Fluoroquinolones and sulfonamides: features of their determination in water. A review

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ABSTRACT
Sulfonamides and fluoroquinolones are two group of antibiotics widely used in the treatment of bacterial infections. Monitoring these residues in live animals and animal products is commonly legislated; however, the environmental occurrence and fate is still sporadically assessed. The development of adequate analytical methods is a key issue to provide accurate data on concentrations of antimicrobial compounds and their residues in different organic matters. This review presents an overview of proposed methods published between 2008 and 2013 for analysis of fluoroquinolones and sulfonamides with special emphasis on sample preparation and detection systems employed. The coupling of high-performance liquid chromatography and mass spectrometry has been the most widely used method to determination of sulfonamides and fluoroquinolones, concomitantly to other antibiotics residues, due to high sensitivity and selectivity. The main drawbacks of this technique are the high cost and the equipment complexity. The coupling high-performance liquid chromatography and fluorescence detection has also been used to fluoroquinolones determination. Their fluorescent properties allowed the development of several methods with limits of detection in ng L\(^{-1}\) range. Sample preparation is an important tool to reach lower detection limits and the online solid-phase extraction has a broader use. The hydrophilic–lipophilic balance polymeric are the mostly applied sorbents for sulfonamides and fluoroquinolones preconcentration. These sorbents have allowed reaching better recoveries and sensitivity improvement. Physico-chemical properties of these antibiotic groups in addition to trends on papers occurrence and frequency of analysis in different types of water (surface water, groundwater, drinking water and wastewater) are discussed.

1. Introduction
Environmental chemicals are substances present in the environment as a result of human action, intentional or unintentional [1,2]. According to the Food and Agricultural Organization (FAO), antibiotics are compounds of natural or synthetic origin that have the capacity to kill or to inhibit the growth of microorganisms. Antibiotics are
among the emerging micropollutants in water, because of their potential effects on the natural ecosystems [3]. The spread of resistance genes in natural ecosystems can cause imbalance of the population dynamics and the physiology of natural microbial populations. Thus, the release of high concentrations of antimicrobial agents into the ecosystem may be harmful to natural microorganisms and in particular of the aquatic environment [4,5], as water plays a central role in antibiotic resistance dispersion [6]. Many of the antibiotic groups are routinely used for treatment of human infections while also used for treatment and prophylaxis of companion and food-producing animals in EU. Moreover, in some countries including China, Brazil and United States, the veterinary antibiotics are still used as growth promoters. With the exponential population growth worldwide, activities related to agriculture, aquaculture and livestock for food production have increased substantially in the last decades. Antibiotics of veterinary use, their metabolites or degradation products reach the terrestrial and aquatic environment through the use of manure or slurry in agricultural activities, or by pasture-reared animals excreting directly on the land, followed by surface run-off and leaching into deeper layers of the earth [1].

Inputs of antibiotics from these activities are estimated to be at the same order as from human medicinal use (either from hospital or ambulatory settings). In September 2009, the EMA (European Medicines Agency) launched the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project, aiming to develop adequate and homogeneous collection of data, on the use of antimicrobials in veterinary medicine from the EU Member countries. At the second and third ESVAC reports [7,8], the total sales of antimicrobial agents for food-producing animals during 2010 and 2011, in 19 EU countries, were 3246 mg/PCU (population correction unit).

Fluoroquinolone (FQs) antibiotics act by targeting the bacterial topoisomerase II and IV enzymes [9]. Moreover, FQs are one of the antimicrobial groups classified as Critically Important Antimicrobials (CIAs), according to World Health Organization (WHO). The other targeted group includes the bacteriostatic agents sulfonamides (SAs). They act by inhibiting folic acid synthesis by the microorganisms. SAs are currently used both for the prevention and treatment of disease under specified conditions within the EU [10], and are also considered as CIAs in intensive agricultural practice [11,12].

It has been demonstrated in prioritisation exercises and monitoring programmes [13–15] that the knowledge of antibiotics concentration in the environment (soil, sediment and aquatic compartments) is fundamental for establishing the fate of veterinary antibiotics, for devising effective risk-based monitoring programmes. While a confirmatory method should, by definition, result in an unequivocal identification of a compound with high selectivity; a screening method should aim on a wide range of compounds, eventually allowing for a compromised selectivity. Intensive research has been conducted to develop new methods for FQs, SAs and their metabolites in water. The majority of new methods involve the use of high-performance liquid chromatography and mass spectrometry (LC-MS) detection due to their low limits of detection. However, they are still expensive and not adequate for in situ determination. The objective of this review is a critical analysis of detection and quantification methods for evaluation of these antibiotic groups, published between 2008 and 2013.

The selection of the members of the two antibiotic groups (SAs and FQs) to be included in this work was based on the presence of these in one of the following situations: (a) in
prioritisation exercises [13,16], (b) on sales reports from ESVAC, (c) in ATCvet list (Anatomical Therapeutic Chemical classification system for veterinary medicinal products), and (d) in comprehensive review paper [17]. All selected SAs are used in human and veterinary practice, except for sulfachloropyridazine, sulfadoxine, and sulfquinine. These are used exclusively in veterinary practice. In the case of the FQs, some of them are indicated for veterinary use only (marbofloxacin, danofloxacin, difl oxacin, enrofloxacin, and sarafloxacin) and the other selected FQs for this review are for both human and veterinary use.

2. Physico-chemical properties of sulfonamides and fluoroquinolones

As analytical method features are strongly dependent of the analyte physico-chemical properties, a brief account of FQ and SA compounds is given here. Nalidixic acid as the first quinolone resulted as a reaction product during the synthesis of chloroquine in 1962 [18]. FQs result from the addition of the 7-piperazinyl group and a fluorine atom at position 6 (Figure 1). The presence of FQs as environmental contaminants is particularly important, due to their mechanism of action and the considerable capacity to accumulate in solid particles [19]. FQs are naturally highly fluorescent compounds and thus fluorimetric detection can be suitable for the assessment of their degradation and accumulation.

The SAs are analogues of PABA (para-aminobenzoic acid). Their structure (Figure 1) is constituted by a 4-aminobenzene core linked to the SA functional group (SO$_2$NH$_2$). The SAs differ between each other in the N-substituent of the SA linkage. Different side moieties lead to distinct physico-chemical properties, such as the compounds solubility and acidity.

Data on physico-chemical properties of antimicrobial agents selected from the group of FQs and SAs are summarised in Table 1, available as supplementary data (Tables S1 and S2). Partition coefficient (log Kow) and bioconcentration factor (BCF) values for both antibiotic groups described can be considered potentially low values. This indicates that these substances have a low potential for bioaccumulation [20]. The FQs have amphoteric character and most of them are poorly soluble in water at pH 6–8. The SAs are

![Figure 1](image)

**Figure 1.** General chemical structures of fluoroquinolones (a) and sulfonamides (b).
amphoteric but their acidic properties are more pronounced than their basic properties. SAs generally present two pKa values that are related to the formation of cationic form of the amino group (at pH 2–3) and anionic form (at pH 5–11). These compounds are also poorly soluble in water at neutral pH and their solubility increase with rising pH. Most of these compounds are soluble in alkali solutions.

3. Analytical methods applied to environmental water

The results of the literature survey on analytical methods for determination of SA and FQ compounds (from 2008 to 2013) are presented in Tables S3 and S4, available as supplementary data. The survey was conducted via SCOPUS® and Web of science™ databases on 5 February 2014. The search term, applied to title, abstract, and keywords, is specified as follows: ((anti-infective OR antibiotics OR biocide) AND (sulfa* OR *floxacin) AND (‘natural water’ OR ‘surface water’ OR river OR lake OR pond OR ‘ground water’ OR ‘well water’ OR stream OR spring OR ‘wastewater’ OR ‘reclaimed water’ OR ‘treated water’ OR ‘drinking water’) AND (‘determination of’ OR ‘detection of’)). The search was restricted by five years timespan (from 2008 to 2013) and excluding also articles dealing with microbial resistance in general. Reviews summarising literature data up to 2008–2009 focusing on the subject of pharmaceuticals in the environment have been published previously [4,5,21,22]. Therefore, our search was focused on the two groups of antibiotics, commonly used in human and veterinary practice, and more detailed information was collected on the applied analytical methods.

Using the above detailed search criteria, 128 hits were obtained. Data collection was focused on recent methods of detection and their application to various types of water, including drinking water, groundwater, surface water, and wastewater. Data from semi-solid and solid material wastewater products (sewage sludge) were excluded. The criteria of inclusion was based on works related to: analytical method, description of matrix sample preparation, analytical data (detection limit and/or quantification limit and/or linear range) presented at least for one type of water sample. Hence, the selection of articles was reduced to a number of 76. Information about the method application (type of water analysed and concentration found) was also compiled.

3.1. Analytical methods for determination of FQs and SAs

In order to attain the limits of detection (LOD) and limits of quantification (LOQs) adequate to perform environmental analysis, most of the available methodologies must be coupled to some sort of sample treatment to foster the removal of sample

| Antimicrobial class | log Kow | pKa | BCF |
|---------------------|---------|-----|-----|
| FQs                 | −2.92 – 1.60 | 5.62 – 9.30 | 1.00 – 11.75 (pH 5.5) |
|                     |         |     | 1.00 – 3.83 (pH 7.4) |
| SAs                 | −1.22 – 1.68 | 4.18 – 10.10 | 1.00 – 7.21 (pH 4) |
|                     |         |     | 1.00 – 1.78 (pH 7) |

Table 1. Summary data about physico-chemical properties of FQs and SAs.
matrix interferences and also analyte enrichment. Figure 2 shows a schematic representation of sample treatment strategies and their connection to analytical methods.

According to the analysis data (Tables S3 and S4), the most frequently used sample preparation method was the solid-phase extraction (SPE, about 80% of reports). This one has been widely used due to the advantages over other methods, like liquid–liquid extraction, including the lower consumption of organic solvents and the wide variety of solid phases available. The application of online SPE became more frequent and the automation of the sample preparation process brings new capacities for the whole analytical procedure, mostly in terms of sample throughput. The polymeric cartridges are the most commonly used SPE sorbents. The hydrophilic–lipophilic sorbents, such as the reversed-phase Oasis® hydrophilic–lipophilic balance (HLB) cartridge, have been widely used for determination of pharmaceuticals, including FQs and SAs. The Oasis® HLB composition (polymer with lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidone groups) allows the extraction of a wide range of acidic, basic, and neutral compounds. In fact, the most used eluent to FQ extraction in HLB cartridges is methanol [23–33]. Meanwhile, another eluents and mixture have been applied with Oasis® HLB sorbent, such as acetonitrile [34,35], methanol:formic acid (96:4, v/v) [36], 2% formic acid in methanol [37], 1% ammonia in methanol [38,39], dichloromethane:methanol (70:30, v/v)[40], and phosphate buffer at pH 3 [41]. Concerning the SA extraction with Oasis® HLB sorbent, methanol is also the eluent most commonly applied [24–29,31–33,42–45]. Among other eluents and mixtures, application of dichloromethane:acetone (3:2, v/v)[46], dichloromethane:methanol (70:30, v/v)[40], 1% formic acid in methanol (v/v) and methanol:ethylacetate (1:1, v/v)[47], methanol:acetonitrile (1:1, v/v) [48], acetonitrile [34], acetonitrile and methanol [49], methanol and 0.1% ammonia pH 9 in methanol (v/v) [50], and ammonia:methanol (1:19, v/v) [51] can be cited.

Application of Oasis® HLB sorbent with previous clean-up step has been applied for selective extraction of organic matter in water samples. For example, Sturini et al. developed an SPE method based on extraction with Oasis® WAX (weak anion-exchange) followed by Oasis® HLB sorbents for FQ extraction. Better chromatogram resolution due
to lower matrix effect was obtained as compared with other sorbent strategies studied (such as HLB and WCX). Concentration factor value of 100 was determined for both analysed compounds (marbofloxacin and enrofloxacin). Recoveries in the range of 90–116% were achieved [41]. A clean-up step in the SPE procedure has also been applied using SAX (strong anion-exchange) in tandem with Oasis® HLB for extraction of FQs from wastewater. Separation and determination of FQs were performed by LC-fluorescence detection (FD). Recoveries in wastewater were in the range of 75–121% and LODs in the range of 8.5–85 ng L⁻¹ [30].

The Oasis® HLB has also been applied to Prospekt™ system, which allows the full automation of online SPE [52]. The association between Oasis® HLB and Oasis® MCX (mixed-mode, reversed-phase/strong cation-exchange cartridge) has been used for pre-concentration of various antibiotics, including FQs and SAs. These cartridges connected in series used methanol as eluent and presented better recoveries of SAs as compared with recoveries using single Oasis® HLB cartridge. The recoveries of sulfacetamide, sulfadiazine, and sulfathiazole have increased from 66.8%, 64.2%, and 51.3% to 88.9%, 88.9%, and 67.4%, respectively [53]. Meanwhile, Diaz-Cruz et al. [54] have evaluated the single MCX and HLB sorbents and the combination of both to extraction of SAs in four types of water (surface water, wastewater, groundwater, and drinking water). Better recoveries were obtained using single HLB cartridges. For example, SA extraction from surface water samples presented recovery values in the range of 97.1–139%, 55–110% and 75.6–92.49% for HLB, MCX, and tandem HLB-MCX sorbents, respectively [54]. When comparing the effect of Oasis® HLB, Strata-X, SDB-XC (poly-styrene divinylbenzene copolymer) and MCX sorbents associated with a capillary electrophoresis (CE) method to SA detection, it was observed that better resolution and sensitivity were obtained with the HLB sorbent [55].

Other commercially available sorbents, such as HySphere™ C18 HD (end-capped, silica-based phase with a high loading of octadecyl chains) [56] and Strata-X (polymeric reversed phase) [57–59], were applied to preconcentrate FQs. A comparison between weak cation-exchange Oasis® WCX and Oasis® HLB sorbents was performed and it was observed that better recoveries have been obtained with HLB sorbent: namely, 89.1, 101.7 and 99.7% for ofloxacin, norfloxacin, and ciprofloxacin, respectively [60]. However, the HLB sorbent showed a better precision (4%) compared to WXC (12%), while the WXC presented lower LOQs, specifically 3.5–5.8 ng L⁻¹ compared to HLB (10–16.5 ng L⁻¹). Concerning method selectivity, higher peak areas were obtained for analysed FQs (ciprofloxacin, ofloxacin, and norfloxacin) using WXC as compared with HLB sorbent.

Moreover, the silica-based MEP sorbent (Anpel™), consisted of divinylbenzene and vinylpyrrolidone copolymer, has been applied for extraction of FQs from wastewater. Recoveries of FQs in wastewater ranged from 79 to 109% [37].

Other examples of sorbents used to SA extraction are the HyperSep Retain PEP (polystyrene divinylbenzene modified with urea functional groups) [61], Oasis® MCX [62,63] and Strata-X cartridges [64,65]. SPE columns have also been used to extract FQs, including the reversed phases based on octadecyl moieties [66] and extraction of multiclass drugs, including sulfamethoxazole, with Strata-X sorbent [67].

Alternative methods to SPE for sample preparation have also been used to FQs and SAs. Montesdeoca et al. applied micellar desorption (MD) in two different methods for determination of five FQs (levofloxacin, ciprofloxacin, norfloxacin, enrofloxacin, and
saraflloxacin) in water. A comparison between MD and traditional desorption in organic phase, namely methanol, was performed. Separation and determination of the FQs were performed by LC-FD in both studies. One of these was based on solid-phase microextraction (SPME) with MD. The SPME was performed using a Carbowax/ Templated Resin fibre saturated with NaCl. MD was performed with polyoxyethylene 10 lauril ether (POLE). This SPME with MD presented lower LODs, which ranged 0.01–0.03 ng mL$^{-1}$ compared to LODs from methanol desorption (0.02–0.18 ng mL$^{-1}$) [68]. Montesdeoca et al. have also applied MD to an SPE procedure for FQ extraction. In this case, the SPE extraction was conducted with HLB sorbent at a pH value of 3.0. The LODs were lower using POLE (0.010–0.034 ng mL$^{-1}$) as compared with the LODs using methanol (0.072–0.200 ng mL$^{-1}$) [69]. Magnetic solid-phase extraction (MSPE) based on magnetic poly(vinylimidazole-co-divinylbenzene) sorbent was applied to determination of five FQs, including enrofl oxacin, marbofl oxacin, and lomefl oxacin, in surface water. Recoveries obtained varied from 52.1 to 104.5% [70].

As illustrated in Figure 2, other strategies for sample treatment have been employed. In fact, a dispersive liquid–liquid microextraction (DLLME) method was developed to determine FQs in water samples. The application of pH 7.6, 685 μL of CHCl$_3$ (as extraction solvent) and 1250 μL of ACN (as disperser) was selected as optimal conditions for DLLME procedure. Accuracy study was performed and values of 90–108% were obtained for mineral water and 87–116% were attained for run-off water [71].

A method based on dispersive solid-phase extraction (DSPE) using oxidised multi-walled carbon nanotubes (o-MWCNTs) for extraction of FQs from water has been developed. 150 mg of o-MWCNTs was used for FQ extraction from 250 mL of spiked water sample (pH 5.0). Elution was performed with 25 mL of 3:1 (v/v) acetone/methanol. Recoveries ranging 62.3–116% with RSD<7.7% were obtained [72]. Moreover, three different studies have applied supported liquid membrane extraction (SLME) as sample preparation method of FQs, SAs, and other antibiotics [73–75]. SLME is based on a structure with an organic membrane phase immobilised in a porous of polymeric support that separates two aqueous phases. The analyte molecules present in the donor phase are dissolved into membrane phase and migrate to the acceptor phase to finally be entrapped in this phase [74].

All SLME studies reported here have applied a Q3/2 Accurel PP polypropylene as hollow fibre (HF). Poliwoda et al. developed an SLME for determination of ciprofloxac in, enrofloxac in, norfloxac in, and danofloxac in in surface water. Optimal conditions for the SLME were as follows: 20% (w/w) di-(2-ethylhexyl) phosphoric acid (carrier) in di-n-hexyl ether as membrane phase. Aqueous pH 6 with 10 mL sample volume and 1.0 M HCl were used as donor and acceptor phases, respectively. Recoveries between 70 and 80% were achieved [74].

Supported liquid membranes have also been used by Payan et al. An HF was soaked with 1-octanol and placed between a 2 M Na$_2$SO$_4$ aqueous solution (pH 7.0) as donor phase and aqueous solution (pH 12.0) as acceptor phase. The following enrichment factors were obtained: 95 for marboflo xacin, 60 for norfloxac in, 50 for ciprofloxac in, 200 for danofloxac in and enrofloxac in, and 900 for flumequin. LC combined with diode array detector (DAD) and FD were applied to determine FQs in water. Lower LODs with FD detection were obtained (0.3–16 ng L$^{-1}$) as compared with DAD detection (7–20 ng L$^{-1}$) [73].
SLME has also been developed for determination of multi-class antibiotics: namely, FQs, SAs, macrolides, and tetracyclines, in water. In total, 20% (w/v) Aliquat 336 (carrier) in dihexyl ether was applied as membrane phase. The extraction employed 20 mL of sample (donor) solution (pH 8.0) and 20 μL of acetic acid as acceptor phase (pH 4.0). Enrichment factors were in the range of 10.9–21.5 for SAs and 30.6–69.2 for FQs [75].

Molecularly imprinted polymer (MIP) extraction has also been used as an alternative for selective SPE of FQs in water [76–78]. Magnetic MIPs were used as sorbent for selective extraction of FQs in wastewater and surface water samples. These magnetic MIPs have been prepared with ciprofloxacin as template molecule, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linking agent and Fe₃O₄ magnetite as magnetic component. Recoveries obtained were 76.3–94.2% [76]. The application of MIPs as selective material for microextraction by packed sorbent (MEPS) has been described for determination of three FQs (ciprofloxacin, norfloxacin, and ofloxacin) by LC-MS/MS, with concentrations detected at ng L⁻¹ level. A comparison between MIP-MEPS and MIP-SPE method was performed. Recoveries of MIP-MEPS were higher (93–115%) as compared to MIP-SPE (87–92%). LODs of MIP-MEPS strategy were lower (0.8–3.8 ng L⁻¹) as compared to MIP-SPE (2.0–8.1 ng L⁻¹) [77].

Rodriguez et al. [78] synthesised monodispersed MIP microspherical beads for online SPE coupled with LC-FD for determination of FQs in water. MIPs were synthesised via precipitation polymerisation using enrofloxacin as the template. These MIPs possess a decreased particle size, which can enhance the resolution and efficiency of SPE extraction.

Ion-pair-based surfactant-assisted microextraction (IP-SAME) was evaluated for extraction of ciprofloxacin and ofloxacin in water and urine samples. The FQs were converted into their ion-pair complexes with Aliquat 336. Then, these complexes were extracted into 1-octanol dispersed in aqueous solution. After extraction and phase separation, 20 μL of the top layer of organic solvent were collected into a syringe and it was directly injected into an LC instrument. Preconcentration factors of 368 and 324 for ciprofloxacin and ofloxacin, respectively, were obtained [79].

MEPS has also been applied for quinolones extraction from groundwater and urine samples [80]. MEPS procedure used silica-C18 as sorbent. Determination of analytes was performed in an LC-UV system at a wavelength of 280 nm. Recoveries of the MEP-LC-UV method ranged 72–98.9%.

Concerning SAs, an MSPE method was applied to extract SAs from surface and wastewater samples. Silica-coated magnetite/graphene was used as adsorbent. Recoveries were in the range of 74.2–89.3% for wastewater and 76.4–104.1% for surface water samples [81]. An SLME methodology was developed for determination of SAs (sulfadiazine, sulfamethazine, sulfamerazine, and sulfamethoxazole) and their metabolites: N4-acetyl-sulfadiazine (NSDZ), N4-acetyl-sulfamethazine (NSM), N4-acetyl-sulfamerazine (NSMR), N4-acetyl-sulfamethazine (NSMT), and N4-acetyl-sulfamethoxazole (NSMZ). A Q3/2 Accurel KM polypropylene HF was soaked with 1-octanol with aqueous solution (pH 12.0) and the donor phase was 2 M Na₂SO₄ aqueous solution (pH 4.0). Concentration factors calculated were higher for primary compounds (200 for sulfadiazine, 250 for sulfamethazine, 1000 for sulfamerazine and sulfamethoxazole) as compared to their metabolites (175, 400, 500, and 600) for NSMT, NSDZ, NSMR, and NSMZ, respectively [82].
Room temperature ionic liquids are solvents with good solubility for inorganic and organic compounds. Tao et al. developed a method based on SLME with ionic liquid as membrane phase for extraction of SAs from surface and wastewater. 1-Octyl-3-methylimidazolium hexafluorophosphate (ionic liquid) with 14% of tri-n-octylphosphine oxide was used as membrane phase. KH_2PO_4 with 2 M Na_2SO_4 (pH 4.5) and NaOH (pH 13) were applied as donor and acceptor phase, respectively. Concentration factors were calculated: 128 for sulfadiazine, 135 for sulfamerazine, 100 for sulfamethazine, 73 for sulfamethoxazole, and 58 for sulfadimethoxine [83].

A method based on DLLME for simultaneous extraction of FQs and SAs has been proposed. Analyte samples with 20% (w/v) of NaCl were adjusted at pH 7.6. CHCl_3 (685 μL) and acetonitrile (1250 μL) were used as extractant and disperser solvent, respectively. Recoveries of 78–117% (RSD 1–20%) for mineral water, and 80–117% (RSD 1–16%) for run-off water were obtained [84].

Within the analyte separation methods for SA and FQ antibiotics, LC was the most widely used method. Since the successful coupling of LC-MS from the 90s, this hyphenation has been widely used for the analytical determination of compounds. Concerning the FQs, the main transitions in the LC coupled to tandem mass spectrometry (MS/MS) include the loss of H_2O and CO_2 from the protonated molecular ion [23,25,28]. Common transitions as an example for ciprofloxacin include m/z 332 > 314 ([M + H–H_2O]^+) and 332 > 288 ([M + H–CO_2]^+) [28,31,40,85] and for norfloxacin, m/z 320 > 302 ([M + H–H_2O]^+) and 320 > 276 ([M + H–CO_2]^+) [28,31,38,40]. In addition, losses of the piperazine substituent in different extent are important for FQ identification purposes [23,28]. A typical transition in high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) for SAs correspond to the cleavage of the S–N bond forming the stable sulfanilamide moiety [M–RNH_2]^+. The transition [M + H]^+>156 resulting from these cleavage has been selected for quantification [54] and qualification of SAs [25,46]. Other characteristic transitions include [M + H]^+>92 ([M–RNH_2–SO_2]^+) [28,32] and [M + H]^+>108, which corresponds to loss of SO from the fragment m/z 156 [43,53,61]. Other methods, such as CE [71], turbulent flow chromatography (TFC) [85], and LC-UV [86], together represented 3% of the total reports.

Concerning methods of detection for FQs and SAs, the election method of detection for both groups (SAs and FQs) was the mass spectrometry. Its widespread use is due to increased selectivity and sensitivity, which provides lower LODs. In fact, the distribution of LOD values obtained for determination of ciprofloxacin and sulfamethoxazole, presented in Figure 3, reveals that 20% of the reported methods (Tables S2 and S3) provided LOD values c.a. 1 ng L\(^{-1}\) for ciprofloxacin and c.a. 0.1 ng mL\(^{-1}\) for sulfamethoxazole. More than 60% of the methods presented LOD values < 10 ng mL\(^{-1}\), for both species.

With regard to the FQs, FD is the second most used method. FD methods for FQ determination are commonly associated with LC separation and have been achieved good sensitivity values with LODs in ng L\(^{-1}\) scale [30,37,41]. For example, Yiruhan et al. obtained LODs in the range of 0.28–0.83 ng L\(^{-1}\) by application of an SPE-LC-FD method for determination of FQs in water [35]. Concerning the fluorescent characteristics of FQs, it has been reported that the acidity of the medium has a large effect on fluorescence of these compounds. The fluorescence intensity decreases gradually with the increasing of pH value [87,88]. It was reported that the luminescence reaches its maximum in a
monoprotonated form for the FQs: norfl oxacin, ciprofl oxacin, and pefl oxacin. The typical transitions of the FQs are the $\pi^*\rightarrow\pi$ for all other protolytic forms [88]. The reported wavelengths most commonly used to FQ detection in aqueous media are 278 or 280 nm for excitation wavelength, and 445 or 450 nm for emission wavelength [30,35,37,41,68]. Payan et al. have measured the excitation and emission wavelength to FQs, respectively: 278 and 445 nm for norfl oxacin; 280 and 456 nm for ciprofl oxacin, danofl oxacin, and enrofl oxacin; 300 and 515 nm for marbofl oxacin; 315 and 368 nm for flumequine [73]. Rodriguez et al. [78] have used the same excitation wavelength for different FQs and two emission wavelengths (440 nm for norfl oxacin, ciprofl oxacin, danofl oxacin and enrofl oxacin, and 515 for levofl oxacin), affording similar sensitivity, with LODs in the range of 1–11 ng L$^{-1}$ and 1–12 ng L$^{-1}$ for drinking water and for wastewater, respectively.

The less used methods are included in the category corresponding to ‘Others’, for each group of antibiotics. Among these a potentiometric method was developed to determination of sulfamethoxazole in aquaculture water, with LOD of 3 μg mL$^{-1}$ [89]. Studies have been applied to DAD detection with LC separation for determination of FQs and SAs. In most studies, sensitivity has been obtained in a μg L$^{-1}$ scale [26,74,84,87]. As an example of DAD application, Pavlović et al. [26] have developed an SPE-LC-DAD method for multiple class drugs, including SA and FQ. LODs obtained were 0.9 μg L$^{-1}$ for sulfamethoxazole and 3.9 μg L$^{-1}$ for ciprofl oxacin. Surface plasmon resonance (SPR) was coupled with MIP and indirect inhibitive immunoassay for determination of sulfamethoxazole in surface water. The LOD for sulfamethoxazole was 0.01 μg L$^{-1}$ [90].

Concerning methods of detection of FQs less frequently applied, a flow injection analysis based on chemiluminescence and application of selective MIPs was developed for determination of enrofl oxacin in surface water. The LOD for enrofl oxacin was 0.27 μg L$^{-1}$ [91]. An electrochemical approach was developed to determine ofloxacin in wastewater, the LOD obtained was $1.6 \times 10^{-7}$ mol L$^{-1}$ [92]. Voltammetry was applied in an
eletrochemical immunobiosensor for detection of enrofloxacin in surface water. The lowest detected concentration of free enrofloxacin was 10 pg mL\(^{-1}\) [93].

Other methods with immunoassays features have also been applied for SA and FQ detection. Pastor-Navarro et al. developed immunoassays in the formats, ELISA in polystyrene 96-well plate, and high-density microimmunoassay on compact disc format. Immunoreagents based on the hapten 4-amino-N-(3-hidroxypropil)-benzenosulfonamide with ovalbumin were applied to both assays to determination of sulfamethoxazole in wastewater. ELISA plate assay presented lower LOD (0.001 ng mL\(^{-1}\)) compared to microimmunoassay on compact disc format (0.09 ng mL\(^{-1}\)) [94]. An indirect inhibitive immunoassay using SPR coupled with MIP was also applied for analysis of sulfamethoxazole. For this purpose, anti-SMZ monoclonal antibodies (MAbs) were used. LOD of sulfamethoxazole was 10 ng L\(^{-1}\) [90]. Zhang et al. [95] developed a time-resolved fluoroimmunoassay (TRFIA) using coating antigens (SA-ovalbumin) and Europium-labelled MAbs for determination of sulfamethoxazole, sulfamethazine, and sulfadiazine. LODs and LOQs ranged 5.4–9.1 ng L\(^{-1}\) and 14.7–61.5 ng L\(^{-1}\), respectively. Concerning FQs, a sensitive indirect competitive enzyme-linked immunosorbent assay (icELISA) was developed for analysis of norfloxacin in water. LOD of 0.016 μg L\(^{-1}\) was achieved. In this immunoassay, a specific polyclonal anti-norfloxacin antibody was applied [96].

### 3.2. Application to water samples

There is undoubtedly a growing interest in determining SA and FQ compounds in water. The frequency distribution of analysis of individual SA and FQ compounds (Figure 4) demonstrates that sulfamethoxazole was the most frequently studied substance, corresponding to 21% of occurrences. This can be related with its generalised use, mainly in human therapy [90,94].

Sulfadiazine (frequency of 9%), considered of high priority in three prioritisation studies of veterinary medicines [13,16,97] to environmental concerning, is listed as the third most recurrent from the SAs. Ciprofloxacin, norfloxacin, and enrofloxacin, which are widely used FQs, had a major frequency of determination, corresponding to 47% of all reports analysed. In fact, enrofloxacin, a veterinary antimicrobial agent, was considered as a high-priority substance in environmental monitoring according to [16] and [13], and was represented 14% of FQ occurrence. Ciprofloxacin, the FQ with major occurrence (19%), is one of the most used FQs in human treatment. In a prioritisation work by de Voogt et al., ciprofloxacin was considered to be a high-priority compound to a study on pharmaceuticals in water management, since it fulfils more than four of the seven criteria applied (regulation, consumption/sales, physico-chemical properties, degradability/persistence, resistance to treatment, toxicity, relative risk approach (PEC/PNEC)/occurrence in wastewater) [98].

Regarding the data distribution according to water sample types, shown in Figure 5, there are a larger proportion of reports on the determination of SAs and FQs in surface water (c.a. 43–44%). The determination of these antibiotic groups in wastewater is also frequent (34–36% of occurrences). In fact, according to the biological or disinfection process applied, the antibiotics removal efficiencies in wastewater treatment plants (WWTP) can vary significantly. The physico-chemical properties (chemical structure, solubility, Kow, pKa), the consumption patterns and the
operational conditions can also have an effect on the removal efficiency [99–101]. For example, at the disinfection process (e.g. chlorine, chlorine dioxide, ozone), several disinfection by-products from FQs and SAs are formed. These may retain antibacterial activity [101]. As an example to FQs, enrofloxacin originates ciprofloxacin as metabolism product [13]. As regard to SAs, the metabolites can deconjugate, such as N4-acetyl sulfamethoxazole into sulfamethoxazole during the wastewater treatment [99], which can contribute to the antibiotic permanence in the surface water. In fact in the analysed reports, data on determination of SA metabolites were found. Examples of

Figure 4. Frequency of occurrence of sulfonamide (a) and fluoroquinolone (b) compounds. NSDZ: N4-acetyl sulfadiazine; NSFP: N4-acetyl sulfapyridine; NSMR: N4-acetyl sulfamerazine; NSMT: N4-acetyl sulfamethazine; NSMZ: N4-acetyl sulfamethoxazole; SBZ: sulfabenzamide; SCP: sulfachloropyridazine; SCT: sulfacetamide; SDM: sulfadimethoxine; SDZ: sulfadiazine; SFD: sulfadoxine; SFG: sulfaguanidine; SFN: sulfanilamide; SFP: sulfapyridine; SFQ: sulfamethoxazine; SFT: sulfathiazole; SME: sulfameter; SMP: sulfamethoxypyridazine; SMR: sulfamerazine; SMT: sulfamethazine; SMZ: sulfamethoxazole; CIP: ciprofloxacin; DIX: difloxacin; DNF: danofloxacin; ENX: enrofloxacin; FLU: flumequin; LMX: lomefloxacin; MBX: Marbofloxacin; NFX: norfloxacin; OFX: ofloxacin; PFX: pefloxacin; SFX: sarafloxacin.
these compounds are cited as follows: N4-acetyl sulfadiazine [49], N4-acetyl sulfamer-azine [49,82], N4-acetyl sulfamethazine [43,45,48,49,54,82], N4-acetyl sulfamethoxazole [45,49,65,82], and N4-acetyl sulfapyridine [49]. The data from determination of these transformation products were included in this review. These metabolites presented a analysis frequency of 6%. Regarding the concentration levels of these compounds in water: in 61% of the cases the concentration was <10 ng L\(^{-1}\), and in 39% of the cases was in the range between 10 and 1000 ng L\(^{-1}\).

We can also observe that SAs and FQs present 12% and 6%, respectively, in groundwater (Figure 5). The drinking water analysis was the less representative to both antibiotic groups. This must be related to the lower levels found of these antimicrobials in this type of water. The previous treatments in WWTPs makes it less probable to find pollutants in drinking water.

Only a part of the reports for method development analysed exhibit application data in real samples. SAs have been detected in surface water, wastewater, and groundwater. Sulfamethazine was also detected in drinking water.

A distribution frequency analysis of the most frequently studied antibiotics from both FQ and SA groups (ciprofloxacin and sulfamethoxazole, respectively) was performed. The concentrations found of each antimicrobial agent were divided into four ranges (in ng L\(^{-1}\)): <10, 10–100, 100–1000, and >1000. According to the data obtained, sulfamethoxazole and ciprofloxacin presented the following distribution frequency, according to the level range, respectively: <10 ng L\(^{-1}\) (34% and 29%), 10–100 ng L\(^{-1}\) range (31% and 34%), 100–1000 ng L\(^{-1}\) range (27% and 25%), and >1000 ng L\(^{-1}\) (8% and 12%). To both substances, the concentration levels >1000 ng L\(^{-1}\) were found. The type of water distribution frequency in the four ranges cited above was also analysed. For sulfamethoxazole, the analysis distribution frequency presented a higher proportion of analysis in wastewater and surface water, corresponding to 75% and 85% to levels <10 ng L\(^{-1}\) and at 10–100 ng L\(^{-1}\) range, respectively. At 100–1000 ng L\(^{-1}\) range and concentration levels >1000 ng L\(^{-1}\), the levels found were only represented of wastewater and surface water. For ciprofloxacin, the analysis distribution frequency shows bias to wastewater and surface water concomitantly, corresponding to 81%, 95%, and 93% of

Figure 5. Distribution per type of water sample concerning SAs (a) and FQs (b) analysis.
the concentrations <10 ng L\(^{-1}\), between 10–100 ng L\(^{-1}\) and 100–1000 ng L\(^{-1}\) range, respectively. The concentration levels >1000 ng L\(^{-1}\) were determined only in wastewater.

According to the presented data, the frequency of detection of the FQs in groundwater was very low. It was observed that ofloxacin, lomefloxacin, and marbofloxacin were detected in a single report, in concentrations <1 \(\mu\)g L\(^{-1}\) [32]. This low frequency of detection in groundwater may be associated with the high sorption capacity of FQs to soils and sediments, which can delay their degradation [19]. Mechanisms such as cation bridging, electrostatic interactions, and hydrogen bonding are proposed to explain the sorption of FQs on soils [102]. The low frequency of detection of both antibiotic groups in groundwater and drinking water may be also related with the smaller proportion of reports analysing these compounds in these types of water.

4. Conclusions

Challenges concerning analysis of SAs and FQs in water are still open. Current methods allow low detection limits, but still require major sample treatment. Furthermore, long-term strategies for environmental monitoring, such as the application of passive sampling devices, should be pursued. According to the data analysed, the coupling of LC to MS/MS has been the most widely used method to determination of SAs and FQs, concomitantly to other antibiotics residues.

This study also shows that screening methods for in situ application are still needed for determination of antibiotic residues. Most of the currently available methods were developed to be applied in laboratory settings, and they are not suitable for field deployment. Research in this area is most welcome to environmental analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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