Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for:

Simultaneous determination of multiclass antibiotics in sewage sludge based on QuEChERS extraction and liquid chromatography-tandem mass spectrometry

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CONTENTS

Table S1: Name, CAS number and some relevant physicochemical properties of the target antibiotics

	Compound	CAS No	Molecular Formular	Molecular Weight	Log K_{ow} [a]	Log K_d [b]	pK_a [c]
Fluoroquinolones	**Ciprofloxacin**	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.34	-0.28	4.3	5.8, 8.6
	Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.37	-0.39	4.2	5.5, 6.2
	Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.33	-1.03	4.2	5.8, 8.7
Sulfonamides	**Sulfamethoxazole**	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	0.89	2.1 - 2.6	2.0, 7.6
	Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.33	1.63	-	2.0, 6.9
	Sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278.33	0.19	-	2.0, 7.0
	Sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310.33	0.70	-	2.3, 6.1
Tetracyclines	**Oxytetracycline**	79-57-2	$C_{22}H_{24}N_2O_9$	460.43	-0.90	-	3.27, 4.6
	Tetracycline	60-54-8	$C_{22}H_{24}N_2O_8$	444.43	-1.30	3.9	3.3, 4.6
Macrolides	**Azithromycin**	83905-01-5	$C_{38}H_{72}N_2O_{12}$	748.98	4.02	2.5 -2.7	8.7
	Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	3.06	2.2	8.9
	Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	747.95	3.16	2.5-2.6	9.0
Bacteriostatic	**Trimethoprim**	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	0.91	-	7.1
Amphenicol	**Thiamphenicol**	15318-45-3	$C_{12}H_{15}Cl_2NO_5S$	356.22	-0.27	-	7.7

Penicillins (β-Lactams)	**Amoxicillin**	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.40	0.87	-	2.4, 7.4
	Flucloxacillin-Na	1847-24-1	$C_{19}H_{16}ClFN_3NaO_5S$	475.85	-1.44	-1	-
	Penicillin G-Na	6957-8	$C_{16}H_{17}N_2NaO_4S$	356.37	1.83	-	-

a-KOWWIN v 1.67 (EPI Suite, USEPA), b- ref. 1, c - ref. 2, (-) – data not available

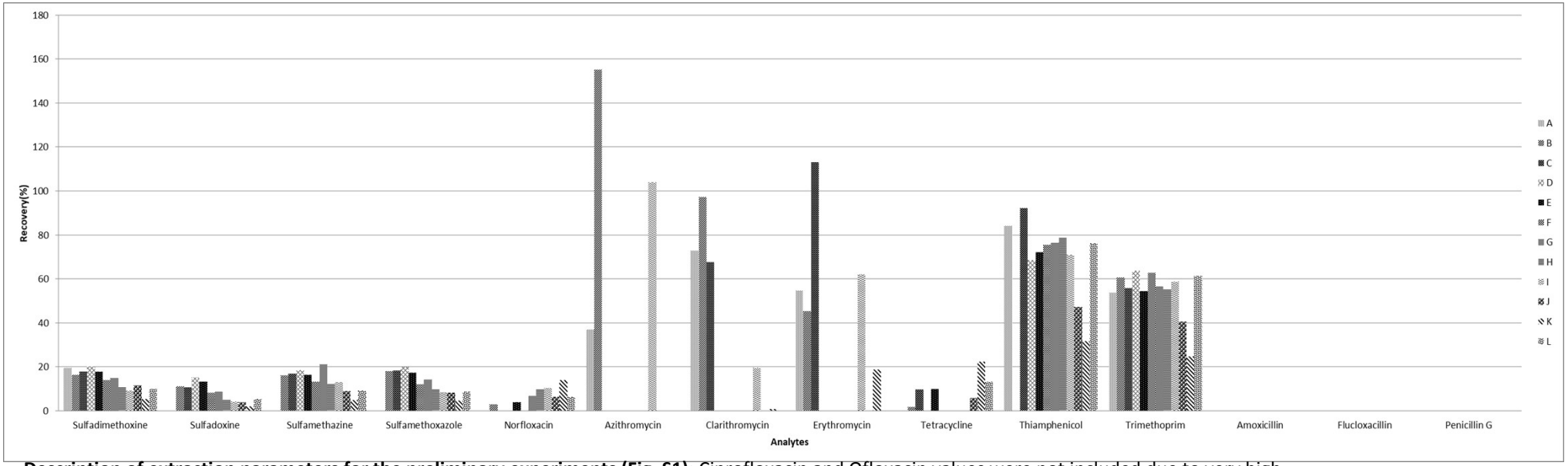
Preliminary assessment of extraction solvents and buffers

From the preliminary experiments almost all the extraction solvents in combination with a buffer or non-buffered salt provided good recoveries for trimethoprim, thiamphenicol and the four sulfonamides with the exception of methanol/acetonitrile/0.1M Na_2EDTA-Mcllvaine buffer (2 mL:8 mL:10 mL) + non-buffered salt with which a reduction in recovery was observed (J and K in Fig. S1). Modification of organic solvent (acetonitrile) with 2 mL methanol slightly reduced their recoveries using 0.1 M Na_2EDTA-Mcllvaine buffer. Though comparable extraction recoveries were observed for sulfonamide antibiotics with all the extraction solvents and buffer combination, 0.1 M Na_2EDTA-Mcllvaine buffer generally presented much lower recoveries. Modification of organic solvent (acetonitrile) with 1% acetic acid however, slightly improved the recoveries of sulfonamides using the 0.1 M Na_2EDTA-Mcllvaine buffer. Citrate and acetate buffers provided highest recoveries for sulfonamides.

For the fluoroquinone norfloxacin, extraction solvents involving 0.1 M Na_2EDTA McIlvaine buffer yielded good recoveries (Fig. S1, experiments G to K). The presence of Na_2EDTA and ultrasonication obviously improved the recovery of norfloxacin. Na_2EDTA was found to improve the extraction recovery of fluoroquinolones from sewage sludge and other related environmental matrices in previous studies.[2,3] (Gago-Ferrero et al 2015, Huang et al. 2013). Generally low recoveries were obtained for norfloxacin with acetate buffer. Peysson and Vulliet also found low recoveries for fluoroquinolones with acetate buffer in their work.[4] However, the authors discontinued to test further the possibility of obtaining better recoveries for fluoroquinolones with citrate buffer. Based on our preliminary studies, citrate buffer proved to be a prospective better candidate for the extraction of fluoroquinolones than acetate buffer (experiments B and E in Fig. S1).

Extraction with acetonitrile/water only using any of the buffer or non-buffered salts yielded good recoveries for the macrolides (clarithromycin, azithromycin and erythromycin). Overall, citrate buffer provided best recoveries for azithromycin and clarithromycin (exp. B), for erythromycin it was non-buffered salt (exp. C). Moreover, citrate buffer, non-buffered salt and 0.1 M Na_2EDTA-McIlvaine buffer gave good recoveries for tetracycline. Modification of acetonitrile with methanol when using 0.1 M Na_2EDTA-McIlvaine buffer apparently improved tetracycline recovery (experiment K). Modification of extraction solvents with Na_2EDTA when using citrate buffer and its addition to McIlvaine buffer also led to improvement of tetracycline recovery. Bourdat-Deschamps and co-workers also added EDTA to extraction solvents in order to improve the recovery of tetracyclines.[5] It is worthy to note that β-lactam

antibiotics (amoxicillin, penicillin and flucloxacillin) were not recovered by any of the combination of extraction solvents and buffers. Further attempts were made in subsequent experiments in order to improve the recoveries of β-lactam and other target antibiotics. Therefore, based on the results of these preliminary studies, extraction solvent composition (acetonitrile/methanol and Na_2EDTA in water) with citrate buffer and ultrasonication were selected for further experiments.



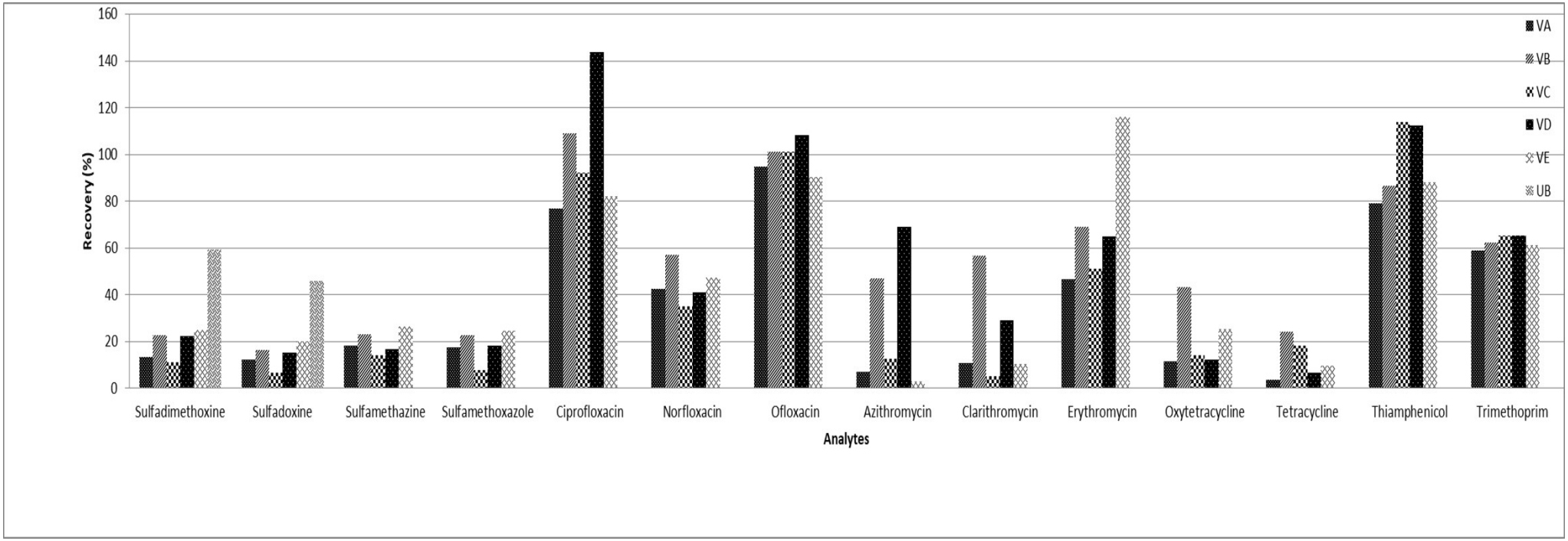
Description of extraction parameters for the preliminary experiments (Fig. S1); Ciprofloxacin and Ofloxacin values were not included due to very high enhancement resulting in corresponding very high recovery values

Experiment	Organic solvent	Aqueous solvent	Buffer salt	Ultrasonication
A	10 mL acetonitrile	10 mL H_2O	acetate	x
B	10 mL acetonitrile	10 mL H_2O	citrate	x
C	10 mL acetonitrile	10 mL H_2O	non-buffered	x
L	10 mL acetonitrile	10 mL 0.1 M Na_2EDTA in H_2O	non-buffered	x
D	10 mL acetonitrile modified with 1% acetic acid	10 mL 0.1 M Na_2EDTA in H_2O	acetate	x
E	10 mL acetonitrile modified with 1% acetic acid	10 mL 0.1 M Na_2EDTA in H_2O	citrate	x
F	10 mL acetonitrile modified with 1% acetic acid	10 mL 0.1 M Na_2EDTA in H_2O	non-buffered	x
G	10 mL acetonitrile modified with 1% acetic acid	10 mL 0.1 M Na_2EDTA-McIlvaine buffer	non-buffered	x
H	10 mL acetonitrile	10 mL 0.1 M Na_2EDTA-McIlvaine buffer	non-buffered	x
I	10 mL acetonitrile	10 mL 0.1 M Na_2EDTA-McIlvaine buffer	non-buffered	ultrasonication
J	8 mL acetonitrile/2 mL methanol	10 mL 0.1 M Na_2EDTA-McIlvaine buffer	non-buffered	x
K	8 mL acetonitrile/2 mL methanol	10 mL 0.1 M Na_2EDTA-McIlvaine buffer	non-buffered	ultrasonication

Fig. S1 Preliminary assessment of extraction solvents and buffers

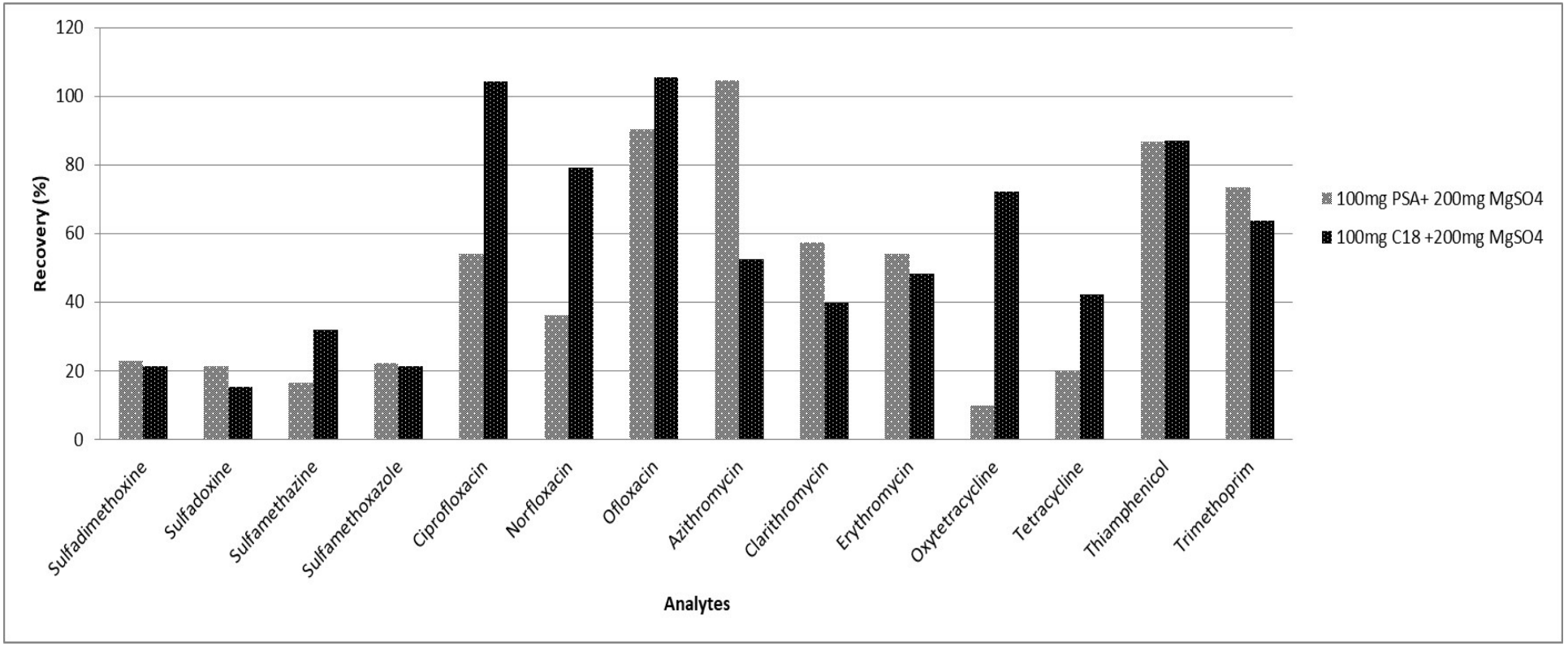
Influence of methanol and Na2EDTA on the extraction recovery

Considering the tremendous influence of methanol and Na_2EDTA on the recoveries of most target antibiotics in the preliminary experiments (Fig. S1), some experiments were further carried out to investigate possible improvement in the recoveries by increasing the concentration of Na_2EDTA in the aqueous solution to 0.2M. Results are presented in Fig. S2. In comparison with 0.1M Na_2EDTA slightly better recoveries were obtained with 0.2M Na_2EDTA aqueous solution for all target antibiotics using 2mL methanol as organic modifier, except the β-lactam antibiotics which were not recovered. However, modification of organic solvent with 1mL methanol gave rise to a general decrease in the recoveries of sulfonamides, macrolides and tetracyclines thus indicating the necessity of optimizing methanol contents for the recovery enhancement of these antibiotics. Finally, taking into account the desirable influence of methanol and Na_2EDTA on the recoveries of most target antibiotics, 2mL methanol + 8 mL acetonitrile + 0.2M Na_2EDTA aqueous solution was chosen as the extraction solvents, employing citrate buffer salt for partitioning followed by ultrasonication.



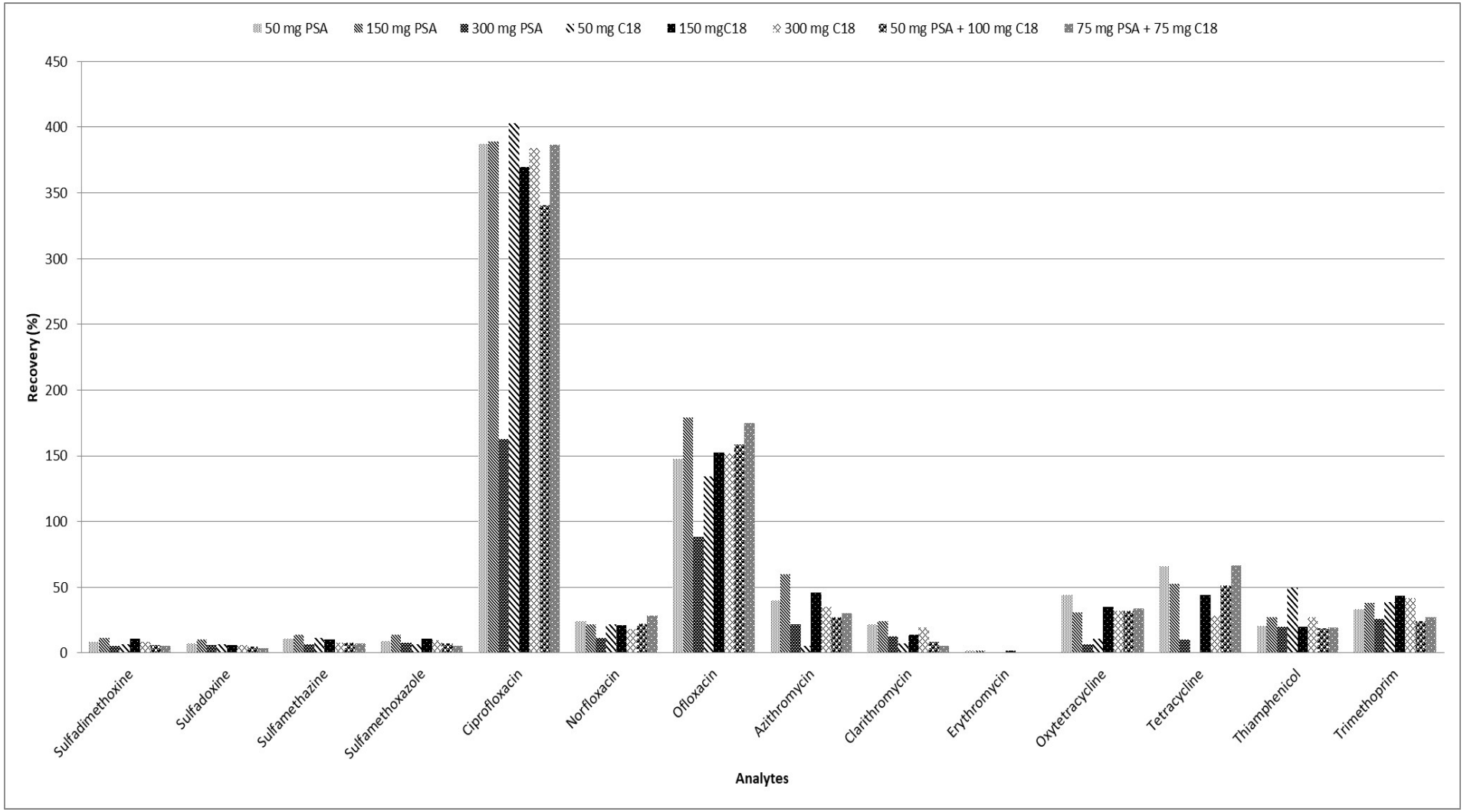
Extraction involved the use of citrate buffer salt for each extraction solvent composition followed by ultrasonication.
Extraction solvent composition: 10 mL organic solvent + 10 mL water at varied composition of organic solvent, acetic acid and Na_2EDTA;
VA: 8 mL ACN + 2 mL MeOH+10 mL 0.1M Na_2EDTA; VB: 8 mL ACN + 2 mL MeOH + 10 mL 0.2M Na_2EDTA; VC: 9 mL ACN + 1 mL MeOH + 10 mL 0.2M Na_2EDTA; VD: 9 mL ACN + 1 mL MeOH + 10 mL 0.1M Na_2EDTA; VE: 8 mL 1% acetic acid in ACN + 2 mL MeOH + 10 mL 0.1M Na_2EDTA; UB: 6 mL ACN + 4 mL MeOH + 10 mL 0.1M Na_2EDTA

Fig. S2 Influence of methanol and Na2EDTA on the extraction recovery



Extraction solvent composition: 9 mL ACN + 1 mL MeOH + 10 mL H_2O; + citrate buffer followed by ultrasonication

Fig. S3 Preliminary assessment of d-SPE sorbents efficiency



Extraction solvent composition: 8 mL ACN + 2 mL MeOH + 10 mL 0.1M Na_2EDTA; + citrate buffer followed by ultrasonication. Each d-SPE sorbent composition contained 200 mg $MgSO_4$

Fig. S4 Recovery studies with different amounts of d-SPE sorbent(s)

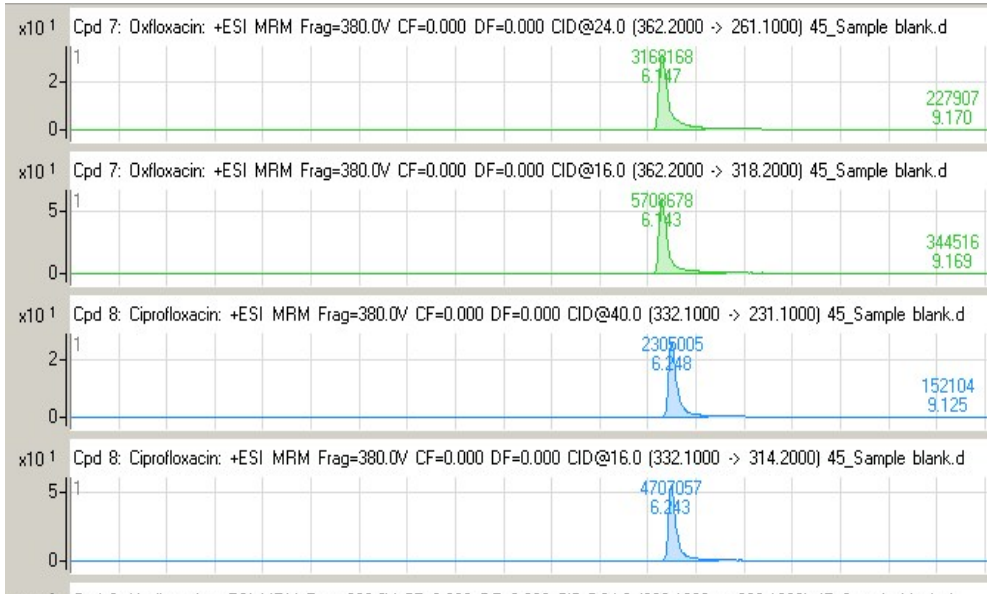


Fig. S5 MS/MS chromatograms of ofloxacin and ciprofloxacin in sample blank: high values of peak areas depicting the presence of the two antibiotics in significant amounts

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