was performed by PLE, mixing the sieved sludge samples with EDTA and Hydromatrix. A further clean-up step was carried out by SPE with an Oasis HLB cartridge. The

recoveries obtained were lower than 67%, with large RSDs ranging from 9% to 43% (at spiking levels of 1 μg g^{-1}).

Better recoveries were reported by Barron et al. [71] using a similar method based on PLE followed by SPE for the analysis of 27 pharmaceuticals, including four antibiotics (TMP, sulfamethoxazole, sulfamethazine and triclosan) from biosolid-enriched soils and digested sludge. Different commercial SPE cartridges were tested for clean-up, including Waters Oasis HLB, Phenomenex Strata X, Varian Focus, Merck LiChrolut EN and Phenomenex Strata X-CW. Final method recoveries of ≥60% were obtained with the HLB cartridges in soils and digested sludge. The sample extracts were analyzed by LC-MS^2, and it was found that the ion suppression effects arising from matrix components were more pronounced in digested sludge samples than in soils.

Nieto el al. [66] reported a method for the extraction of three MCs, five SAs, ranitidine, omeprazole and TMP in sewage sludge using PLE followed by LC-ESI-MS without further clean-up. Recoveries ranged between 54% and 95% for the analysis of a lyophilized sludge sample spiked at 2 mg/kg. The inclusion of an SPE step that used Oasis HLB cartridges after PLE to preconcentrate the extracts before chromatographic analysis did not improve the antibiotic recoveries, which were lower than 50% with the exceptions of sulfapyridine and tylosin (>68%). This method was applied to the analysis of sludge samples from two STPs, and concentrations of between 1.3 and 4 mg kg^{-1} of roxithromycin and tylosin were obtained. Some others were found in several samples at levels below the limit of quantification, and could be determined with more sensitive detectors such as MS–MS.

An alternative extraction technique has been optimized by Löffler et al. [63] using USE for the determination of three MCs, three SAs and TMP in river sediments. Antibiotics were extracted first with MeOH, and then by acetone and ethyl acetate, and the collected supernatants were rotaevaporated and reconstituted with deep ground water, which is supposed to be free of organic contaminants. The extracts were spiked with a constant amount of humic acids to favor the extraction of the MC antibiotics, and preconcentrated using a mixture of LiChrolute EN and LiChrolute C_{18} as sorbent [56]. The SPE extracts were analyzed by LC-MS^2. The performance of the method at low spiking levels (3 ng/g) was very poor, except for sulfadiazine. Anyhow, the main disadvantages of USE over PLE for extraction from sludge and soil samples include the higher organic solvent consumption per sample (180 mL [63], versus ~22 mL for PLE [69]) and the lower sample throughput (~90 min for USE [63], versus ~20 min for PLE [69]), although USE is a simpler and cheaper approach.

Göbel et al. have published a comparative evaluation of the performances of USE and PLE for the extraction of MCs, SAs and TMP from activated and digested sewage sludge [69]. For the PLE method, a MeOH/H_2O mixture (50:50, *v/v*) was selected as extraction solvent using an extraction temperature of 100 °C, higher than that used in other applications [66, 71]. For USE, the samples were ultrasonicated for 5 min with MeOH and acetone, and the combined extracts were evaporated. The extracts were reconstituted with local groundwater for SPE using Oasis HLB cartridges. SA antibiotic retention on the SPE cartridges was not affected by the pH of the loading samples, as reported for wastewater samples [37]; however, higher RSDs (up to 33%) were observed when the pH of the sample was adjusted to 4.0 prior to SPE, due to increased clogging of the cartridges, which did not allow the enrichment of the total sample volume in some cases. The precision ranged between 2% and 8% for PLE and between 7% and 20% for USE. The lower precision of USE could be attributed to the higher amount of matrix extracted with the solvents used in this method and to the higher automation of the PLE procedure in comparison to USE. However, USE seemed to be equally or slightly less efficient for the extraction of MCs and TMP, while significantly lower extraction efficiencies were obtained for SAs compared to PLE.

Jacobsen et al. [72] reported the simultaneous extraction of two TCs, two MCs and one SA from agricultural soils. They carried out a PLE extraction of all of the antibiotics, followed by a clean-up and preconcentration step using SPE and then LC-MS^2 determination. Two cartridges in tandem were applied for sample clean-up and preconcentration: a SAX cartridge was employed to remove negatively charged humic material and to reduce matrix interferences, thus avoiding clogging, contamination and overloading of the HLB cartridge that retained the antibacterial agents. PLE extractions were performed at room temperature and 1500 psi to avoid the conversion of TCs into their epi or anhydrous form when heated. The sample, with an average moisture content of approximately 5%, was mixed with an equal amount of Otawa sand. The extraction solvent was a 1:1 mixture of MeOH and 0.2 M citric acid buffer (pH 4.7). The addition of the chelating agent favored the extraction of TCs in particular, as they form strong complexes with di- and trivalent cations in the clay mineral interlayers or with hydroxy groups at the surface of the soil particles. For samples with low concentrations of antibiotics, three PLE extracts were combined prior to preconcentration to improve method sensitivity. The recoveries varied depending on the spiked concentration level and the type of soil, although the extraction procedure was optimized for TCs and so higher recoveries could probably be achieved for MCs and SAs under different conditions.

Food matrices

The most common analyzed food matrices include milk, honey, animal tissues and eggs. Sample preparation involves procedures for deproteinization, defatting, and sugar hydrolysis for honey samples [33, 83]. Deproteinization is usually achieved with organic solvents such as acetonitrile or methanol, and, when necessary, sample extracts are further defatted with hexane.

"Dilute and shoot" is the simplest sample preparation strategy, especially when designing multiclass methods, where the selectivity of SPE is somewhat of a disadvantage. Dilution of the extracts can reduce matrix effects to a certain degree, but extensive maintenance of the LC-MS system is needed to ensure reproducible chromatograms and MS sensitivity through column regeneration and MS ion-source cleaning and/or the use of a divert valve.

As an example, Chico et al. [84] developed a simple method for the analysis of 39 antimicrobials (TCs, Qs, PCs, SAs and MCs) in animal muscle tissues. It consisted of an extraction with MeOH/H_2O (70:30, *v/v*) containing EDTA to improve the extraction of TCs followed by the dilution of the extracts before injection into the chromatographic system. Matrix-matched standards were used for correct quantification of the samples. The simplicity of the sample preparation procedure along with the use of UPLC enabled a high sample throughput to be realized. The method was successfully applied in an Official Public Health Laboratory for the routine analysis of 1012 samples over a six-month period [84]. Moreover, the method was applied in several interlaboratory studies with good results.

In another application, Granelli et al. [85] developed a method for the extraction of a total of 19 antimicrobials, including TCs, SAs, Qs, β-LCs and MCs, from muscle or kidney samples. Extraction was performed with 70% MeOH, and the extracts were then diluted fivefold with water before LC-MS injection. The method was only suitable for screening purposes at the MRLs. Matrix effects were more relevant in kidney samples than in muscle, especially for TCs and MCs, which were affected by signal suppression, resulting in poorer precision for these two antimicrobial classes. This can be attributed to the fact that during extraction with 70% MeOH, urine salts are coextracted with the analytes, causing suppression. Moreover, lower recoveries were also observed for TC, MCs and Qs in kidney samples (<66%) compared to muscle.

A recent work has described, for the first time, a "dilute-and-shoot" strategy for the simultaneous extraction of a wide variety of residues and contaminants (pesticides, mycotoxins, plant toxins and veterinary drugs) from different food (meat, milk, honey and eggs) and feed matrices [86]. Several antimicrobial classes were included (SAs, Qs, LCs, MCs, IPhs, TCs and NMZs) in the analytical methodology. Sample extraction was performed with H_2O/ACN or acetone/1% formic acid, but instead of diluting the extracts before analysis by ultraperformance liquid chromatography with tandem MS (UPLC-MS^2), small extract volumes (typically 5 μL) were injected to minimize matrix effects. Despite the absence of clean-up steps and the inherent complexity of the different sample matrices, adequate recoveries were obtained for the majority of the analyte/matrix combinations (typical values for antimicrobials were in the range 70–120%). Moreover, the use of UPLC allows high-speed analysis, since all analytes eluted within nine minutes.

Sample preparation strategies known as "QuEChERS" (quick, easy, cheap, effective, rugged and safe), that were previously used for pesticide analysis [87], have recently been applied to the analysis of multiclass veterinary drugs in foodstuffs. The conventional QuEChERS strategy is based on ACN extraction/partitioning of the analytes followed by the removal of water and proteins by salting out with sodium chloride and magnesium sulfate. Afterwards, dispersive SPE (d-SPE), which involves the addition of small amounts of a bulk sorbent to the extracts, is usually applied [87]. The application of this methodology provides—among other advantages—high recovery rates for analytes covering a wide polarity range, and allows the use of smaller amounts of organic solvents.

For instance, Aguilera-Luiz et al. described a simple and fast procedure for the extraction of SAs, Qs, MCs and TCs from milk samples based on a buffered QuEChERS liquid extraction methodology [88]. The target compounds were extracted from milk with acidified ACN in the presence of EDTA to increase the recoveries of MCs and TCs. After this step, water and proteins were removed by adding a mixture of magnesium sulfate and sodium acetate followed by centrifugation and filtration of the organic phase; the diluted extracts were analyzed directly without further clean-up. No denaturing of proteins was required prior to extraction and fat removal, which was performed in a single step, so the proposed method is less time-consuming and easier to perform than the currently available procedures. Furthermore, extraction times were lower than 10 min per sample, with recovery values for the antimicrobials ranging between 73% and 108%, and separation of the analytes by UPLC was achieved in less than 10 min, so this method could be applied in routine laboratories.

In another work, Stubbings et al. [89] demonstrated the applicability of a QuEChERS procedure for the analysis of multiclass antimicrobials (SAs, Qs, FQs, IPhs and NMZs) in animal tissues. Although the conventional QuEChERS liquid extraction method is performed under neutral extraction conditions, the use of buffered acidic conditions was selected for this work in order to improve the extraction efficiency of Qs/FQs. Thus, extraction was

performed with ACN containing 1% (*v/v*) acetic acid in the presence of anhydrous sodium sulfate, followed by d-SPE with a Bondesil-NH_2 sorbent. An aliquot of the extracts was evaporated to dryness and redissolved in ACN/H_2O (90:10, *v/v*) before LC-MS^2 analysis. Determination of NMZs required additional clean-up after d-SPE with Bond Elut SCX cartridges. Validation was performed on chicken muscle samples, using matrix-matched standards because of the MS suppression signal observed for many of the target analytes. Besides the abovementioned antimicrobial classes, recent progress in this line of research has demonstrated that the method is also applicable to other antimicrobial classes, namely MCs and LCs.

Yamada et al. [90] have described a method for the simultaneous screening of 61 antimicrobials of different classes along with other 69 veterinary drugs in bovine, porcine and chicken muscle. The samples were first homogenized with sodium sulfate and then extracted with ACN/MeOH (95:5, *v/v*). After centrifugation, the extracts were defatted with *n*-hexane saturated with ACN, evaporated to dryness, and the residue was dissolved in MeOH before LC/MS^2 analysis. The great majority of the analytes showed recoveries of between 70 and 110%, and only ciprofloxacin and norfloxacin among the antimicrobials yielded recoveries of below 70%. Although no significant matrix effects were observed, poor precisions (RSDs>20%) were obtained for AMPs and SAs.

Didier et al. have recently developed a generic milk sample preparation procedure for the comprehensive screening of 150 veterinary drugs by UPLC-TOF-MS [91]. Among the antimicrobials, β-LCs, MCs, NMZs, Qs, SAs and TCs along with other veterinary medicinal products and metabolites were analyzed by the proposed methodology. To ensure high sample throughput, the sample treatment was kept as simple as possible. It consisted of protein precipitation with a solution of 0.1% formic acid in ACN, followed by ultrafiltration using cut-off membranes of 3 kDa. In this way, it was possible to screen more than 50 samples per day.

A substantial matrix effect was observed that was very compound dependent. Thus, Qs and TCs suffer significant signal enhancement (up to 1300% for enrofloxacin), whereas considerable signal suppression was observed for avermectins. In fact, many accuracy values exceeded the 120% level, and in some particular cases they even reached 807%. So, from a quantitative point of view, the method possesses an important limitation, since matrix effects must be controlled to avoid important quantitative errors. Despite that, the signal enhancement was quite beneficial for screening purposes, as it allows better detection limits. The method was successfully applied to the screening of more than 150 raw milk samples as part of the Swiss National plan for residue monitoring.

Honey is a complex matrix that contains sugars, pigments and phenolic compounds that must be removed prior to LC-MS analysis [83]. Finding a generic extraction procedure for multiclass antibiotic analysis in this type of matrix is not an easy task. For instance, acid hydrolysis is usually required to dissociate sugar-bound SAs and TCs. In contrast, MCs are usually extracted under basic conditions because they are not stable at acid pH, and the addition of EDTA is required to avoid the complexation of these antimicrobials with metal ions.

Thus, a rather laborious sample preparation procedure consisting of four subsequent LLE steps has been described for the extraction of 37 multiclass antimicrobials (TCs, MCs, AGs, β-LCs, AMPs and SAs) from honey samples [92]. Despite analyzing the extracts independently or pulling them together (which causes β-LC loss due to the presence of acid in two of the extracts), the authors performed a single LC-MS^2 analysis using a stacking injection method. Thus, 10 μL of each of the four honey extracts were successively injected, giving a final injection volume of 40 μL. The gradient elution and MS/MS acquisition was started after the injection of the last honey extract. The extract injection order was a very important influence on the β-LC loss during LC-MS^2 acquisition. Up to twelve honey samples can be prepared within five hours using the abovementioned sample preparation procedure.

Nevertheless, most of the sample preparation methods for multiclass antibiotic analysis in food matrices have adopted SPE clean-up prior to LC-MS determination, either to remove matrix interferences or to concentrate the antimicrobials in order to achieve sub-μg kg^{-1} level sensitivities. Typical sorbents for SPE include Oasis HLB (hydrophilic–lipophilic balanced) and Strata X, among others. Oasis HLB cartridges have been preferred due to their good retentions and highly reproducible recoveries of a wide range of compounds, whether polar or nonpolar (due to their combined hydrophobic–hydrophilic retention mechanism). Strata X cartridges, which have a similar functionality to Oasis HLB cartridges, provide comparable results.

SPE is generally performed in offline mode, but in some cases coupling of online SPE with LC-MS through a column-switching valve has been used for semi-automated sample preparation in order to improve sample throughput. Thus, online SPE provides a solution for high sample throughput and could be more effective when analyzing a large number of samples in a limited time. However, some disadvantages of online SPE clean-up include the gradual deterioration of reusable cartridges and the risk of sample cross-contamination after analyzing incurred samples.

Among the multiclass methods reported in the literature, a procedure that involves sample dissolution with EDTA under mildly acidic conditions (pH 4.0) followed by SPE with Oasis HLB cartridges has been applied for the simultaneous analysis

of MCs, TCs, Qs and SAs in honey samples [93]. Separation and determination by UPLC-MS^2 enabled the analysis of 17 compounds in less than 5 min. Mean recoveries ranged from 70 to 120%, except for three compounds (doxycycline, erythromycin and tilmicosin), which had recoveries of >50%. The method has been applied to the analysis of honey samples obtained from different beekeepers and local supermarkets, and it found residues of erythromycin, sarafloxacin and tylosin in three of them.

Other authors have proposed a sample preparation procedure that involves the dissolution of honey with water, centrifugation and filtration, followed by SPE with Strata X cartridges [94]. In this way, 15 antimicrobials from seven different classes, namely TCs, FQs, MCs, LCs, SAs, AGs and CAP, could be quantified at very low ng mL^{-1} levels, whereas erythromycin and monensin were only detected and confirmed—not quantified—due to the very low recoveries (~30%) obtained for these two analytes. The method has several drawbacks: the analysis of AGs (e.g., streptomycin) has to be performed separately due to the particular extraction and chromatographic conditions required, such as the use of ion-pairing reagents; CAP is assayed in a second chromatographic run because this antibiotic is detected in ESI negative ion mode. Obviously, sample processing and analysis then becomes very time consuming, resulting in a low sample throughput.

In another work, Turnipseed et al. described a method for the multiclass residue determination of β-LCs, SAs, TCs FQs and MCs in milk and dairy products [95]. The sample preparation combines extraction with ACN, clean-up with Oasis HLB cartridges, and ultrafiltration using molecular weight cut-off filters to improve the overall performance of the analysis. Acceptable recoveries were obtained for SAs, MCs and Qs (>70%); however, they were rather low for TCs (50–60%) and β-LCs (<50%). Despite the extensive clean-up procedure, a large degree of matrix ion suppression was observed for many compounds, making it necessary to include matrix-matched calibration standards for quantification purposes.

Stolker et al. [96] developed a method that was useful for screening more than 100 veterinary drugs in milk, including antibacterials of different classes, namely MCs, PCs, Qs, SAs, TCs, NMZs, IPhs and AMPs. After protein precipitation with ACN, centrifugation and further clean-up with Strata X cartridges, the extracts were analyzed by UPLC-TOF-MS. The results obtained were satisfactory in terms of repeatability (RSDs<20% for 86% of the compounds), reproducibility (RSDs<40% for 96% of dthe compounds) a,nd accuracy (80–120% for 88% of the compounds). However, identification criteria for TOF-MS detectors are not yet included in the EU 2002/657/EC guidelines [97], so the method can only be used for screening purposes, and those samples that are suspected to be positive must be confirmed by a tandem MS technique.

In the same way, Kaufmann et al. have reported a method based on UPLC-TOF-MS for the analysis of more than 100 veterinary drugs, including the most relevant antibiotic classes, in different animal tissues (muscle, kidney and liver) [98]. The sample preparation proposed by these authors is rather extensive. It consisted of a liquid–liquid–solid extraction technique (so-called bipolarity extraction) which enables polar, medium polar and apolar compounds to be recovered, followed by SPE clean-up with Oasis HLB cartridges. The use of aqueous solvents failed to extract the most apolar compounds, whereas organic solvents showed poor recoveries for polar PCs and TCs. Thus, an extraction combining two solvents of different polarities, such as ACN and aqueous McIlvaine buffer (0.1 M citric acid, 0.2 M disodium phosphate), was performed in the presence of high amounts of ammonium sulfate, which induces phase separation. The formation of an emulsion favors the simultaneous extraction of a wider range of analytes in terms of polarity than the use of ACN dissolved in aqueous buffer. Subsequent extract clean-up using Oasis HLB cartridges allowed the removal of major matrix food components.

Despite the fact that the sample preparation approach was rather laborious, recoveries of over 74% were obtained for the most of the analytes. Nevertheless, β-LCs were recovered at lower rates from liver and kidney than from muscle, probably due to the high enzymatic activity in these matrices, which is partly responsible for such losses.

A high-throughput method that combines online extraction and determination by LC-MS^2 has been developed for the screening of 13 multiclass antibacterials (MCs, FQs, LCs, and TMP) in different animal muscle tissues [99]. After sample deproteinization with ACN, the sample extracts were directly loaded onto the SPE cartridge, packed with an Oasis HLB sorbent, and connected through a switching valve device to a short LC analytical column. In this way, a complete cycle of SPE clean-up and LC determination can be performed in only 6 min. The method has shown excellent selectivity, as no interfering peaks were observed in the retention windows of any of the target compounds. Furthermore, the performance of the extraction cartridge was found remain consistent over 100 injections. The only precaution taken was to flush ACN and MeOH over the cartridge once the clean-up step was finished, in order to remove residual tissue matrix.

Heller et al. developed a method to screen antibacterial residues in table eggs [100]. A total of 29 analytes belonging to four antibacterial classes (SAs, TCs, FQs, β-LCs) were analyzed. The extraction of the antimicrobials from the matrix was achieved by adding succinate buffer and centrifugation; afterwards, the cloudy extracts required

a clean-up step with Oasis HLB cartridges. Recoveries for each drug class were as follows: SAs (70–80%), TCs (45–55%), FQs (70–80%), and β-LCs (25–50%). Indeed, the reproducibility of the method varied widely (from 10% to >30%). Therefore, the results provide an estimated concentration range and the method could be useful for screening purposes, but not for quantitation.

Pressurized liquid extraction (PLE) possesses great potential as a sample preparation technique in food analysis, since it combines the benefits of high throughput, automation and low solvent consumption, although expensive lab equipment is required [101, 102].

Recently, Carretero et al. described a multiclass method for the analysis of 31 antibacterials (including β-LCs, MCs, LCs, Qs, SAs, TCs, NMZs and TMP) in meat samples by PLE-LC-MS^2 [103]. Meat samples were homogenized and blended with EDTA-washed sand, and then extracted with water by applying 1500 psi, 70 °C and one extraction cycle (10 min). Besides the automation of the extraction procedure, a drawback of the method is the large volumes of extract (40 mL) obtained, which required evaporation to reduce the extract volume to 10 mL and thus increase sensitivity. This evaporation step considerably increases the time required for sample preparation. The proposed methodology has been applied to the analysis of 152 samples of cattle and pig tissues, fand the presence of Qs, TCs and SAs was found in 15% of the samples, although at concentrations below the MRLs.

Tables 3 and 4 summarize the sample preparation procedures as well as the analytical methods used to determine multiclass antibiotics in foodstuff samples.

LC-MS analysis

The majority of the LC methods used for multiclass antimicrobial analysis in environmental and food matrices (Tables 2 and 4) make use of reversed-phase chromatography with stationary phases based on octadecyl (C_{18}) or octyl (C_8) silane. Some compounds (e.g., AGs), due to their highly polar nature, require the use of hydrophobic ion-pair reagents to improve the peak shape and retention in reversed-phase chromatography. It is known that ion-pair reagents can cause ion suppression and produce significant analyte signal loss, decreasing sensitivity for many compounds. For these reasons, it is usually difficult to separate out AGs using current multiclass methods.

Recently, the introduction of UPLC, a fast chromatographic technique based on the use of stationary phases containing small particles (typically <2 μm in size), has enabled multiclass separations to be performed with higher resolutions, sensitivities and reduced analysis times (around three times shorter than classical LC separation). The current trend towards more generic and less selective sample preparation procedures for multiclass analysis increases the risk of spectral interferences and ion suppression. The use of UPLC could help to reduce matrix effects produced by isobaric co-eluting sample compounds because of the enhanced chromatographic resolving power provided by UPLC in comparison to conventional LC. Although the application of UPLC is still scarce (Tables 2 and 4), it is growing in popularity.

In the 1990s, LC-MS^2 operated with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources became mandatory as confirmatory techniques for the analysis of target veterinary drug residues, especially in the field of food safety [104]. Nowadays, besides conventional tandem mass analyzers, time of flight mass spectrometry (TOF) is emerging as a powerful and complementary technique for the identification of non-target contaminants (e.g., unknown degradation products and metabolites) [105].

Among the tandem mass analyzers applied to multiclass antimicrobial analysis (Tables 2 and and 4), the triple quadrupole (QqQ) is the one that is the most widely used for quantitative analyses of target compounds due to its high specificity and sensitivity, especially when operated in the multiple reaction monitoring (MRM) mode [106]. According to EU criteria (2002/657/EC) [97] for confirming veterinary drug residues in foodstuffs, at least two MRM transitions (in the correct ion ratios) must be recorded, usually corresponding to those from the most abundant precursors to the most abundant product ions.

The major limitation of QqQ working in the MRM mode is that a complete mass spectrum cannot be obtained, so unknown or non-target compounds are missed, thus making it difficult to detect the presence of novel residues, metabolites or transformation products. Furthermore, despite the technological advances in the latest QqQ analyzers, they are limited in terms of the number of MRM transitions that can be monitored, and thus in their ability to determine a large number of analytes (over 100) in a single LC-MS^2 run.

On the other hand, the ion-trap (IT) analyzer provides high sensitivity in full-scan mode and has the ability to perform multiple-stage fragmentations (MS^n), which is important for the structural elucidation of unknown metabolites or degradation products, but its sensitivity is one order of magnitude lower than that of QqQ instruments.

Nowadays, in the field of multiresidue analysis, there is a clear trend towards the use of accurate mass full-scan MS techniques (TOF) [107, 108]. Full-scan MS approaches offer the ability to screen a virtually unlimited number of analytes, including both target and non-target compounds. In the latter case, the possibility of retrospectively evaluating the MS spectra and the isotopic patterns is a useful tool

Table 3 Summary of sample preparation procedures for the determination of multi-class antibiotics in food samples

Compounds	Matrix	Extraction and clean-up	Recoveries (%)	References
SAs, Qs, MCs, TCs, PCs, TMP	Muscle	Extraction with MeOH/H_2O (70:30, *v/v*) containing EDTA, followed by dilution with H_2O and filtration	60.5–96.5%	[84]
SAs, Qs, MCs, TCs, β-LCs	Muscle, kidney	Extraction with 70% MeOH and fivefold dilution with H_2O	47–121% (muscle); 26–107% (kidney)	[85]
SAs, Qs, LCs, MCs, NMZs, IPhs, TCs	Honey, milk, eggs, muscle	Extraction with H_2O/ACN or acetone/1% formic acid (*v/v*)	70–120% (>80% of analytes)	[86]
SAs, Qs, MCs, TCs	Milk	"QuEChERS" liquid extraction with acidified ACN-EDTA in the presence of magnesium sulfate and sodium acetate for removal of water and proteins. Centrifugation and filtration of the organic phase and dilution of extracts	73–108%	[88]
SAs, Qs, IPhs, NMZs	Chicken muscle	"QuEChERS" liquid extraction with 1% (*v/v*) acetic acid/ACN and sodium sulfate, followed by d-SPE clean-up with a Bondesil-NH_2 sorbent (plus additional strong cation exchange clean-up with Bond Elut SCX cartridges for NMZs)	55–89%	[89]
SAs, Qs, MCs, AMPs, LCs, IPhs	Muscle	Extraction with ACN/MeOH (95:5, *v/v*) and defatting with *n*-hexane saturated in ACN, followed by evaporation and dissolution in MeOH	70–110%	[90]
βLCs, MCs, NMZs, Qs, SAs, TCs	Milk	Deproteinization with a solution of 0.1% (*v/v*) formic acid in ACN, followed by ultrafiltration for 1 h with cut-off membranes of 3 kDa. Finally, the extracts were partially evaporated and centrifuged and the supernatants collected	Only useful for screening; accuracy and precision do not fulfill EU guidelines	[91]
SAs, MCs, TCs, β-LCs, AGs, AMPs	Honey	Four sequential L-L extraction steps: (1) ACN; (2) 10% (*v/v*) TCA + ACN; (3) NFPA + ACN; (4) hydrolysis + ACN. The four extracts were individually re-suspended in MeOH/H_2O (20/80, *v/v*), vortexed, sonicated, and filtered	TCs and AMPs (68–118%); AGs, MCs, SAs and β-LCs (26–104%)	[92]
MCs, TCs, Qs, SAs	Honey	Extraction with aqueous EDTA under milk acidic conditions (pH 4.0) followed by clean-up with Oasis HLB cartridges	70–120%, except DOXI, ERY A and TIL (>50%)	[93]
TCs, FQs, MCs, LCs, SAs, AGs, CAP	Honey	Dissolution of honey with water, centrifugation and filtration. An aliquot of the supernatant was diluted with water for AG determination. The remaining portion was submitted to a clean-up with Strata X cartridges	65–104%, except ERY A (~30%)	[94]
βLCs, SAs, TCs, FQs, MCs	Milk	Extraction with ACN/0.1% formic acid (*v/v*) and clean-up with Oasis HLB cartridges. Partial solvent evaporation for 20 min, followed by ultrafiltration for another 20 min with cut-off membranes	SAs, MCs and Qs (>70%); TCs (50–60%) and β-LCs (<50%)	[95]
MCs, PCs, Qs, SAs, TCs, NMZs, IPhs, AMPs	Milk	Deproteinization with ACN (shaking for 30 min) followed by centrifugation for a further 15 min. Tenfold dilution of the supernatants with H_2O and clean-up with Strata X cartridges	80–120% (for 88% of analytes)	[96]
SAs, Qs, MCs, TCs, LCs, β-LCs	Muscle, kidney, liver	"Bipolarity" extraction with ACN-aqueous McIlvaine buffer/ammonium sulfate, followed by clean-up with Oasis HLB cartridges	48–148%; lower recovery rates for some β-LCs in kidney and liver than in muscle	[98]
MCs, FQs, LCs, TMP	Muscle	Homogenization, deproteinization with ACN, followed by centrifugation, partial evaporation of the extracts, and defatting with *n*-hexane. After centrifugation and filtration, the extracts underwent clean-up with an Oasis HLB short column coupled online to LC	>80%	[99]
SAs, TCs, FQs, β-LCs	Eggs	Extraction with sodium succinate buffer, centrifugation, and clean-up with Oasis HLB cartridges	45–80%, except β-LCs (<25%)	[100]
β-LCs, MCs, LCs, Qs, SAs, TCs, NMZs, TMP	Muscle	PLE extraction: solvent H_2O, pressure 1500 psi, temperature 70 °C, flush volume 60%, static time 10 min, 1 extraction cycle	70–99%	[103]

AGs, aminoglycosides; *AMPs*, amphenicols; *β-LCs*, β-lactams; *d-SPE*, dispersive SPE; *DOXI*, doxiciclin; *ERY A*, erythromycin A; *FQs*, fluoroquinolones; *IPhs*, ionophores; *LCs*, lincosamides; *MCs*, macrolides; *NMZs*, nitroimidazoles; *NFPA*, nonafluoropentanoic acid; *PCs*, penicillins; *PLE*, pressurized liquid extraction; *Qs*, quinolones; *SAs*, sulfonamides; *TCs*, tetracyclines; *TIL*, tilmicosin; *TCA*, trichloroacetic acid; *TMP*, trimethoprim

Table 4 Summary of multiclass LC-MS methods used for antibiotic residue determination in food samples

Compounds	Matrix	LC		MS		Sensitivity		References
		Column	Mobile phase	Type	Ionization	LOD or CCα	LOQ or CCβ	
SAs, Qs, MCs, TCs, PCs, TMP	Muscle	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm)	0.2% formic acid with 1 mM oxalic acid/ACN with 0.1% formic acid (gradient)	QqQ	ESI (+)	4–318 μg kg^{-1}	7–337 μg kg^{-1}	[84]
SAs, Qs, MCs, TCs, β-LCs	Muscle, kidney	Genesis C_{18} (50×2.1 mm, 4 μm)	ACN/0.2% formic acid with 0.1 mM oxalic acid (gradient)	QqQ	ESI (+)	2–15 μg kg^{-1}	–	[85]
SAs, Qs, LCs, MCs, NMZs, IPhs, TCs	Honey, milk, eggs, muscle	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm), Symmetry C_{18} (150×3 mm, 5 μm)	1 mM formate with formic acid/H_2O:MeOH (5:95 *v/v*) with 1 mM formate/formic acid (gradient)	QqQ	ESI (+)/(−)	0.01–0.05 mg/kg^{-1}	–	[86]
SAs, Qs, MCs, TCs	Milk	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm)	MeOH/0.01% formic acid (gradient)	QqQ	ESI (+)	1–4 μg kg^{-1}	3–10 μg kg^{-1}	[88]
SAs, Qs, IPhs, NMZs	Chicken muscle	Synergi Fusion C_{18} (100×2 mm, 2.5 μm)	0.1% formic a cid/MeOH/ACN (gradient)	QqQ	ESI (+)	0.3–444 μg kg^{-1}	0.45–487 μg kg^{-1}	[89]
SAs, Qs, MCs, AMPs, LCs, IPhs	Muscle	TSK-gel C_{18} 100 S (150×2.1 mm)	10 mM NH_4Ac with 0.3% HAc/ACN-MeOH (20:80, *v/v*) (gradient)	QqQ	ESI (+)/(−)	0.03–3 ng g^{-1}	0.1–10 ng g^{-1}	[90]
βLCs, MCs, NMZs, Qs, SAs, TCs	Milk	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm)	0.1% formic acid in H_2O/0.1% formic acid in ACN (gradient)	TOF	ESI (+)	0.2–222 μg L^{-1}	0.3–246 μg L^{-1}	[91]
SAs, MCs, TCs, β-LCs, AGs, AMPs	Honey	Zorbax SB C_{18} (50×2.1 mm, 1.8 μm)	1 mM NFPA with 0.5% formic acid/ACN–MeOH (50:50, *v/v*) with 0.5% formic acid (gradient)	QqQ	ESI (+)	29–81 μg kg^{-1}	–	[92]
MCs, TCs, Qs, SAs	Honey	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm)	MeOH/0.05% formic acid (gradient)	QqQ	ESI (+)	–	0.3–3.3 μg kg^{-1}	[93]
TCs, FQs, MCs, LCs, SAs, AGs, CAP	Honey	Synergi Polar C_{18} (50×2.0 mm, 4 μm)	0.1% formic acid in ACN/0.1% formic acid in H_2O (gradient)	QqQ	ESI (+)/(−)	–	–	[94]
βLCs, SAs, TCs, FQs, MCs	Milk	YMC-AQ C_{18} (100×2 mm, 3 μm)	0.1% formic acid/ACN (gradient)	QqQ	ESI (+)	–	0.02–25 μg L^{-1}	[95]
MCs, PCs, Qs, SAs, TCs, NMZs, IPhs, AMPs	Milk	Acquity UPLC C_{18} (100×2.1 mm, 1.7 μm)	0.1% formic acid/H_2O:ACN (10:90, *v/v*) with 0.1% formic acid (gradient)	TOF	ESI (+)	–	1.7–142 μg L^{-1}	[96]
SAs, Qs, MCs, TCs, LCs, β-LCs	Muscle, kidney, liver	UPLC HSS T3 (100×2.1 mm, 1.8 μm)	0.3% formic acid in ACN:H_2O (5:95, *v/v*)/0.3% aqueous formic acid (gradient)	TOF	ESI (+)	–	0.1–30 μg kg^{-1}	[98]
MCs, FQs, LCs, TMP	Muscle	Luna C_{18} (50×2.1 mm, 5 μm)	ACN/0.1% formic acid in water/MeOH (gradient)	QqQ	ESI (+)	0.1–8.4 μg kg^{-1}	–	[99]
SAs, TCs, FQs, β-LCs	Eggs	Phenyl cartridge column (50×4 mm, 3 μm)	0.1% formic acid/ACN (gradient)	IT	ESI (+)	10–50 μg kg^{-1}	–	[100]
β-LCs, MCs, LCs, Qs, SAs, TCs, NMZs, TMP	Muscle	XTerra MS C_{18} (100×2.1 mm, 3.5 μm)	10 mM formic acid in H_2O/10 mM formic acid in MeOH (gradient)	QqQ	ESI (+)	12–308 μg kg^{-1}	14–327 μg kg^{-1}	[103]

AGs, aminoglycosides; *AMPs*, amphenicols; *β-LCs*, β-lactams; *CCα*, decision Limit; *CCβ*, detection capability; *ESI*, electrospray ionization; *FQs*, fluoroquinolones; *IPhs*, ionophores; *IT*, ion trap; *LCs*, lincosamides; *LOD*, limit of detection; *LOQ*, limit of quantification; *MCs*, macrolides; *NMZs*, nitroimidazoles; *NFPA*, nonafluoropentanoic acid; *PCs*, penicillins; *Qs*, quinolones; *QqQ*, triple quadrupole; *SAs*, sulfonamides

in addition to accurate mass determination for detecting and identifying non-target compounds.

Thus, the coupling of UPLC to high-speed TOF analyzers provides an excellent analytical tool for the multiclass screening of hundreds of different compounds in complex matrices [104, 107]. This is particularly interesting for environmental or food safety laboratories that have to confirm the presence of a large number of contaminants or residues in environmental samples or food commodities.

The TOF analyzer offers improved selectivity due to its high mass accuracy (1–3 ppm error) and medium mass resolving power (typically 10,000 FHWM), which allows the identification of mass interferences that have the same nominal masses and chromatographic retention times as analytes. The disadvantage of the TOF analyzer is its narrow linear dynamic range, which somewhat limits its applicability to quantitative analysis.

On the other hand, the new hybrid quadrupole time-of-flight (QqTOF) spectrometer can be described as a QqQ in which the last quadrupole has been replaced with a TOF analyzer. A QqTOF can be simply operated as a TOF analyzer in full-scan mode, or as a tandem mass spectrometer in the product-ion scan mode. This imparts unique features—such as the acquisition of full MS spectra with high sensitivity, mass accuracy and medium-range high resolution—that favor multiresidue screening and the structural elucidation of unknown compounds, as well as accurate mass product-ion spectra that allow the unequivocal identification or confirmation of compounds of interest.

However, the sensitivities of TOF and/or QqTOF analyzers are generally 1–2 orders of magnitude lower than that of a QqQ operated in the MRM mode, compromising their applicability, especially in environmental fields, considering the very low concentrations of antimicrobials normally present in these samples. Table 5 summarizes the main characteristics of the different MS analyzers used for LC-MS determinations of multiclass antibiotics.

Unfortunately, despite the potential of accurate-mass LC-MS technologies for multiclass analysis, the European Commission Decision 2002/657/EC [97] does not describe criteria for confirming veterinary drug residues in foodstuffs using these medium-range, high-resolution mass analyzers. Decision 2002/657/EC does not take into account an important parameter, mass accuracy, for the confirmation of a chemical contaminant. However, different authors have proposed that additional LC-MS criteria should be implemented in Decision 2002/657/EC [107]. Thus, some have suggested that three TOF ions should be collected in order to earn the 4.0 identification points (IPs) usually required for confirmation when using common tandem MS analyzers [107]. By contrast, others have proposed a criterion based on the use of either absolute or relative mass errors for IP assignment, rather than resolution power [109]. Thus, for substances with MRLs, at least two ions must be monitored to achieve a minimum of three IPs for satisfactory confirmation with mass errors of between 2–10 mDa or ppm.

Conclusions

Recent approaches in antimicrobial analysis have focused on the development of multiclass analytical methods that allow a realistic evaluation of the fates, bioavailabilities and ecotoxicological effects of these drugs in the environment, and enable the food supply to be monitored for regulated veterinary residues and contaminants, in response to the demands of regulatory agencies, while minimizing the time and costs involved.

Table 5 Summary of the different MS analyzers used for LC-MS determinations of multiclass antibiotics

MS analyzer	Characteristics	Detection capabilities
QqQ	High specificity and sensitivity in MRM mode, but complete mass spectra cannot be obtained. Low mass resolution. Wide linear dynamic range	Outstanding advantages for quantitative determinations of target analytes, but not appropriate for identifying unknowns or non-target compounds. Limitations when determining a large number of analytes (over 100) in a single run
IT	High sensitivity in full scan and multistage fragmentation (MS^n) capabilities. Low mass resolution. Narrow linear dynamic range	Useful for screening and identifying unknowns or metabolites. Lacks adequate sensitivity to perform quantitative trace analysis, especially of banned drugs
QqLIT	Combines the selectivity of a QqQ with the full-scan high sensitivity of an IT. Low mass accuracy. Wider dynamic range than IT	Identification and quantification of target compounds. Identification of unknowns from fragmentation patterns. Ideal for the confirmation and quantification of compounds that exhibit poor fragmentation
TOF	Acquisition of full mass spectra. Mass accuracy (1–3 ppm) and high mass resolution (10,000 FHWM). Narrow linear dynamic range. Lower sensitivity (1–2 orders) than QqQ	Identification of unknowns based on accurate mass and isotopic profile evaluation. Possibility of retrospective analysis
QTOF	Acquisition of full or MS^2 spectra. Mass accuracy (5 ppm) and medium-range high mass resolution (5000 FHWM). Lower sensitivity (1–2 orders) than QqQ	Unequivocal identification or confirmation of unknowns based on accurate mass and isotopic profile evaluation of product-ion spectra. Possibility of retrospective analysis

IT, ion trap; *MRM*, multiple reaction monitoring; *QqQ*, triple quadrupole; *QqLIT*, quadrupole–linear ion trap; *QTOF*, quadrupole time of flight; *TOF*, time of flight

The method of choice for multiclass antimicrobial analysis is liquid chromatography coupled to MS and LC-MS^2. However, despite the high sensitivities and selectivities of these techniques, especially LC-MS^2, the complexity of many food and environmental matrices necessitates clean-up steps or dilution of the extracts prior to the chromatographic analysis.

Sample preparation procedures for single-class residue analysis aim to obtain maximum recovery rates of the analytes in a specific matrix. However, the main goal of the optimization of multiclass methods is the simultaneous extraction of the largest number of analytes, from multiple classes, using simple methods in order to achieve high sample throughput. This requires a compromise between the different solid phases that provide the best recoveries for each class of compounds. However, the wide variations in the physicochemical properties of the different antibiotic families and the fact that they are usually present at low concentration levels in environmental and food matrices considerably complicate sample preparation, and this issue constitutes one of the main challenges for future research in this field.

SPE is the preferred extraction technique for sample clean-up and preconcentration in these samples. Although offline methods are applied in most cases, recent developments include the optimization of online procedures that facilitate method automation, minimize sample manipulation, and reduce analytical time and organic solvent consumption.

Regarding chromatographic separations, the introduction of UPLC has facilitated multiclass separations with higher resolutions and sensitivities as well as reduced analysis times. The availability of this technique is still low, but it is a promising method for future developments. Besides conventional tandem mass analyzers, recent mass analyzers such as time-of-flight mass spectrometers (TOF-MS) and new hybrid quadrupole time-of-flight (QqTOF) spectrometers are emerging as powerful and complementary techniques for the identification of new contaminants, although their sensitivities are generally 1–2 orders of magnitude lower than that of a QqQ operated in the MRM mode, meaning that they may not be applicable to some samples.

Our current knowledge of the impact of antimicrobials in environmental and food samples focuses mainly on individual compounds; however, we also need more scientific research aimed at understanding how the presence of complex antibiotic mixtures affects human health and environmental sustainability.

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References

1. Kümmerer K (2009) Chemosphere 75:417–434
2. EU (2003) Council Regulation No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Off J Eur Commun L268:29
3. Wise R (2002) J Antimicrob Chemother 49:585–586
4. Kümmerer K (2003) J Antimicrob Chemother 52:5–7
5. ESAC (2009) European surveillance of antimicrobial consumption website. http://www.esac.ua.ac.be/
6. Kümmerer K (2008) Pharmaceuticals in the environment. A brief summary; antibiotics in the environment. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 3–17–75–88
7. Cha JM, Yang S, Carlson KH (2006) J Chromatogr A 1115:46–57
8. Boxall ABA, Fogg LA, Kay P, Blackwell PA, Pemberton EJ, Croxford A (2004) Rev Environ Contam Toxicol 182:1–91
9. Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Sci Total Environ 225:109–118
10. Montfort MHMM (2008) Methodological aspects concerning the environmental risk assessment for medicinal products; research challenges. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 399–410
11. Castensson S (2008) Pharmaceutical waste. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 490–499
12. Ye Z, Weinberg HS, Meyer MT (2007) Anal Chem 79:1135–1144
13. Boxal ABA (2008) Fate of veterinary medicines applied to soils. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 103–119
14. Kemper N (2008) Ecolog Indicat 8(1):13
15. Thiele-Bruhn S (2003) J Plant Nutrit Soil Sci 166:145–167
16. EU (2001) Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal products for human use. Off J Eur Commun L311:67
17. EU (1981) Council Directive 81/852/EEC on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products. Eur Off J L317:16–28
18. EMEA (2000–2006) Guideline on environmental impact assessment (EIAS) for veterinary medicinal products phase 1/final (CVMP/VICH/592, SWP/4447/00). European Agency for the Evaluation of Medicinal Products, London
19. FDA (1998) Guidance for industry. Environmental assessment of human drugs and biologics applications. Food and Drug Administration, Washington, DC
20. VICH (International Cooperationon Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products). Environmental impact assessment (EIAs) for veterinary medicinal products (VMPs)-phase I. VICH Topic GL6 (Ecotoxicity Phase I), Step 7, June 2000. Available from: http://vich.eudra.org/pdf/2000/Gl06_st7.pdf
21. VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) (2004) Environmental impact assessment (EIAs) for veterinary medicinal products (VMPs), phase II (VICH Topic GL38, Ecotoxicity Phase II, Step 7, October 2004). http://vich.eudra.org/pdf/10_2004/GL38_st7.pdf
22. US Food and Drug Administration (1986) General principles for evaluating the safety of compounds in food producing animals. FDA, Rockville
23. EU (1999) Commission Regulation 508/1999/EC amending Annexes I to IV to Council Regulation (EEC) No 2377/90 laying

down community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Off J Eur Commun L224:16 (http://europa.eu.int)
24. EU (1990) Council Regulation 2377/90/EEC laying down a community procedure for the establishment of maximum residue limits for veterinary medicinal products in foodstuff of animal origin. Off J Eur Commun L224:1
25. Lupin JR (2002) Critic Rev Food Sci Nutr 42:403
26. Backhaus T, Scholze M, Grimme LH (1999) Aquat Toxicol 49:49–61
27. Munch Christensen A, Ingerslev F, Baun A (2006) Environm Toxic Chem 25:2208–2215
28. Backhaus T, Sumpter J, Blanck H (2008) On the ecotoxicology of pharmaceutical mixtures. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 257–273
29. Cunningham V (2008) Special characteristics of pharmaceuticals related to environmental fate. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risk, 3rd edn. Springer, Berlin, pp 23–34
30. Hernández F, Sancho JV, Ibáñez M, Guerrero C (2007) Trends Anal Chem 26:466–485
31. Fatta D, Nikolaou A, Achilleos A, Meric S (2007) Trends Anal Chem 26:515–533
32. Kümmerer K (2009) J Environm Manag 90:2354–2366
33. Marazuela MD, Bogialli S (2009) Anal Chim Acta 645:5–17
34. Petrovic M, Hernando MD, Díaz-Cruz MS, Barceló D (2005) J Chromatogr A 1067:1–14
35. Gros M, Petrovic M, Barceló D (2006) Anal Bioanal Chem 386:941–952
36. Babic S, Asperger D, Mutavdzic D, Alka Horvat JM, Kastelan-Macan M (2006) Talanta 70:732–738
37. Göbel A, McArdell CS, Suter MJF, Giger W (2004) Anal Chem 76:4756–4764
38. Senta I, Terzic S, Ahel M (2008) Chromatographia 68:747–758
39. Batt AL, Aga DS (2005) Anal Chem 77:2940–2947
40. Ye Z, Weinberg HS (2007) Anal Chem 79:1135–1144
41. Dodd MC, Shah AD, Gunten U, Huang CH (2005) Environ Sci Technol 39:7065–7076
42. Peng X, Wang Z, Kuang W, Tan J, Li K (2006) Sci Total Environ 371:314–322
43. Peng X, Tan J, Tang C, Yu Y, Wang Z (2008) Environ Toxicol Chem 27:73–79
44. Tamtam F, Mercier F, Bot BL, Eurin J, Dinh QT, Clément M, Chevreuil M (2008) Sci Total Environ 393:84–95
45. Feitosa-Felizzola J, Chiron S (2009) J Hydrol 364:50–57
46. Miao XS, Bishay F, Chen M, Metcalfe CD (2004) Environ Sci Technology 38:3533–3541
47. Castiglioni S, Bagnati R, Calamari D, Fenelli R, Zuccato E (2005) J Chromatogr A 1092:206–215
48. Stolker AAM, Niesing W, Hogendoorn EA, Versteegh JFM, Fuchs R, Brinkman UAT (2004) Anal Bioanal Chem 378:955–963
49. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) Talanta 74:1299–1312
50. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) Anal Bioanal Chem 391:1293–1308
51. Wu C, Spongberg AL, Witter JD (2008) Int J Environ Anal Chem 88:1033–1048
52. Hilton MJ, Thomas KV (2003) J Chromatogr A 1015:129–141
53. Pozo OJ, Guerrero C, Sancho JV, Ibañez M, Pitarch E, Hogendoorn E, Hernández F (2006) J Chromatogr A1103:83–93
54. Lindberg R, Jarnheimer PA, Olsen B, Johansson M, Tysklind M (2004) Chemosphere 57:1479–1488
55. Renew JE, Huang CH (2004) J Chromatogr A 1042:113–121
56. Hirsch R, Ternes TA, Haberer K, Mehlich A, Ballwanz F, Kratz KL (1998) J Chromatogr A 815:213–223
57. Renew JE, Huang CH (2004) J Chromatogr A 1042:113–121
58. Gros M, Petrovic M, Barceló D (2009) Anal Chem 81:898–912
59. Gómez MJ, Petrovic M, Fernández-Alba AR, Barceló D (2006) J Chromatogr A 1114:224–233
60. Gros M, Petrovic M, Barceló D (2006) Talanta 70:678–690
61. Petrovic M, Gros M, Barceló D (2006) J Chromatogr A 1124:68–81
62. Kaufmann A, Butcher P, Maden K, Widmer M (2007) Anal Chim Acta 586:13–21
63. Löffler D, Terness TA (2003) J Chromatogr A 1021:133–144
64. Yang S, Cha J, Carlson K (2005) J Chromatogr A 1097:40–53
65. Peng X, Tan J, Tang C, Yu Y, Wang Z (2008) Environ Toxicol Chem 27:73–79
66. Nieto A, Borrull F, Marcé RM, Pocurull E (2007) J Chromatogr A 1174:125–131
67. Tolls J (2001) Environ Sci Technol 35:3397–3406
68. Golet EM, Strehler A, Alder AC, Giger W (2002) Anal Chem 74:5455–5462
69. Göbel A, Thomsen A, McArdell CS, Alder AC, Giger W, Theiβ N, Löffler D, Termes TS (2005) J Chromatogr A 1085:179–189
70. Díaz-Cruz MS, de Alda MJ López, Barceló D (2006) J Chromatogr A 1130:72–82
71. Barron L, Tobin J, Paull B (2008) J Environ Monit 10:353–361
72. Jacobsen AM, Halling-Sorensen B, Ingerslev F, Hansen SH (2004) J Chromatogr A 1038:157–170
73. Reverté S, Borrull F, Pocurull E, Marcé RM (2003) J Chromatogr A 1010:225–232
74. Lindsey ME, Meyer M, Thruman EM (2001) Anal Chem 73:4640–4646
75. Vanderford BJ, Pearson RA, Rexing DJ, Snyder SA (2003) Anal Chem 75:6265–6274
76. Hao C, Lissemore L, Nguyen B, Kleywet S, Yang P, Solomon K (2006) Anal Bioanal Chem 384:505–513
77. Sorensen LK, Elbaek TH (2004) Chromatographia 60:287–291
78. Cachill JD, Furlong ET, Burkhardt MR, Kolpin D, Anderson LG (2004) J Chromatogr A 1041:171–180
79. Kim SC, Carlson K (2007) Environ Sci Technol 41:50–57
80. Feitosa-Felizzola J, Temime B, Chiron S (2007) J Chromatogr A 1164:95–104
81. Tamtam F, Mercier F, Eurin J, Chevreuil M, Le Bot B (2009) Anal Bioanal Chem 393:1709–1718
82. Tong L, Li P, Wang Y, Zhu K (2009) Chemosphere 74:1090–1097
83. Kujawski MW, Namiésnik J (2008) Trends Anal Chem 27:785–793
84. Chico J, Rúbies A, Centrich F, Companyó R, Prat MD, Granados M (2008) J Chromatogr A 1213:189–199
85. Granelli K, Branzell C (2007) Anal Chim Acta 586:289–295
86. Mol HGJ, Plaza-Bolaños P, Zomer P, de Rijk TC, Stolker AAM, Mulder PPJ (2008) Anal Chem 80:9450–9459
87. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenk FJ (2003) J AOAC Int 86:412–431
88. Aguilera-Luiz MM, Martínez-Vidal JL, Romero-González R, Garrido-Frenich A (2008) J Chromatogr A 1205:10–16
89. Stubbings G, Bigwood T (2009) Anal Chim Acta 637:68–78
90. Yamada R, Kozono M, Ohmori T, Morimatsu F, Kitayama M (2006) Biosci Biotechnol Biochem 70:54–64
91. Ortelli D, Cognard E, Jan P, Edder P (2008) J Chromatogr B (in press) doi:10.1016/j.jchromb.2009.03.006
92. Hammel YA, Mohamed R, Gremaud E, LeBreton MH, Guy PA (2008) J Chromatogr A 1177:58–76
93. Martínez-Vidal JL, Aguilera-Luiz MM, Romero-González R, Garrido-Frenich A (2009) J Agric Food Chem 57:1760–1767
94. Lopez MI, Pettis JS, Barton I, Chu PS (2008) J Agric Food Chem 56:1553–1559
95. Turnipseed SB, Andersen WC, Karbiwnyk CM, Madson MR, Miller KE (2008) Rapid Commun Mass Spectrom 22:1467–1480

96. Stolker AAM, Rutgers P, Oosterink E, Lasaroms JJJP, Peters RJB, van Rhijn JA, Nielen MWF (2008) Anal Bioanal Chem 391:2309–2322
97. EU (2002) Commission Decision 2002/657/EC of 12th August 2002, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of the results. Off J Eur Union L221:8–10
98. Kaufmann A, Butcher P, Maden K, Widmer M (2008) J Chromatogr A 1194:66–79
99. Tang HP, Ho C, Lai SS (2006) Rapid Commun Mass Spectrom 20:2565–2572
100. Heller D, Nochetto CB, Rumel NG, Thomas MH (2006) J Agric Food Chem 54:5267–5278
101. Carabias-Martínez R, Rodríguez-Gonzalo E, Revilla-Ruiz P, Hernández-Mendez J (2005) J Chromatogr A 1089:1–17
102. Mendiola JA, Herrero M, Cifuentes A, Ibáñez E (2007) J Chromatogr A 1152:234–246
103. Carretero V, Blasco C, Picó Y (2008) J Chromatogr A 1209:162–173
104. De Brabander HF, Noppe H, Verheyden K, Vanden Bussche J, Wille K, Okerman L, Vanhaecke L, Reybroeck W, Ooghe S, Croubels S (2009) J Chromatogr A (in press) doi:10.1016/j.chroma.2009.02.027
105. Picó Y, Barceló D (2008) Trends Anal Chem 27:821–835
106. Blasco C, Picó Y (2007) Liquid chromatography–mass spectrometry. In: Picó Y (ed) Food toxicants analysis. Techniques, strategies and developments. Elsevier, Amsterdam, pp 509–559
107. Stolker AAM, Zudeima T, Nielen MWF (2007) Trends Anal Chem 26:967–979
108. van der Heeft E, Bolck YJC, Beumer B, Nijrolder AWJM, Stolker AAM, Nielen MWF (2009) J Am Soc Mass Spectrom 20:451–463
109. Wang J (2009) Mass Spectrom Rev 28:50–92