SUPPLEMENTARY MATERIAL

Multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE–LC–MS/MS and matrix–matched standards

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Optimization of SPE

In this study, analyte retention capabilities of three different sorbents were compared (Oasis HLB, Oasis MCX and Oasis MCX) analyte retention capability using the 3 cc/ 60 mg SPE Cartridges. Cartridges were preconditioned with 3 mL MeOH followed by 3 mL Ultrapure water. Prior to sample loading, the pH was adjusted to correspond with the basic protocols for the individual sorbents [1]. In summary, pH was adjusted to 3, 7 and 11 for the MCX, HLB and MAX, respectively using either 2 M formic acid or 2 M NH_4OH. 100 mL ultrapure water spiked at 10 µg/L concentration for target analytes was loaded in triplicate to the sorbent material under test. The elution for MCX, HLB and MAX was done using 4 mL of MeOH/ 2 M NH_4OH 90/10 (v/v), MeOH and MeOH/ 2 M HCOOH 90/10 (v/v), respectively and then reconstituted for analysis. The results prompted the choice of Oasis HLB as for subsequent analysis (See section 3.2).

In determination of the optimum sample pH, 5 duplicate sets 100 mL surface water containing 2.0 µg/L of target analytes were separately adjusted to pH 3, pH 5, pH 7, pH 9 and pH 11 using either 2 M NH_4OH or 2 M formic acid. The samples were loaded to Oasis HLB (3 cc, 60 mg) cartridges then washed with 6 mL ultrapure water at the corresponding loading pH before elution with MeOH. The optimum recovery was achieved at pH 5, which was used in the subsequent stages.

In determination of the elution solvent, 100 mL surface water adjusted to pH 5 and containing 2.0 µg/L of target analytes was loaded into 4 triplicate sets of Oasis HLB (3 cc, 60 mg) cartridges and eluted with 4 mL of the solvents under study. The solvents considered in this study included MeOH, ACN, MeOH/ACN 1/1 (v/v), MeOH/ACN/ 2 M NH_4OH 45/45/10 (v/v).

Optimum elution volume was evaluated using Oasis HLB (6 cc, 200 mg) and 500 mL surface water containing 1.0 µg/L of mixed standards in 3 triplicate sets of cartridges. Each set was separately eluted with 2 mL, 4 mL and 6 mL elution solvent volume. Cartridges were then washed with 6 mL ultrapure water at pH 5.

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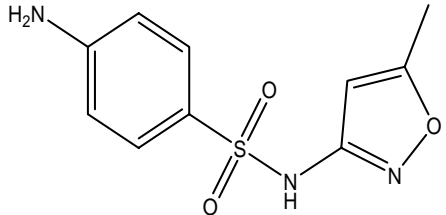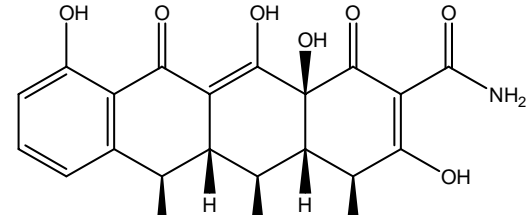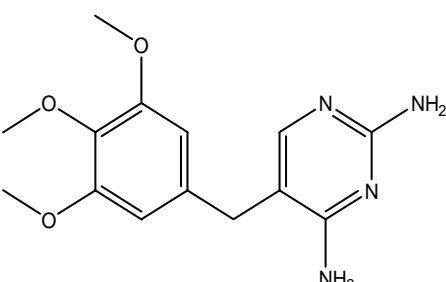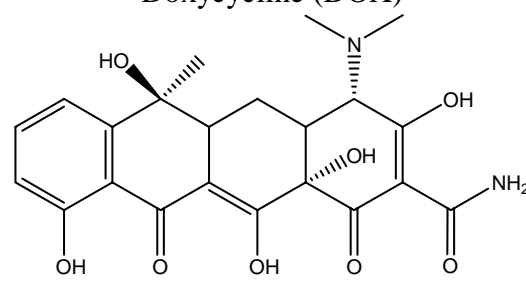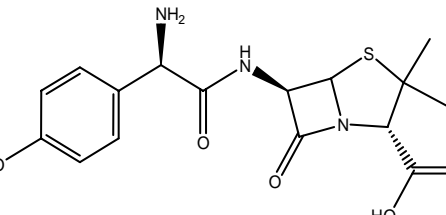
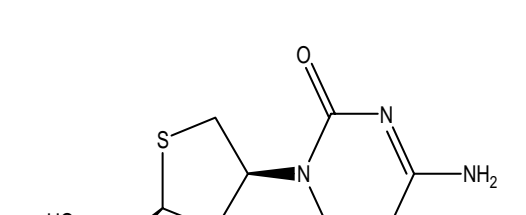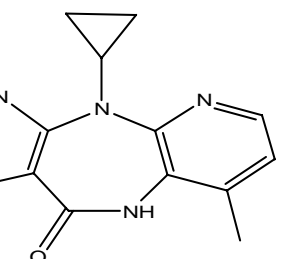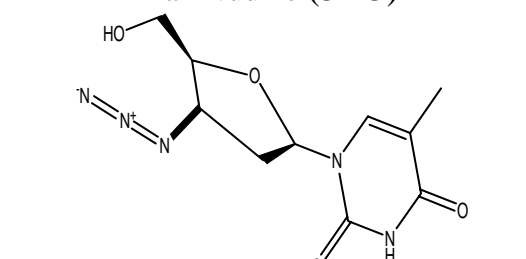**Table S1: Structures of the analytes**
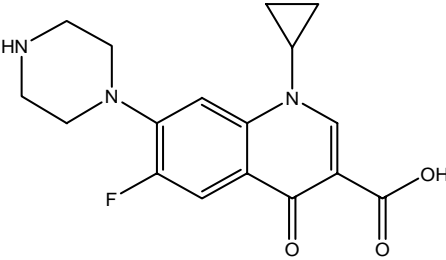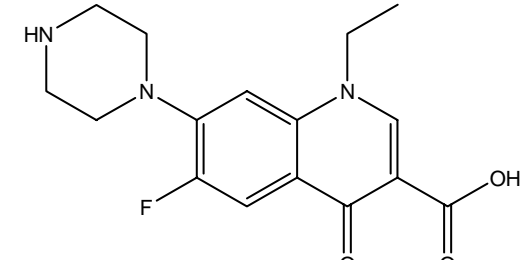
Sulfamethoxazole (SMX)

Doxycycline (DOX)

Trimethoprim (TMP)

Tetracycline (TET)

Ciprofloxacin (CIP)

Norfloxacin (NOR)

Amoxicillin (AMO)

Lamivudine (3TC)

Nevirapine (NVP)

Zidovudine (ZDV)

Table S2: Instrumental repeatability (Precision) at three concentrations of working standards dissolved in the mobile phase (n=6) expressed as % RSD

Analyte	Intraday (RSD)			Interday (RSD)		
	40 µg/L	200 µg/L	1000 µg/L	40 µg/L	200 µg/L	1000 µg/L
SMX	2.8	2.7	3.1	5.3	3.1	6.0
TMP	3.7	1.2	3.7	5.8	3.1	4.6
CIP	4.3	4.0	2.9	6.1	6.6	3.5
NOR	4.7	6.2	5.4	6.6	6.9	6.9
DOX	7.5	6.3	4.4	6.0	4.4	9.3
TET	4.7	5.9	4.0	10.1	9.0	8.6
AMO	6.2	6.3	4.2	11.5	9.8	9.4
3TC	2.1	3.8	9.8	3.1	4.4	6.3
ZDV	7.2	3.7	6.3	8.3	4.8	8.4
NVP	2.0	3.0	2.6	3.9	3.9	4.5

Table S3: Method repeatability at three concentration levels interday (n=3) expressed as % RSD

Analyte	0.1 µg/L	0.5 µg/L	2 µg/L
SMX	4.5	5.2	5.7
TMP	3.2	4.6	7.4
CIP	6.4	7.1	7.9
NOR	8.0	8.2	10.2
DOX	5.1	7.6	7.1
TET	5.8	6.5	8.2
AMO	7.5	10.5	11.5
3TC	4.5	8.4	9.4
ZDV	9.1	10.7	10.9
NVP	2.9	4.3	3.2

Table S4: Instrumental detection limit (IDL) and quantification (IQL)

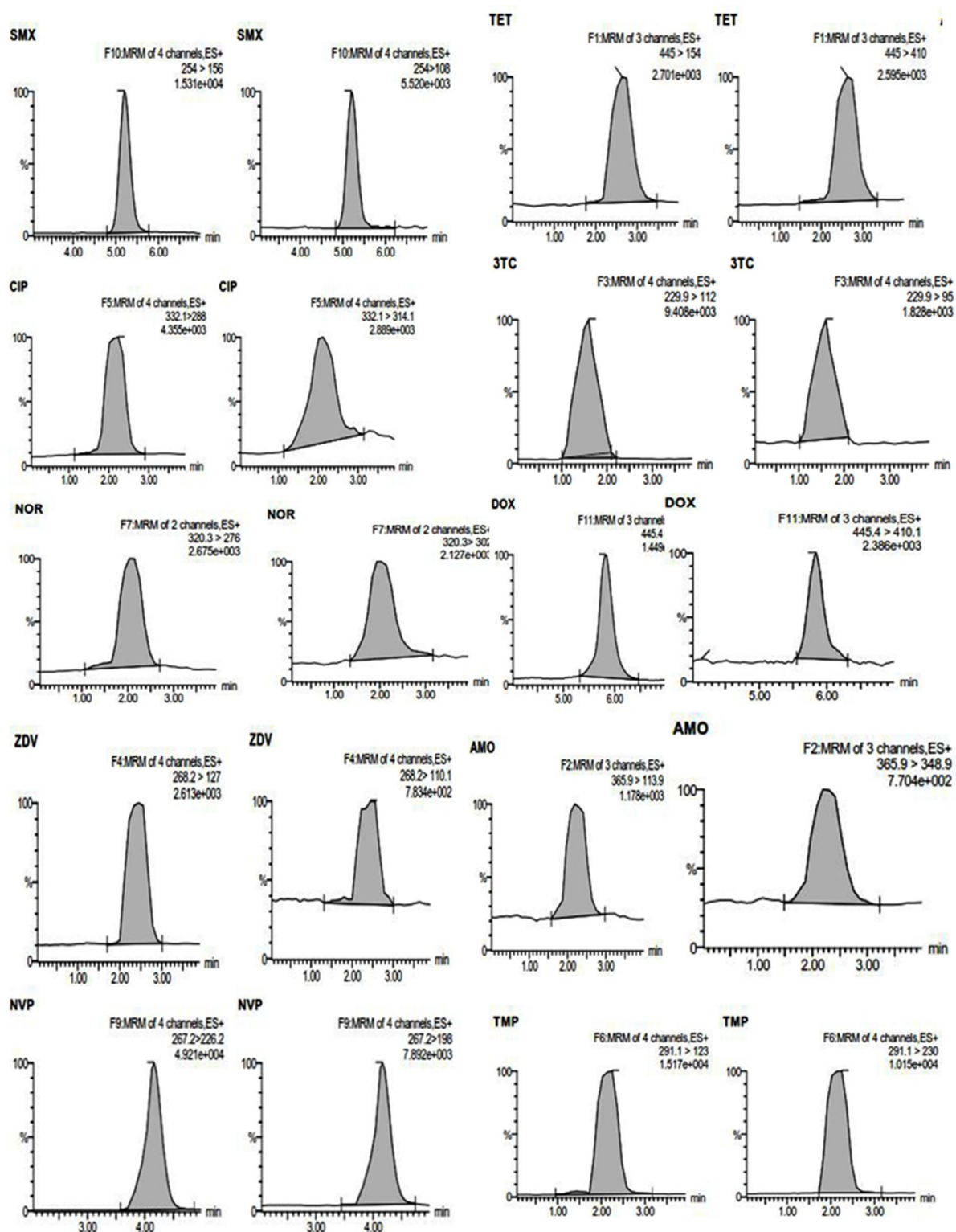| Analyte	IDL (µg/L)	IQL (µg/L)
SMX	0.9	3.3
TMP	1.7	5.9
CIP	1.1	3.8
NOR	2.2	7.6
DOX	2.4	7.9
TET	2.1	7.5
AMO	7.5	23.3
3TC	1.1	3.7
ZDV	6.9	21.1
NVP	1.2	3.9

Figure S1: LC–MS/MS chromatogram showing the retention time and the two MRM transitions for the selected pharmaceuticals spiked in surface water at 100 ng/L and extracted by SPE
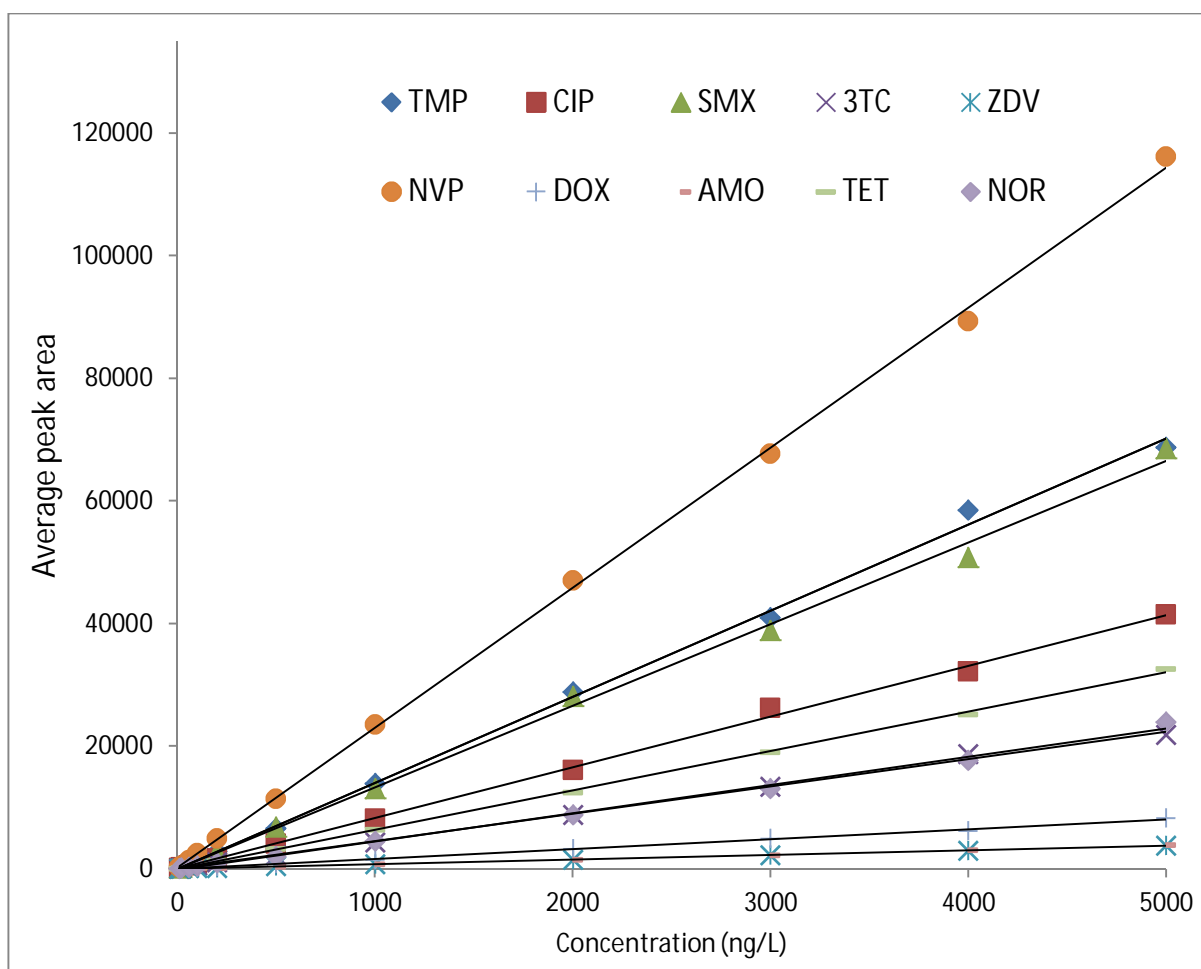
Figure S2: Matrix–matched calibration curves for the selected pharmaceuticals constructed by extracting 500 mL surface water spiked at concentration levels between 10 ng/L–5000 ng/L (11 points)

References

1   J. Arsenault, *Beginner's guide to SPE*, Waters Corporation, Milfold, 2012.