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Analytical challenges and occurrence of antibiotics in biosolids from municipal sewage treatment plants in North Spain

A. Barros, G. Castro, M. Ramil, I. Rodríguez *

Department of Analytical Chemistry, Nutrition and Food Sciences, ARCUS – Aquatic One Health Research Centre, Universidade de Santiago de Compostela, R/ Constantino Candeira, 5, 15782 Santiago de Compostela, Spain

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ABSTRACT

Antibiotics are among the most concerning pharmaceutical contaminants released from municipal sewage treatment plants (STPs), occurring both in treated effluents and in dewatered biosolids. This study examines key analytical challenges during the determination of ten antibiotics in sewage wastes using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Furthermore, results of their distribution in dewatered biosolids from different STPs are presented. Fluoroquinolones were identified as the most problematic compounds due to sorption on glassware, strong interaction with sample matrix, and signal suppression effects during LC-MS/MS analysis. Optimized extraction, based on sonication of freeze-dried samples with a buffered acetonitrile–water solution (pH 4.4, 1:1), yielded average recoveries between 74% and 108%, with moderate variability across sludge types. During simultaneous quantification of multiclass antibiotics, fluoroquinolones exhibited moderate to high signal attenuation depending on the matrix. For this group of compounds, signal suppression could be mitigated by fractionating extracts using mixed-mode (reversed phase and cation exchange) sorbents. Combined with isotopically labelled surrogate standards, solvent based calibration enabled accurate quantification of all targeted compounds, achieving limits of quantification below 5 ng g^{-1}. Azithromycin, clarithromycin, norfloxacin, ciprofloxacin and ofloxacin were ubiquitous in dewatered biosolids, with median concentrations ranging from 7 ng g^{-1} (clarithromycin) to 1761 ng g^{-1} (ofloxacin). A mass balance assessment of emissions through treated wastewater and biosolids highlighted azithromycin, ciprofloxacin, norfloxacin, and ofloxacin as the priority antibiotics for monitoring in final dewatered solid waste streams (biosolids) from STPs.

1. Introduction

Antibiotics are among the most concerning groups of emerging pollutants due to their ability to promote the development of antibiotic resistance genes (ARGs) and the potential incorporation of these genes into bacterial genomes [1–3]. Municipal wastewater constitutes the main route by which antibiotics for human use enter the environment [4]. Their fate during sewage treatments is driven by the balance between degradation, or transformation, in the aqueous phase and sorption to sludge [5]. Despite their moderate (e.g. macrolides) to high polarity and amphoteric properties (e.g. fluoroquinolones), residues from various families of antibiotics have been consistently detected in final dewatered sludge (hereafter biosolids) from municipal sewage treatment plants (STPs), regardless the employed sludge treatment technologies [6,7].

Antibiotic residues may affect microbial communities involved in key biochemical pathways, including those controlling the production of biogas during anaerobic digestion [8], and the biodegradation of other concerning pollutants at STPs [9]. Furthermore, the fraction of these compounds remaining in biosolids and/or derived products, such as compost [10], vermicompost [11] and sludge digestates [12], raises concern regarding the environmental impacts associated with their application as fertilizers in agricultural or forestry soils.

From the perspective of control laboratories, the intrinsic complexity of biosolids, together with the need of specific sample preparation conditions for the effective extraction of certain families of compounds [12], represents a major barrier to elucidate the mass balance of antibiotics at municipal STPs. Two critical steps during the sensitive determination of antibiotics in sludge and biosolids are: (1) the effective, simultaneous extraction of groups of compounds that differ greatly in their physico-chemical properties, and (2) mitigating matrix-induced signal suppression that compromises their detectability by liquid

* Corresponding author.
E-mail address: isaac.rodriguez@usc.es (I. Rodríguez).

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chromatography tandem mass spectrometry (LC-MS/MS).

Pressurized liquid extraction (PLE), ultrasound assisted extraction (UAE) and QuEChERS have been tested for the extraction of antimicrobial agents from biosolids, sludge and related matrices, such as manure. Solvent mixtures comprising polar organic solvents and ultrapure water buffered, or simply adjusted at acidic pH, were proposed for PLE extraction of fluoroquinolones from freeze-dried sludge and sludge-amended soils [13–16]. For less polar antibiotics such as macrolides, sulfonamides and trimethoprim, higher extraction efficiencies have generally been reported for pure organic solvents (e.g. methanol) than for their aqueous solutions under similar PLE conditions [14].

Solid-liquid extraction approaches based on QuEChERS protocols have also yielded quantitative recoveries during extraction of macrolides and sulfonamides from moisturized sludge [17]. However, inconsistent results have been described for fluoroquinolones. While some studies reported low extraction efficiencies, in the range from 10% to 20% [18,19], modifications to the QuEChERS protocol, including its combination with a sonication step, improved the recoveries of fluoroquinolones, albeit at the cost of reducing those for certain macrolides (clarithromycin and erythromycin) and sulfonamides [20].

The aims of this study were: (i) to identify critical factors affecting the performance of LC-MS/MS determination of antibiotics from five antimicrobial classes (macrolides, quinolones, sulfonamides, lincosamides and diaminopyrimidines); (ii) to evaluate the influence of different variables on the efficiency and selectivity of their extraction; (iii) to propose a streamlined sample preparation workflow achieving limits of quantification (LOQs) low enough to monitor their levels in biosolids and different types of sludge produced in municipal STPs; and (iv) to underscore those antibiotics that must be determined in biosolids based on their relative emissions through dewatered sludge and treated wastewater.

2. Material and methods

2.1. Solvents, sorbents and standards

Ultrapure water was obtained in the laboratory using a Geni U system (Rephile, Shanghai, China). LC-MS grade acetonitrile (ACN), methanol (MeOH), formic acid (FA), NaOH and citric acid were provided by Merck (Darmstadt, Germany). Polypropylene (PP) tubes (15 mL and 50 mL) and sorbents for QuEChERS extraction (AOAC and EN modalities) [21] were purchased from Waters (Milford, MA, USA). Solid-phase extraction (SPE) cartridges (OASIS HLB, 200 mg; and OASIS MCX, 150 mg) were also obtained from Waters. Graphitized carbon and C_{18}, as bulk sorbents, were provided by Merck. An aqueous buffer, at pH 4.4, was prepared by mixing a 0.2 M solution of citric acid with the required volume of NaOH 1 M.

Standards for native antibiotics (azithromycin, clarithromycin, erythromycin, clindamycin, ciprofloxacin, ofloxacin, norfloxacin, enrofloxacin, sulfamethoxazole and trimethoprim) and the isotopically labelled analogues (azithromycin-d_3, clarithromycin-d_3, ciprofloxacin-d_8, levofloxacin-d_3, sulfamethoxazole-$^{13}C_6$ and trimethoprim-d_9) were purchased from Merck and Cymit Quimica (Barcelona, Spain), respectively. CAS numbers and chemical structures of targeted compounds are provided as supplementary information, **Table S1**. Individual solutions of each compound were prepared in MeOH [20,22]. Further dilutions and separate mixtures of native and labelled compounds were made in the same solvent. Individual standards and their mixtures were stored in PP tubes, maintained at −20 °C. Calibration solutions containing increasing concentrations of native antibiotics from 0.050 ng mL^{-1} to 100 ng mL^{-1} (n = 9) and a fixed concentration of labelled compounds (8 ng mL^{-1}) were prepared in 1:1 mixture of ACN: H_2O, buffered at pH 4.4.

2.2. Samples and sample preparation

Biosolids were obtained from 23 STPs processing sewage from municipalities in the North of Spain. Four plants include an anaerobic digestion unit, operating under mesophilic conditions, previously to sludge dewatering. The rest of facilities did not apply any treatment in the line of sludge, except a centrifugation step to reduce the percentage of water in biosolids to a value of 70–72%. In addition to the final biosolids, different types of sludge were obtained from a large STP including a thermal hydrolysis unit, followed by anaerobic sludge digestion previously to dewatering. Treated wastewater (time proportional integrated samples, obtained at intervals of 30 min during 24 h) and biosolids were also obtained from three STPs. All samples were directly provided by companies operating municipal STPs. After reception semi-solid samples (sludge and biosolids) were frozen, freeze-dried, homogenized using a mortar, and stored at −20 °C until extraction. Treated wastewater was maintained at −20 °C until analysis.

During method development, different extraction techniques and conditions were assessed. In these assays, 0.5 g of a pooled matrix prepared by combining freeze-dried biosolids from several STPs, were spiked with a mixture of nine antibiotics (enrofloxacin was not available during these preliminary experiments) and stored overnight before extraction.

QuEChERS extractions were carried out using 5 mL of ACN, containing 2.5% of FA. Samples were first moisturized with 4 mL of ultrapure water followed by addition of acidified ACN and salts corresponding to AOAC (magnesium sulphate, sodium chloride and sodium acetate) or EN (magnesium sulphate, sodium chloride, disodium hydrogen citrate and trisodium hydrogen citrate) modalities of the QuEChERS protocol [23], respectively. After manual agitation and centrifugation, the organic phase was recovered and filtered before analysis. Pressurized liquid extraction (PLE) was conducted in 11 mL stainless steel cells, using an ASE200 extractor from Dionex (Sunnyvale, CA, USA), under similar conditions to those employed in previous studies [16]. In brief, 0.5 g of sample was placed above 1 g of diatomaceous earth in the extraction cell. The remaining free volume was filled with same sorbent. Extractions were conducted at 90 °C, with cells pressurized at 1500 psi, in two cycles of 5 min. Buffered (pH 4.4) and non-buffered mixtures of ACN:H_2O (1:1) were tested as extraction solvents. Extracts were adjusted at final volume of 25 mL before analysis. As for ultrasound assisted extraction (UAE), experiments were conducted in an ultrasonic bath, using different volumes (10 and 25 mL) of MeOH:H_2O and ACN:H_2O (pH 4.4) solutions.

As for clean-up step different strategies were tested: i) SPE concentration of the extracts, previously diluted with ultrapure water, using reversed-phase HLB cartridges; ii) fractionation of undiluted extracts with mixed-mode (MCX) cartridges; and iii) dispersive SPE (d-SPE), adding graphitized carbon and/or C_{18} sorbents to a fraction of the extract.

The extraction efficiencies (EEs, %) of the tested techniques were calculated as the ratio between the difference of responses for each antibiotic in the extracts from spiked (2000 ng g^{-1}) and non-spiked fractions of the pool of biosolids, divided by the difference in responses for post-extraction spiked and non-spiked extracts, and multiplied by 100. Matrix effects (MEs, %) during LC-MS/MS analysis were estimated as the normalized ratio between difference of responses for spiked (40 ng mL^{-1}) and non-spiked extracts, divided by the response obtained for the reference standard. Thus, MEs around 100% mean equal ionization efficiencies for sample extracts and solvent-based standards, while normalized ratios below and above 100% correspond to signal suppression and signal enhancement, respectively [24].

Under optimal conditions, 0.5 g of freeze-dried samples were weighed in 50 mL PP tubes followed by addition of 25 mL of ACN:H_2O (1:1), 0.2 M in citric acid (buffered at pH 4.4). Samples were submitted to UAE for 30 min, at room temperature. Thereafter, tubes were centrifuged at 3500 rpm for 10 min. A fraction of the extract (2 mL) was retrieved and vortexed with 40 mg of C_{18}. The supernatant was passed through a 0.22 μm syringe filter before LC-MS/MS analysis.

2.3. Determination conditions

Antibiotics were determined using an Agilent (Wilmington, DE, USA) 1290 model, ultra-high performance liquid chromatography (UHPLC) system coupled to an Agilent 6495D triple quadrupole mass spectrometer (QqQ-MS), equipped with an ion-funnel electrospray (ESI) source. Accurate product ion spectra of target compounds were recorded with a quadrupole time-of-flight (QTOF) MS system, Agilent 6550, also combined with an Agilent 1290 UHPLC system. Chromatographic separations were carried out in a Zorbax Eclipse Plus, C_{18} column (50 mm x 2.1 mm, 1.8 μm), also purchased from Agilent. The column was maintained at 30 °C. The mobile phase consisted of ultrapure water (A) and MeOH (B), both 0.1% in FA, at a flowrate of 0.3 mL min^{-1}, combined as follows: 5% B (0–2 min), 100% B (15–16 min), 5% B (16.1–19 min). The ESI source operated in the positive mode (ESI+) and the injection volume of biosolid and sludge extracts was set at 1 μL. Retention times and transitions corresponding to each compound and the associated surrogate standard (SS) are compiled in Table 1. The link between native compounds and SSs was made attending to structure similarities and vicinity of retention times.

Identification of antibiotics in biosolid extracts and the directly injected treated wastewater samples was based on retention times and ratios between responses for qualification (Q2) and quantification (Q1) ions matching those corresponding calibration standards within limits of ± 0.1 min and ± 30%, respectively. Instrumental quantification limits (iLOQs) were calculated using solvent-based standards, considering a minimum signal to noise ratio of 10 for Q1 and Q2 product ions of each compound. Linearity was assessed correcting responses for the Q1 transition of each compound with that obtained for the assigned SS, Table 1. The concentrations of antibiotics in extracts from biosolids and sludge were calculated using solvent-based standards, containing the same concentration of SSs as those expected in sludge extracts, Table 1. Accuracy of the method was assessed through the recoveries obtained for two different type of biosolids, spiked at two different concentrations. Procedural LOQs (mLOQs) for biosolids were estimated from iLOQs, considering sample intake (0.5 g) and extract volume (25 mL), corrected with the EEs and MEs of the proposed method for biosolids when they were outside the accepted range (from 80% to 100%).

Residues of antibiotics in wastewater were quantified using matrix-matched calibration standards, prepared by addition of increasing concentrations (n = 6) of native antibiotics to each sample of treated wastewater in the range of concentrations from 0.05 ng mL^{-1} to 1.0 ng mL^{-1} Filtered (0.22 μm) aliquots of treated wastewater were analyzed using the same LC-MS/MS conditions as those used for the extracts of biosolids, but increasing the injection volume to 10 μL.

The concentrations of antibiotics measured in biosolids and treated wastewaters from 3 different STPs were employed to assess the relative contribution of each matrix to the emission of antibiotics from municipal STPs.

3. Results and discussion

3.1. LC-MS/MS determination conditions

Among the selection of antibiotics considered in this research, macrolides (azithromycin, clarithromycin, and erythromycin) and fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin and enrofloxacin) exhibited some common characteristics affecting their chromatographic behaviour, stability, ionization, and fragmentation patterns, which impact their detectability by LC-MS/MS. Firstly, the retention of these species in the C_{18} column was affected by the modifier added to the mobile phases. Fluoroquinolones showed tailing peaks when using ammonium acetate (5 mM) in the mobile phase, which increased their LOQs compared to the use of FA (0.1%) as modifier. The chromatographic profiles for the rest of antibiotics were hardly affected by the selected modifier; however, azithromycin and clarithromycin coeluted when using ammonium acetate. As their precursor ions ($[M + H]^+$) differed in 1 Da, and their product ion spectra share several common fragments (ions at nominal m/z values of 83, 116, 158 and 591, see **Fig. S1**), chromatographic co-elution may lead to misidentification problems during the analysis of real samples. The use of FA (0.1%) in the mobile phase shifts the acid-base equilibrium of the macrolides azithromycin and clarithromycin towards their di- and mono-protonated forms, shortening the retention time of the first compound improving their chromatographic separation. At acid pHs, fluoroquinolones stay as positively charged species, showing a lower polarity and higher solubility in the mobile phase than their zwitterionic forms, predominant at neutral pH corresponding to the use of ammonium acetate as modifier, which explain the improvement in the peak shape observed for these compounds.

Stock solutions and mixtures of target compounds in the μg per mL range were prepared in MeOH; however, problems were noticed for standards in the same solvent at the low, and the sub, ng per mL range. **Fig. S2** shows the plots of response versus concentration for calibration standards of ciprofloxacin, prepared in MeOH, stored in glass and PP, 2 mL autosampler vials. In the first case an exponential dependence between response (peak area for the Q1 transition) and concentration (from 0.1 to 100 ng mL^{-1}) was noticed versus the linear trend obtained for standards maintained in PP vials. A similar behaviour was observed for the rest of fluoroquinolones and azithromycin, pointing out to significant sorption of these compounds onto the surface of autosampler glass vials. As glass sorption is particularly relevant at low

Table 1
LC-ESI-MS/MS determination parameters for native compounds and isotopically labelled analogues, linearity, and instrumental limits of quantification (iLOQs).

Compound	Retention time (min)	$[M + H]^+$ion	Q1 (CE, V)	Q2 (CE, V)	Q2/Q1 ratio	R^2 (0.05–100 ng mL^{-1})	iLOQ (ng mL^{-1})
[a]Azithromycin	8.77	749.5	116 (50)	83 (50)	1.1	0.998	0.05
[b]Ciprofloxacin	7.10	332.1	231.0 (42)	314 (21)	1.55	0.999	0.02
[c]Clarithromycin	11.62	748.5	158 (31)	590.5 (15)	0.29	0.999	0.02
[c]Clindamycin	9.38	425.2	126.1 (31)	377.2 (19)	0.08	0.999	0.05
[b]Enrofloxacin	7.28	360.2	316.2 (31)	342.2 (20)	5.07	0.999	0.05
[c]Erythromycin	10.77	734.5	158.1(29)	576.4 (15)	0.46	0.999	0.02
[b]Norfloxacin	6.94	320.2	231.0 (50)	302 (20)	5.5	0.999	0.02
[d]Ofloxacin	6.82	362.0	261.1 (31)	318 (19)	1.4	0.999	0.02
[e]Sulfamethoxazole	6.84	254.1	92 (27)	156 (13)	0.77	0.999	0.1
[f]Trimethoprim	6.16	291.1	230.2 (25)	123 25)	0.6	0.999	0.02
[a]Azithromycin-d_3	8.76	752.5	116 (50)	83 (50)	1.1	-	-
[c]Clarithromycin-d_3	11.6	751.5	161 (31)	593.5 (15)	0.13	-	-
[b]Ciprofloxacin-d_8	7.05	340.2	235.0 (42)	322.2 (21)	1.7	-	-
[d]Levofloxacin-d_3	6.8	365	261.0 (31)	321.0 (19)	0.91	-	-
[e]Sulfamethoxazole-$^{13}C_6$	6.79	260.1	98 (27)	162 (13)	0.78	-	-
[f]Trimethoprim-d_9	6.05	300.2	233.1 (25)	123.1 (25)	1.6	-	-

Codes a to f correspond to the link between target compounds and the deuterated species assigned as surrogate standard (SS).

concentrations, exponential response graphs were obtained. Other compounds (clindamycin, sulfamethoxazole, and trimethoprim) rendered linear responses, whatever the type of autosampler vial, **Fig. S2**. Non-linear responses were detected for calibration standards of fluoroquinolones and azithromycin prepared in MeOH, ACN, or ACN:FA (97.5:2.5), stored in glass vessels, but not in PP ones. This behaviour has been previously reported by Fabregat-Safont et al. [25]. The authors noted that although azithromycin had its own isotopically labelled SS, the relative responses showed poor reproducibility, suggesting that the two compounds did not behave in the same way and therefore the labelled azithromycin could not properly correct the signal decrease along the sequence [25]. Herein, the sorption of these compounds on glass vessels was avoided by diluting the calibration standards in 1:1 mixture of organic solvents (MeOH or ACN) and ultrapure water. Furthermore, only PP material was used during extraction of antibiotics from biosolids, to store concentrated standards prepared in MeOH, and to host calibration standards in the range of concentrations from 0.05 to 100 ng mL^{-1}. Wastewater samples were also stored in high-density polyethylene (HDPE) jars and injection aliquots in PP autosampler vials, respectively.

Another problem reported in some previous studies dealing with LC-ESI(+)-MS/MS analysis of fluoroquinolones (ciprofloxacin and norfloxacin) in wastewater samples was protonation in different functionalities of their structures [25]. The balance between the protomers of the same compound differed depending on the complexity of the considered sample. Furthermore, protomers showed different product ions affecting the reliability of their determination in complex environmental samples [25]. In this research, no differences were observed between the accurate product ion spectra of fluoroquinolones for solvent-based standards and sludge extracts. Fig. 1 shows the accurate masses of product ions of ciprofloxacin (*m/z* of 314.1297, 288.1501, 245.1077 and 231.0560) in QTOF-MS spectra obtained for the extracts from raw sludge (A), final biosolid from a STP equipped with an anaerobic digestion unit (B), and solvent-based standard (C) at two different collision energies (20 and 40 eV).

Under the determination conditions summarized in the *Material and Methods section*, the UHPLC-QqQ-MS system provided linear responses for antibiotics in the range of concentrations from 0.05 to 100 ng mL^{-1}, with determination coefficients (R^2) above 0.998, and iLOQs in the range from 0.02 ng mL^{-1} to 0.1 ng mL^{-1}, considering an injection volume of 1 μL, Table 1.

3.2. Sample preparation conditions

Table 2 summarizes the EEs of nine antibiotics (the standard of enrofloxacin was not available during the preliminary experiments of this research) from a spiked pool of biosolids (spiked concentration 2000 ng g^{-1}), under the different conditions (extraction techniques and solvents) described in Section 2.2. Extraction parameters were selected based on literature data, considering the polar nature of compounds, and avoiding the use of additives with a low compatibility with LC-MS analysis (e.g. phosphate derived buffers) reported in pioneer research using fluorescence detection following their LC separation [4]. Five compounds showed EEs above 70% under all considered conditions. Erythromycin presented a different behaviour than the other two macrolides, with lower EEs during sonication with MeOH:H_2O and QuEChERS extraction in presence of citrate salts, Table 2. The lability of erythromycin may explain the differences in their apparent extraction efficiencies [26]. The EEs of fluoroquinolones were dramatically affected by the selected technique and solvent. QuEChERS displayed very low EEs as well as UAE with MeOH:H_2O (EEs < 35%, Table 2), which agrees with previous data published by some authors [19,27]. EEs above 60% were obtained for the three fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) using ACN:H_2O, buffered at pH 4.4, either under PLE, or using UAE. The buffered solution reduces the polarity of fluoroquinolones by promoting protonation of their carboxylic group

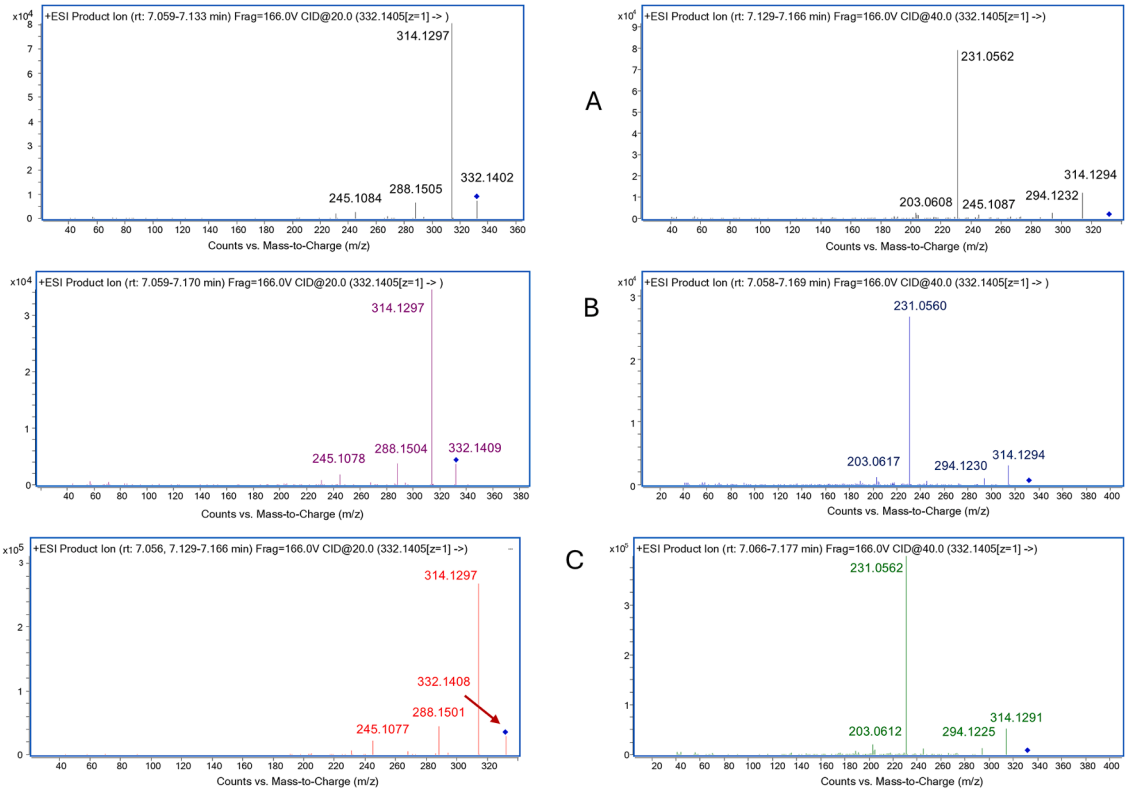

Fig. 1. Product ion spectra of ciprofloxacin in the extracts from raw (non-digested) sludge (A), biosolids after anaerobic digestion and dewatering (B) and a solvent-based standard (C), using collision energies of 20 eV (left) and 40 eV (right).

Table 2
Extraction efficiencies (EEs, %), with standard deviations within parenthesis, as function of technique and solvent, n = 3 replicates. Spiked concentration 2000 ng g^{-1}.

Compound	QuEChERS		UAE		PLE	
	acetate buffer	citrate buffer	MeOH: H_2O	ACN: H_2O (pH 4.4)	ACN: H_2O	ACN: H_2O (pH 4.4)
Azithromycin	77 (1)	70 (6)	80 (11)	126 (4)	73 (8)	95 (12)
Ciprofloxacin	<1	22 (3)	14 (5)	60 (9)	45 (9)	60 (13)
Clarithromycin	79 (3)	74 (7)	88 (11)	82 (2)	71 (10)	94 (15)
Clindamycin	75 (6)	69 (8)	77 (13)	79 (9)	74 (10)	99 (14)
Erythromycin	71 (3)	28 (3)	13 (11)	77 (6)	97 (12)	93 (16)
Norfloxacin	<2	18 (3)	14 (5)	62 (13)	41 (7)	65 (16)
Ofloxacin	<1	35 (4)	20 (4)	90 (3)	64 (4)	78 (12)
Sulfamethoxazole	82 (1)	83 (9)	96 (12)	101 (2)	87 (6)	96 (18)
Trimethoprim	80 (5)	74 (7)	71 (9)	100 (4)	84 (7)	95 (13)

(pKa values between 5.1 and 6.1, see **Table S1**). At pH 4.4, these antibiotics remain in their positively charged form instead of acting as zwitterions, as they would act at neutral pH. In addition to pH adjustment, citrate works as a chelating agent, preventing the formation of complexes between fluoroquinolones and metal cations that could hinder their efficient extraction from biosolids. Based on data compiled in Table 2, UAE using 25 mL of a mixture of ACN:H_2O (1:1), 0.2 M in citric acid (pH 4.4) were selected as extraction technique and solution, respectively. Extractions were carried out in 50 mL disposable PP tubes immersed in an ultrasonic bath during 30 min, at room temperature.

The possibility to purify and concentrate the extracts from sludge samples was firstly investigated after dilution to 100 mL with ultrapure water (4-fold dilution factor), followed by filtration and SPE concentration of a 25 mL aliquot using HLB cartridges [16]. Under these conditions, fluoroquinolones were not quantitatively retained by the SPE sorbent, with percentages of breakthrough in the range from 20% to 25%. This behaviour agrees with the higher polarity of these compounds compared to the rest of antibiotics involved in this research, **Table S1**. Considering that most of compounds contain basic groups in their structure, the use of mixed-mode sorbents (reversed-phase and cation exchange properties) was tested as an alternative clean-up approach. Fractions (2 mL) of UAE extracts from spiked biosolids were passed through a MCX 150 mg SPE cartridge. This sorbent provides similar interactions to those reported for the combination of reversed-phase and ion exchange polymers commonly recommended for concentration of fluoroquinolones from water samples [4]. Thereafter, the cartridge was rinsed with 2 mL of MeOH, followed by 2 mL fractions of MeOH:H_2O:NH_3 (49:49:2) and MeOH:NH_3 (98:2). Normalized responses of each compound in the above fractions are shown in **Fig. S3**. Except for sulfamethoxazole, the rest of compounds were retained in the sorbent by electrostatic interactions, showing negligible, or low (clarithromycin and erythromycin), normalized responses in the buffered extract flowing through the SPE sorbent and the MeOH rinsing fraction (2 mL each). Elution of antibiotics retained in the MCX sorbent required different solvents, in line with their range of polarities. Thus, fluoroquinolones were recovered using 2 mL of a MeOH:H_2O (1:1) solution containing 2% of NH_3, whilst the less polar macrolides, clindamycin and trimethoprim required the use of 2 mL of MeOH:NH_3 (98:2) to effectively break their electrostatic interactions with the positively charged groups in the sorbent and being released from the SPE cartridge. To prevent dilution of the extract during the clean-up step, in a further series of assays, the volume of extract loaded in the MCX cartridge was increased to 4 mL followed by sorbent rinsing with MeOH (4 mL) and the elution of the retained compounds was achieved in a single fraction of 2 mL of MeOH:H_2O:NH_3 (49.5:49.5:1) followed by 2 mL of MeOH:NH_3 (98:2). Under these conditions, the four fluoroquinolones, azithromycin, clindamycin and trimethoprim could be isolated in the 4 mL basic fraction, with similar, or even higher, responses than those obtained for the non-cleaned complex extract, **Fig. S4A**. Pictures of the extract of biosolids flowing through the MCX sorbent, the rinsing and the basic fractions collected from the MCX cartridge support the lower complexity of the latter fraction, **Fig. S4B**. In summary, the MCX fractionation strategy might be a suitable clean-up approach for seven compounds, which could be fractionated from neutral interferences removed in the methanolic fraction. However, it failed for clarithromycin, erythromycin, and sulfamethoxazole. The first two compounds were distributed between several fractions, whilst sulfamethoxazole was not retained in the MCX sorbent, **Fig. S4**.

The final evaluated clean-up strategy involved the use of lipophilic sorbents in the d-SPE mode [19]. The addition of small amounts of C_{18} to UAE sample extracts is expected to remove some strongly lipophilic compounds, without retaining the more polar antibiotics. Data shown in **Fig. S5** confirm that responses of antibiotics did not change between raw extracts and those vortexed with C_{18} (40 mg of sorbent were added to 2 mL of extract). Unfortunately, C_{18} alone did not achieve to reduce the visual complexity of the extracts, **Fig. S5**. Graphitized carbon solved this problem; nevertheless, even small amounts of this sorbent retained most antibiotics from ACN:H_2O extracts, with losses up to 90% for ciprofloxacin and ofloxacin (figure not shown). As the primary aim of this study was the simultaneous determination of the ten selected compounds in biosolids and sludge, unless otherwise stated, dispersive clean-up using 20 mg of C_{18} per mL of extract was employed in further experiments reported in this study.

The composition and properties of solid wastes produced in STPs is affected by the specific treatments applied in the sludge treatment (e.g. thermal hydrolysis and anaerobic digestion) before the final dewatering step [28]. These modifications may modulate the EEs and MEs during sample preparation and determination steps, respectively. Table 3 summarizes the EEs for sludge obtained at the different stages of the same STP: raw sludge from primary and biological water treatment units, sludge after thermal hydrolysis, sludge after anaerobic digestion, and the obtained biosolids after the dewatering step using UAE. Global average EEs are shown in **Fig. S6**. The EEs of azithromycin, clarithromycin, clindamycin, and trimethoprim were hardly affected by the type of sludge, with global average values in the range from 95% to 109% and standard deviations below 10%. Average EEs for the rest of antibiotics varied from 74% for norfloxacin to 91% for sulfamethoxazole, **Fig. S6,** with some differences depending on the type of sludge, particularly in the case of ciprofloxacin, Table 3. EEs compiled in Table 3 for the different types of sludge and biosolids improve those reported for UAE using mixtures of organic solvents with FA, with values below 30% for fluoroquinolones[19].

MEs for the abovementioned sludge types, following clean-up with d-SPE using C_{18} sorbent, are also summarized in Table 3. Variations in ionization efficiency compared to solvent-based standards were compound, and sample dependent. The ionization efficiency of azithromycin and clindamycin did not vary significantly among samples, with normalized responses in the range from 81% to 127% versus a solvent-based standard of same concentration. For the rest of compounds, the extent of signal suppression varied from 20% up to 80%. Enrofloxacin, ofloxacin and sulfamethoxazole were the compounds presenting the highest signal suppression, and sludge obtained after thermal hydrolysis the most complex matrix, with significant variations in the ionization efficiency for most compounds (except azithromycin and clindamycin) versus solvent based standards. MEs compiled in Table 3 are in line with those reported in previous studies, with signal suppression effects in the

Table 3
Summary of extraction efficiencies (EEs, %) and matrix effects (MEs, %) for ultrasound assisted extraction followed by C_{18} clean-up for different kinds of sludge obtained from the same STP equipped with thermal hydrolysis and anaerobic digestion units before the dewatering step. Average values for triplicate extractions are summarized with standard deviations (SD) within parenthesis.

Compound	EEs(%) with SD				MEs(%) with SD			
	Raw sludge	Thermal hydrolysis	Anaerobic digestion	Biosolids	Raw sludge	Thermal hydrolysis	Anaerobic digestion	Biosolids
Azithromycin	112 (4)	114 (1)	112 (2)	93 (6)	98 (5)	85 (1)	104 (6)	127 (2)
Ciprofloxacin	100 (6)	94 (1)	51 (10)	66 (3)	67 (1)	51 (2)	63 (4)	73 (3)
Clarithromycin	93 (3)	90 (13)	98 (1)	97 (5)	75 (10)	57 (3)	66 (17)	71 (3)
Clindamycin	106 (1)	115 (1)	107 (2)	108 (9)	83 (5)	91 (4)	83 (6)	81 (3)
Enrofloxacin	93 (3)	85 (3)	64 (11)	64 (4)	44 (7)	17 (3)	33 (12)	53 (11)
Erythromycin	78 (3)	80 (9)	105 (1)	79 (4)	68 (8)	54 (3)	64 (21)	86 (2)
Norfloxacin	89 (6)	94 (1)	51 (10)	60 (1)	57 (4)	49 (4)	75 (3)	79 (3)
Ofloxacin	92 (3)	91 (3)	61 (8)	72 (2)	44 (7)	28 (3)	37 (12)	72 (7)
Sulfamethoxazole	79 (7)	73 (18)	95 (2)	117 (10)	87 (24)	20 (11)	37 (12)	50 (10)
Trimethoprim	98 (1)	99 (3)	94 (1)	96 (4)	90 (5)	51 (2)	68 (8)	79 (3)

range from 60% to 90% during determination of fluoroquinolone antibiotics in sludge [19,20] and poultry manure [29] by LC-MS/MS. In view of these sample dependent MEs, the use of isotopically labelled analogues becomes mandatory to increase the reliability of the determination of antibiotic residues in different types of sludge, without using the time-consuming matrix-matched calibration for each sample.

The potential for fractionating basic compounds (azithromycin, clindamycin, trimethoprim) and the amphoteric antibiotics (fluoroquinolones) from neutral compounds by passing sludge extracts through an MCX sorbent (**Fig. S4**) was further investigated to determine whether this approach reduces the MEs reported in Table 3. The macrolides clarithromycin and erythromycin, together with sulfamethoxazole, were not retained by MCX sorbent; therefore, this clean-up strategy is not suitable for these compounds. Fig. 2 compares the normalized responses observed in the spiked extract (after correction with a non-spiked aliquot of same sample), versus a reference standard, for the rest of antibiotics involved in the study. Depicted data correspond to the most complex of the investigated matrices: sludge after thermal hydrolysis. The extent of signal suppression of trimethoprim and the four fluoroquinolones was reduced when applying the MCX fractionation strategy compared to d-SPE with C_{18}. The normalized responses of azithromycin and clindamycin remained in the range from 80% to 120% for both approaches.

To summarize, MCX fractionation appears to be a suitable strategy for analytical methods focused exclusively on basic and amphoteric antibiotics; however, it is not appropriate for multi-class methods that also include neutral compounds not retained by this sorbent.

3.3. Method performance

The accuracy of the procedure was investigated in final biosolids obtained from two different STPs, with and without anaerobic digestion treatments applied in the line of sludge, spiked at 50 ng g^{-1} and 200 ng g^{-1}. At each experiment, the added concentrations of azithromycin, ciprofloxacin and ofloxacin were 10 times higher (500 ng g^{-1} and 2000 ng g^{-1}) than those corresponding to the rest of antibiotics, to compensate for differences among their residues in the matrix of biosolids. Accuracy was determined using solvent-based standards. In general, the corrected recoveries were comprised between 73% and 111%, with relative standard deviations (RSD) below 10%. For clindamycin, values between

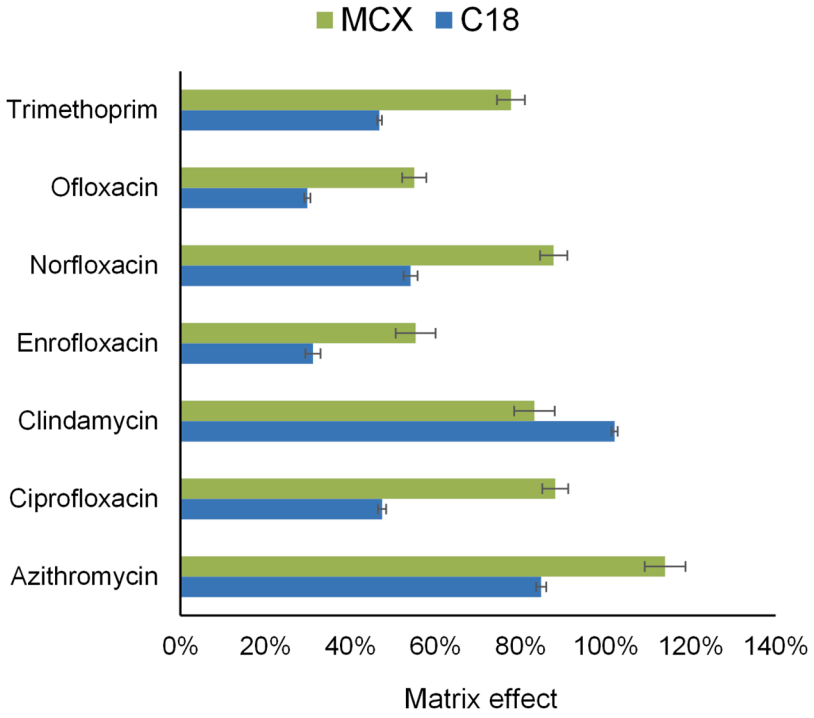

Fig. 2. Matrix effects (MEs), as normalized responses versus a reference standard, for selected antibiotics as function of the clean-up technique. Data corresponding to spiked fractions from the same type of sludge, n = 3 replicates.

65% and 94% were obtained, Table 4. Likely, this higher variability is due to the lack of a deuterated analogue for this compound. Thus, the assigned SS (azithromycin-d_3) showed a limited capability to compensate for changes in the ionization efficiency of clindamycin. Finally, the recovery of norfloxacin could not be calculated for biosolids from the anaerobic STP, at the lowest added concentration (50 ng g^{-1}), due to the high residue existing in this matrix. The mLOQs varied between 1 ng g^{-1} to 5 ng g^{-1}, Table 4, in line with those reported in previous research [5, 17,30], with the advantage of permitting the simultaneous determination of antibiotics from different chemical classes. When compared with established multiclass antibiotic determination methods applied to complex solid matrices (i.e. manure), the proposed approach yielded LOQs one order of magnitude lower [29]. Alternatively, it enabled the implementation of markedly simplified workflows that eliminate the need for concentrating the primary extract [6].

3.4. Antibiotic residues in biosolids

The concentrations of target antibiotics were assessed in the biosolids collected from 23 different STPs. STPs 1 to 4 employ anaerobic digestion of sludge, obtained from primary and biological treatment units, prior to dewatering. The rest of facilities do not apply any treatment to the sludge apart from dewatering. Clindamycin and sulfamethoxazole remained below their mLOQs (2.5 and 5 ng g^{-1}, respectively) in all the studied biosolids. The latter compound was hardly detected in sludge from other STPs in Europe, with a maximum residue of 9 ng g^{-1} and a detection frequency (DF) lower than 10% [17, 22]. Literature values for clindamycin residues ranged from non-detected up to 5000 ng g^{-1}, depending on the STP [22]. Concentrations measured for the rest of antibiotics are provided as supplementary information, **Table S2**. The sum of their concentrations ranged from 1837 ng g^{-1} to 12,513 ng g^{-1}, being the fluoroquinolones ofloxacin and ciprofloxacin, and the macrolide azithromycin the compounds presenting the highest median concentrations, 1761, 1610 and 441 ng g^{-1}, respectively (**Table S2**).

Fig. 3**A** summarizes median and maximum concentrations of each compound above mLOQs and their DFs. Enrofloxacin and erythromycin presented DF below 30% with maximum residues below 100 ng g^{-1}. Enrofloxacin is commonly used as veterinary drug, and it is de-ethylated to ciprofloxacin during wastewater treatments [31], which explains the very low residues of enrofloxacin and the comparatively high concentrations of ciprofloxacin detected in samples from municipal STPs. The DF of trimethoprim and clarithromycin were 87% and 100%, respectively; with median and maximum concentrations in the same range of values as those observed for enrofloxacin and erythromycin. Trimethoprim was not detected in biosolids from the four STPs applying anaerobic digestion; however, it could be quantified in the rest of biosolids (STPs codes 5 to 23) with median concentrations of 18.3 ng g^{-1}, Fig. 3**A**. In a recent survey conducted in the South of Spain, trimethoprim was quantified in dewatered sludge at an average concentration of 4.9 ng g^{-1} [31]. As for the fluoroquinolones norfloxacin, ciprofloxacin and ofloxacin, and the macrolide azithromycin were ubiquitous in biosolids. Together, they represent >95% of total antibiotic residues measured in the set of analyzed samples, Fig. 3**B** Despite biosolids analyzed in the current study were generated in STPs located in the same geographic area (North Spain), the prevalence of fluoroquinolones in the processed samples matches trends reported for STPs in Nordic countries [32], Switzerland [14] and China [6].

3.5. Contribution of biosolids and treated wastewater to antibiotic emissions at STPs

Understanding the relevance of biosolids as a source of antibiotics in municipal STPs and determining the need to control their residues in this matrix, depends largely on their relative emissions via final biosolids compared with the dissolved fraction remaining in treated wastewater. For this comparison, an estimated production of 300 kg of dewatered biosolids per 1000 m^3 of treated wastewater was employed [33]. Antibiotic concentrations measured in final biosolids from the three evaluated STPs, along with those quantified in 24- h composite samples of treated wastewater, are summarized in **Table S3**. The STPs selected for the comparison employ anaerobic digestion of sludge and UV disinfection of treated wastewater.

Erythromycin and enrofloxacin were detected in both matrices at concentrations below their mLOQs (1 to 5 ng g^{-1} in biosolids and 0.01 ng mL^{-1} in treated wastewater). Clindamycin and sulfamethoxazole could be quantified in treated wastewater but remained below detection limits in biosolids, as previously noted in Section 3.4. For mass balance estimations, half of the mLOQ values for biosolids (2.5 and 5 ng g^{-1}, respectively) were used in the calculations. Norfloxacin was quantified in biosolids from all three facilities but not detected in the aqueous phase. Therefore, to facilitate mass balance calculations, a concentration of 0.01 ng mL^{-1} was assigned to the treated wastewater (**Table S3**).

Fig. 4 illustrates the percentage of each compound emitted via biosolids relative to the total mass discharged per 1000 m^3 of treated wastewater. Among the investigated antibiotics, azithromycin, ciprofloxacin, norfloxacin, and ofloxacin were the only compounds for which residues measured in biosolids were significant to assessing their total emissions from urban STPs (mass fraction >20%), Fig. 4**A**. For clarithromycin, the biosolid-associated fraction was approximately 5% of the total emitted amount, while for clindamycin, sulfamethoxazole, and trimethoprim remained below 1%, Fig. 4**B.** The total mass of the investigated antibiotics emitted per 1000m^3 of treated wastewater ranged from 1100 mg for ofloxacin to 46 mg for clindamycin, Fig. 4A and 4B. Data compiled in Fig. 4 indicate that biosolids represent a relevant emission pathway only for four selected antibiotics, whereas for

Table 4
Accuracy of the analytical procedure for spiked samples of biosolids from two STPs without sludge treatment facilities and anaerobic digestion, n = 3 replicates, and procedural LOQs (mLOQs).

Compound	None		Anaerobic digestion		mLOQs
	200 ng g^{-1} Recovery % (RSD)	50 ng g^{-1} Recovery % (RSD)	200 ng g^{-1} Recovery % (RSD)	50 ng g^{-1} Recovery % (RSD)	(ng g^{-1})
Azithromycin	104 (7)	98 (2)	96 (1)	100 (1)	2.5
Ciprofloxacin	102 (6)	73 (3)	99 (2)	92 (6)	2
Clarithromycin	91 (3)	87 (6)	102 (3)	80 (2)	1
Clindamycin	94 (3)	65 (4)	72 (9)	66 (24)	2.5
Enrofloxacin	97 (4)	92 (2)	95 (2)	83 (2)	5
Erythromycin	97 (3)	83 (2)	92 (3)	74 (9)	1
Norfloxacin	131 (6)	91 (7)	111 (6)	n.e.	2
Ofloxacin	103 (3)	85 (2)	101 (2)	90 (4)	2
Sulfamethoxazole	106 (2)	96 (2)	100 (1)	84 (10)	5
Trimethoprim	97 (3)	85 (2)	95 (2)	89 (3)	1

n.e., not evaluated.

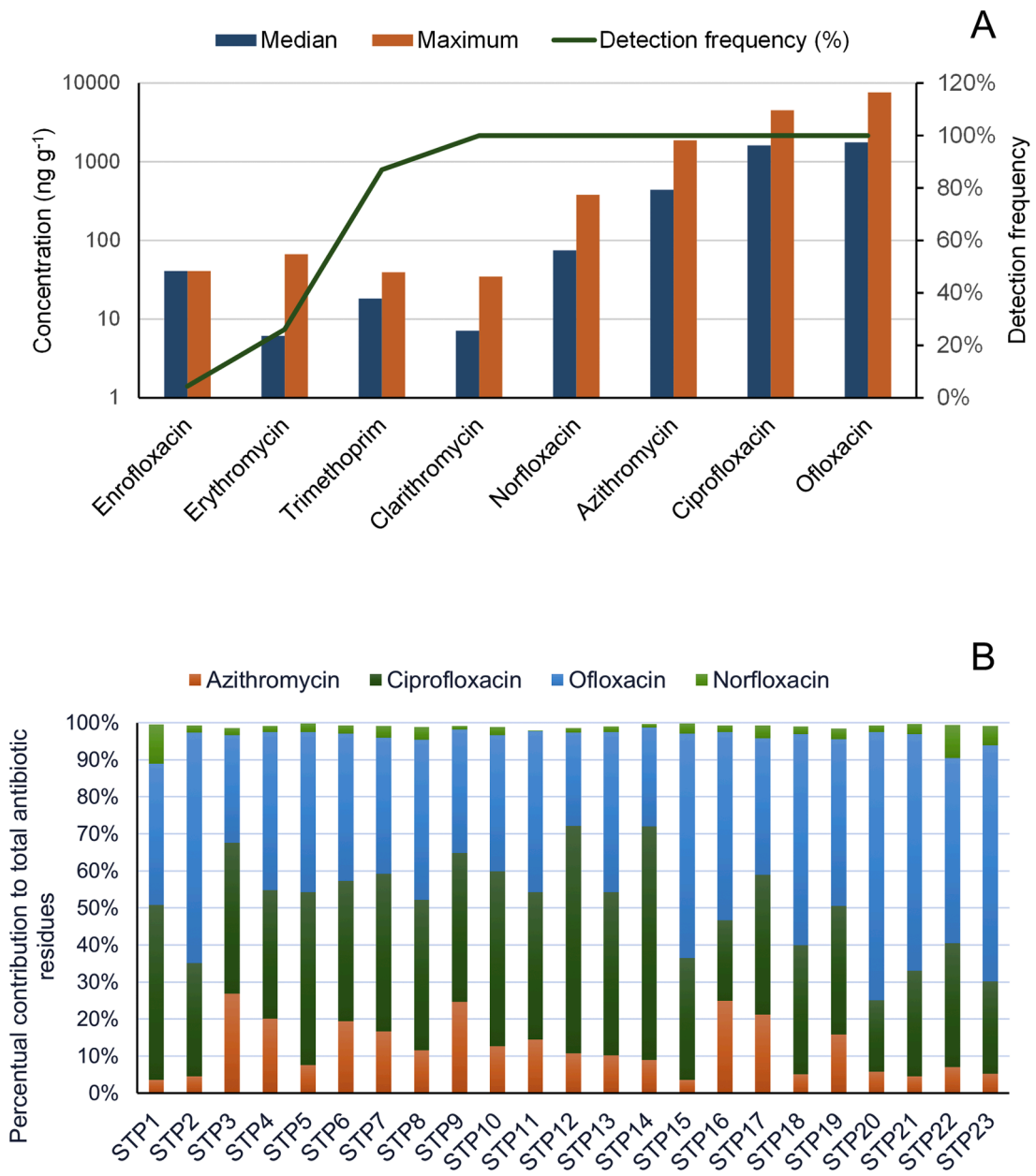

Fig. 3. A, Median, maximum and detection frequencies of antibiotics in dewatered biosolids from 23 STPs. B, relative contributions of azithromycin and three fluoroquinolones to residues of 10 antibiotics in sludge.

most compounds their contribution to total emissions is negligible. Given that these balances are based on concentrations measured in three STPs applying anaerobic digestion and UV irradiation for sludge and wastewater treatment respectively, the findings summarized in Fig. 4 require further confirmation, considering a higher number of STPs with diverse treatment technologies applied to wastewater and sludge before dewatering, in order to compare differences in antibiotic biosolid discharges across treatment types.

4. Conclusions

Residues of antibiotics in biosolids from municipal STPs are dominated by the fluoroquinolones, ciprofloxacin and ofloxacin, and the macrolide azithromycin. While azithromycin was readily extracted with organic solvents based strategies, the efficient recovery of fluoroquinolones required buffered mixtures of ACN and ultrapure water. Under the employed UAE conditions, EEs of antibiotics were moderately affected by the type of sludge; however, matrix effects observed after d-SPE clean-up with C_{18}, were found to be sample-dependent, with pronounced signal suppression for fluoroquinolones. Further extract clean-up optimization is therefore required. In particular, fractionation of UAE extracts using reversed-phase and strong cationic exchange (mixed-mode) sorbents should be explored to mitigate signal suppression problems identified during the determination of fluoroquinolones.

Among the antibiotics considered in this research, erythromycin and enrofloxacin showed low detection frequencies in both biosolids and treated wastewater. Clarithromycin, clindamycin, sulfamethoxazole, and trimethoprim were mainly detected in treated wastewater with biosolids accounting for <5% of their total mass emitted by STPs. In contrast, ciprofloxacin, norfloxacin and ofloxacin were emitted mostly associated to biosolids. Both matrices contributed substantially to the environmental emission of azithromycin. These findings highlight the need for further research on sludge treatment technologies aimed at reducing fluoroquinolones concentrations in biosolids from municipal

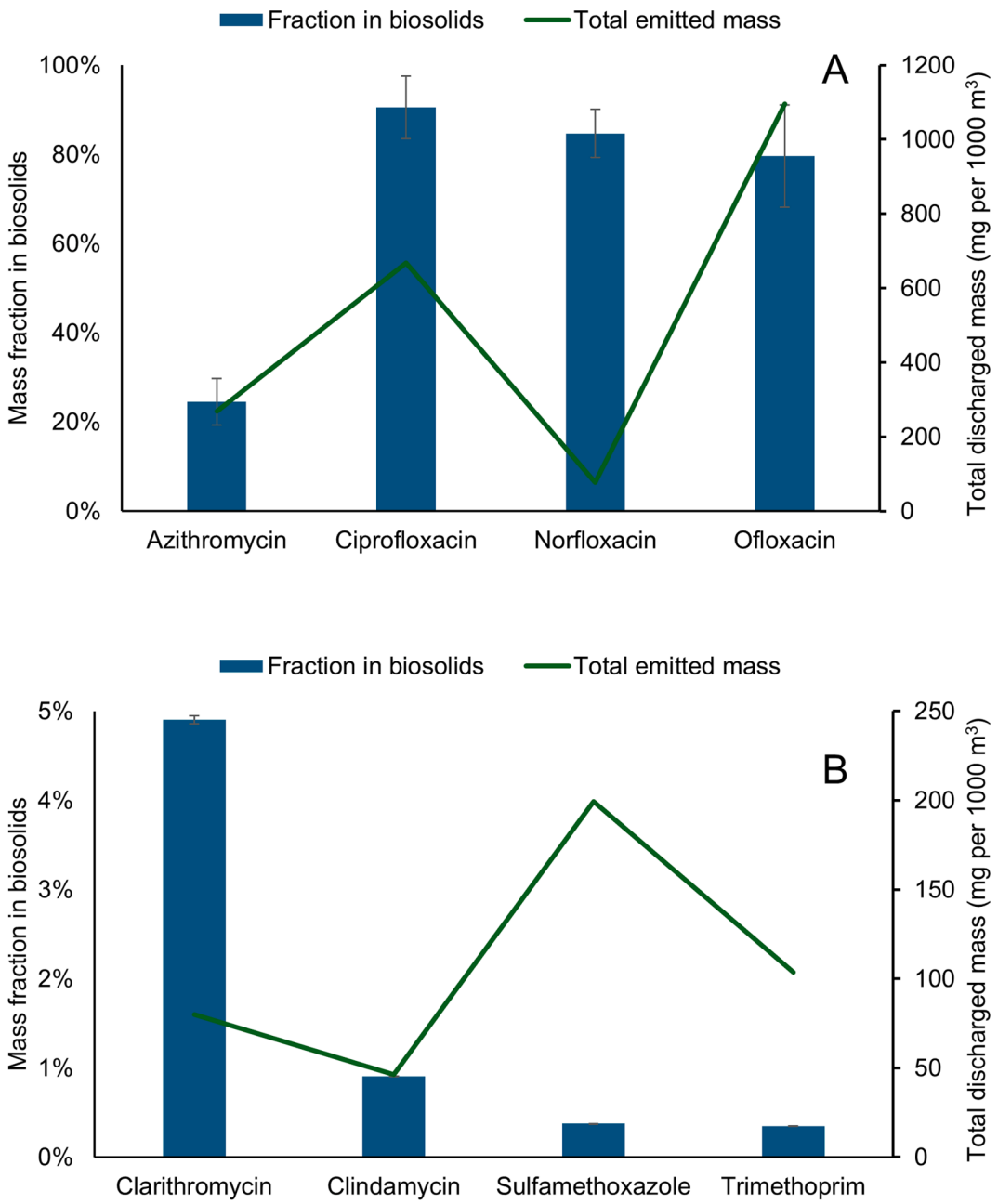

Fig. 4. Average percentages of antibiotics quantified in biosolids versus total emissions through biosolids and treated wastewater. A, data for azithromycin and fluoroquinolones. B, data for minor residue compounds.

STPs, before considering their application as soil fertilizers.

CRediT authorship contribution statement

A. Barros: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **G. Castro:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Ramil:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **I. Rodríguez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2026.467015.

Data availability

Data will be made available on request.

References

[1] R. Rosato, F. Castellani, M. Ricci, A. Manucci, V. Di Simone, G. Scortichini, G. Saluti, Antibiotics in the aquatic environment. A deep voyage across chemical occurrence data, socio-economic relationships and international policies on antimicrobial consumption and resistance within 2014–2024, Sci. Total Environ. 1002 (2025) 180543, https://doi.org/10.1016/j.scitotenv.2025.180543.

[2] X. Ji, Q. Shen, F. Liu, J. Ma, G. Xu, Y. Wang, M. Wu, Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China, J. Hazard. Mater. 235–236 (2012) 178–185, https://doi.org/10.1016/j.jhazmat.2012.07.040.

[3] S.A. Kraemer, A. Ramachandran, G.G. Perron, Antibiotic pollution in the environment: from microbial ecology to public policy, Microorganisms 7 (2019) 180, https://doi.org/10.3390/microorganisms7060180.

[4] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Temes, W. Giger, Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection, Anal. Chem. 73 (2001) 3632–3638, https://doi.org/10.1021/ac0015265.

[5] A. Göbel, A. Thomsen, C.S. McArdell, A. Joss, W. Giger, Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment, Environ. Sci. Technol. 39 (2005) 3981–3989, https://doi.org/10.1021/es048550a.

[6] L. Gao, Y. Shi, W. Li, H. Niu, J. Liu, Y. Cai, Occurrence of antibiotics in eight sewage treatment plants in Beijing, Chemosphere 86 (2012) 665–671, https://doi.org/10.1016/j.chemosphere.2011.11.019.

[7] D.N.D. Samaraweera, X. Liu, G. Zhong, T. Priyadarshana, R. Naseem Malik, G. Zhang, M.S. Khorram, Z. Zhu, X. Peng, Antibiotics in two municipal sewage treatment plants in Sri Lanka: occurrence, consumption and removal efficiency, Emerg. Contam. 5 (2019) 272–278, https://doi.org/10.1016/j.emcon.2019.08.001.

[8] Y. Wu, C. Wu, X. Liu, H. Chen, Z. Deng, D. Wang, Z. Wu, S. Tang, X. Huang, The resistance of hydrogenotrophic methanogenic microorganisms to ofloxacin in sludge anaerobic digestion process, J. Environ. Manage. 365 (2024) 121522, https://doi.org/10.1016/j.jenvman.2024.121522.

[9] N. Wang, L. Peng, Y. Gu, C. Liang, R.W.M. Pott, Y. Xu, Insights into biodegradation of antibiotics during the biofilm-based wastewater treatment processes, J. Clean. Prod. 393 (2023) 136321, https://doi.org/10.1016/j.jclepro.2023.136321.

[10] R.S. Mendonça, A.J. de Souza, R.M.P. Leal, J.F. Osti, R.L. Oliveira, J.B. Regitano, Industrial composting of sewage sludge mitigates antimicrobial resistance risks and preserves bacterial dynamics in tropical soils, J. Environ. Manage. 391 (2025) 126656, https://doi.org/10.1016/j.jenvman.2025.126656.

[11] B. Ravindran, P.N.S. Mnkeni, Identification and fate of antibiotic residue degradation during composting and vermicomposting of chicken manure, Int. J. Environ. Sci. Technol. 14 (2017) 263–270, https://doi.org/10.1007/s13762-016-1131-z.

[12] L. Lehmann, E. Bloem, Antibiotic residues in substrates and output materials from biogas plants – Implications for agriculture, Chemosphere 278 (2021) 130425, https://doi.org/10.1016/j.chemosphere.2021.130425.

[13] E.M. Golet, A. Strehler, A.C. Alder, W. Giger, Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction, Anal. Chem. 74 (2002) 5455–5462, https://doi.org/10.1021/ac025762m.

[14] L. Schinkel, Y. Eberhard, A. Maccagnan, M. Berg, C.S. McArdell, Antibiotics and other micropollutants in Swiss sewage sludge and fecal compost, Chemosphere 375 (2025) 144216, https://doi.org/10.1016/j.chemosphere.2025.144216.

[15] A. Ezzariai, D. Riboul, M.Z. Lacroix, M. Barret, L. El Fels, G. Merlina, A. Bousquet-Melou, D. Patureau, E. Pinelli, M. Hafidi, A pressurized liquid extraction approach followed by standard addition method and UPLC-MS/MS for a fast multiclass determination of antibiotics in a complex matrix, Chemosphere 211 (2018) 893–902, https://doi.org/10.1016/j.chemosphere.2018.08.021.

[16] A. Göbel, A. Thomsen, C.S. McArdell, A.C. Alder, W. Giger, N. Theiß, D. Löffler, T. A. Ternes, Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge, J. Chromatogr. A 1085 (2005) 179–189, https://doi.org/10.1016/j.chroma.2005.05.051.

[17] P. Landová, L. Mravcová, Š. Poláková, P. Kosubová, Application of QuEChERS extraction and LC–MS/MS for determination of pharmaceuticals in sewage sludges sampled across the Czech Republic, Environ. Sci. Pollut. Res. 31 (2024) 63946–63958, https://doi.org/10.1007/s11356-024-35508-8.

[18] A. Bergé, E. Vulliet, Development of a method for the analysis of hormones and pharmaceuticals in earthworms by quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), Anal. Bioanal. Chem. 407 (2015) 7995–8008, https://doi.org/10.1007/s00216-015-8972-z.

[19] C. Mejías, J.L. Santos, J. Martín, I. Aparicio, E. Alonso, Multiresidue method for the determination of critically and highly important classes of antibiotics and their metabolites in agricultural soils and sewage sludge, Anal. Bioanal. Chem. 415 (2023) 7161–7173, https://doi.org/10.1007/s00216-023-04982-3.

[20] A.S. Ajibola, S. Tisler, C. Zwiener, Simultaneous determination of multiclass antibiotics in sewage sludge based on QuEChERS extraction and liquid chromatography-tandem mass spectrometry, Anal. Methods 12 (2020) 576–586, https://doi.org/10.1039/c9ay02188d.

[21] K. Miserli, C. Kosma, I. Konstantinou, Determination of pharmaceuticals and metabolites in sludge and hydrochar after hydrothermal carbonization using sonication—QuEChERS extraction method and UHPLC LTQ/Orbitrap MS, Environ. Sci. Pollut. Res. 30 (2023) 1686–1703, https://doi.org/10.1007/s11356-022-22215-5.

[22] K. Styszko, W. Bolesta, A.P. Daso, B. Kasprzyk-Hordern, Antimicrobial agents in agricultural fertilizers produced from sewage sludge – A cause for concern? Sci. Total Environ. 962 (2025) 178433 https://doi.org/10.1016/j.scitotenv.2025.178433.

[23] K. Miserli, V. Boti, I. Konstantinou, Analysis of perfluorinated compounds in sewage sludge and hydrochar by UHPLC LTQ/Orbitrap MS and removal assessment during hydrothermal carbonization treatment, Sci. Total Environ. 929 (2024) 173650, https://doi.org/10.1016/j.scitotenv.2024.172650.

[24] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in Human plasma at picogram per milliliter concentrations, Anal. Chem. 70 (1998) 882–889, https://doi.org/10.1021/ac971078+.

[25] D. Fabregat-Safont, E. Gracia-Marín, M. Ibáñez, E. Pitarch, F. Hernández, Analytical key issues and challenges in the LC-MS/MS determination of antibiotics in wastewater, Anal. Chim. Acta 1239 (2023) 340739, https://doi.org/10.1016/j.aca.2022.340739.

[26] S.C. Chuo, N. Abd-Talib, S.H. Mohd-Setapar, H. Hassan, H.M. Nasir, A. Ahmad, D. Lokhat, G.M. Ashraf, Reverse micelle extraction of antibiotics using an eco-friendly, Sophorolipids Biosurfactant, Sci. Rep. 8 (2018) 477, https://doi.org/10.1038/s41598-017-18279-w.

[27] W. Peysson, E. Vulliet, Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry, J. Chromatogr. A 1290 (2013) 46–61, https://doi.org/10.1016/j.chroma.2013.03.057.

[28] H. Wen, X. Wang, X. Zhang, Y. He, L. Gu, H. Zhang, P. Wu, Thermal hydrolysis-induced molecular transformations in sludge: implications for photochemical reactivity and dissolved antibiotics photodissipation, J. Hazard. Mater. 491 (2025) 137985, https://doi.org/10.1016/j.jhazmat.2025.137985.

[29] S.N. Weber, N. Ulrich, J. Mühlenberg, N. Engler, M. Nelles, Determination of veterinary antibiotics in German poultry manure by ultrasonic assisted extraction and QuEChERS coupled with LC-MS/MS, Talanta Open 12 (2025) 100592, https://doi.org/10.1016/j.talo.2025.100592.

[30] J. Radjenović, A. Jelić, M. Petrović, D. Barceló, Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS), Anal. Bioanal. Chem. 393 (2009) 1685–1695, https://doi.org/10.1007/s00216-009-2604-4.

[31] C. Mejías, J.L. Santos, J. Martín, I. Aparicio, E. Alonso, Distribution and fate of antibiotics and their main metabolites in sludge stabilization technologies, Chemosphere 385 (2025) 144573, https://doi.org/10.1016/j.chemosphere.2025.144573.

[32] O. Leino, L. Äystö, P. Fjäder, N. Perkola, S. Lehtoranta, Contaminants of environmental concern in sewage sludge in the Nordic countries, Environ. Pollut. 381 (2025) 126604, https://doi.org/10.1016/j.envpol.2025.126604.

[33] T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, A rapid method to measure the solid-water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge, Water Res 38 (2004) 4075–4084, https://doi.org/10.1016/j.watres.2004.07.015.