Supplemental Information for

Occurrence and fate of antibiotics and antibiotic resistance genes in typical urban water of Beijing, China

Xiaohui Liu[a,b], Ying Liu[a], Shaoyong Lu[a*], Pan Qin[a], Xiaochun Guo[a],

Bin Bi[a], Beidou Xi[a], Fengchang Wu[a], Weiliang Wang[c],Tingting Zhang[d]

[a] *State Environmental Protection Scientific Observation and Research Station for Lake Dongtinghu (SEPSORSLD), National Engineering Laboratory for Lake Pollution Control and Ecological Restoration, State Key Laboratory of Environmental Criteria an Risk Assessment, Research Centre of Lake Environment, Chinese Research Academy of Environmental Sciences, Beijing 100012, People's Republic of China.*[1]

[b] *School of Environment, Tsinghua University, Beijing 100084, China.*

[c] *School of geography and environment, Shandong Normal University, Jinan, Shandong, 250358, China;*

[d] *School of Chemical Engineering, Beijing University of Chemical Technology, Beijing, 100029, China*

[1] *Corresponding author: Tel.: +86 10 84935064

E-mail: lushy2000@163.com(S. Lu)

Table S5 Primer pairs used in qPCR assays for specific detection and quantification of ARGs

Table S6 Environmental factors in multiple water bodies of Beijing

Table S1 Detail information of hospital and STPs

	Location	Service population	Type of sewage	Daily average treating quantity	Maximum treating quantity	Hydraulic Retention Time (HRT)	ferric chloride (PIX)	Sewage treatment process	Remarks
Hospital	Haidian District	Theoutpatient volume(2000000/year), Total hospitalization patients (40000people/yerr)		_		_	_	Chlorination	1000beds. professional departments(more than 50)
Sewage treatment plant(STPs)	Haidian District	300000-400000 people	domestic sewage	30000-40000 m^3/d	80000 m^3/d	10-12h	100mg/L	anaerobic/anoxic/oxic (A^2/O) activated sludge +MBR process	
	Chaoyang District		domestic sewage		100000 m^3/d	6-8h	100mg/L	cyclic activated sludge technology (CAST).	
	Miyun District		domestic sewage/industrial wastewater(7:3)		80000 m^3/d	8-12h	100mg/L	anoxic/oxic (A^2O) activated sludge +MBR process	

Sample pretreatment. The water samples (2 L) were filtered through glass microfiber filters (0.45μm) (Whatman GF/F, 0.45μm, USA) to remove suspended particles. Then, the pH value was adjusted to 3 using 0.1mol L^{-1} sulfuric acid, Na_2EDTA (0.5g) and 100μL of 1mg L^{-1} surrogate standards (ciprofloxacin-D_8, erythromycin-^{13}C, phenacetin-^{13}C, Sulfamethazine -$^{13}C_6$) was added to water samples. The treated solutions were extracted using Waters Oasis HLB cartridges (500mg, 6 mL), which were sequentially preconditioned with 10 mL of methanol and 10 mL of ultrapure water, after which the water samples were loaded into the cartridges at a flow rate of 3-5mL min^{-1}. The cartridges were rinsed with 10 mL of a 1% methanol aqueous solution and 10 mL of ultrapure water, and dried for 2h under vacuum. The antibiotics retained on the Oasis HLB cartridges were eluted with 6 mL of methanol and 6mL of a 5% NH_4-H_2O methanol solution, and the eluents were concentrated to near dryness under a gentle stream of nitrogen, after which the resulting residues were re-dissolved in 1 mL of a 10% methanol aqueous solution. After filtration through a 0.22 μm membrane to remove particles, the final extracts were transferred to 2 mL amber glass vials added to 100μL (1mg L^{-1}) Atrazine-$^{13}C_3$ in preparation for analysis.

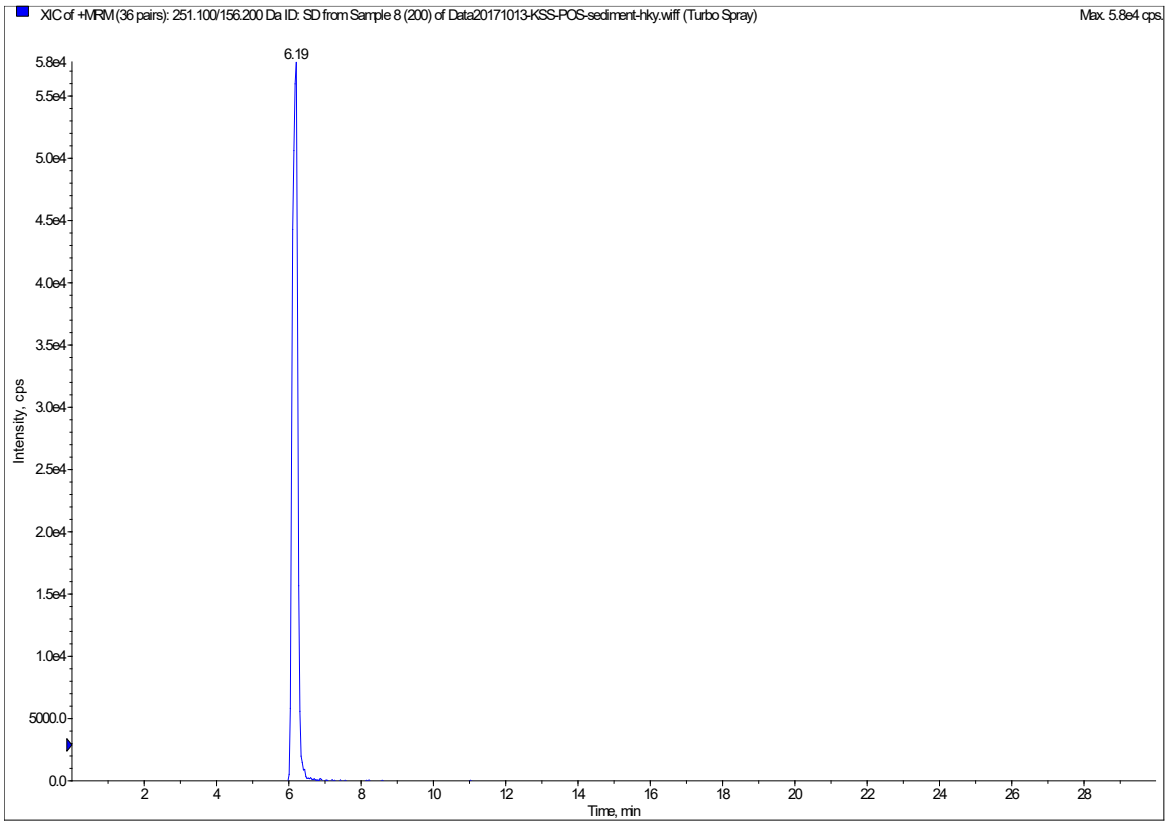
Mass spectrometric analyses. The separation of the antibiotics was achieved with the following gradient program: 0-7 min, 3-15% B; 7-9 min, 15% B; 9-12 min, 15-30% B; 12-13 min, 30% B; 13-18 min, 30-42% B; 18-19 min, 42% B; 19-21 min 42-3% B; 21-29 min, 3% B. Sample acquisition was performed in the multiple reaction monitoring (MRM) mod operated in the positive ionization mode. The nebulizer pressure was set to 40 psi and the flow rate of drying gas was set to 3 L/min. The capillary and nozzle voltages were 5000 and 0 V, respectively. The flow rate and temperature of the sheath gas were 10L/min and 500 °C, respectively.

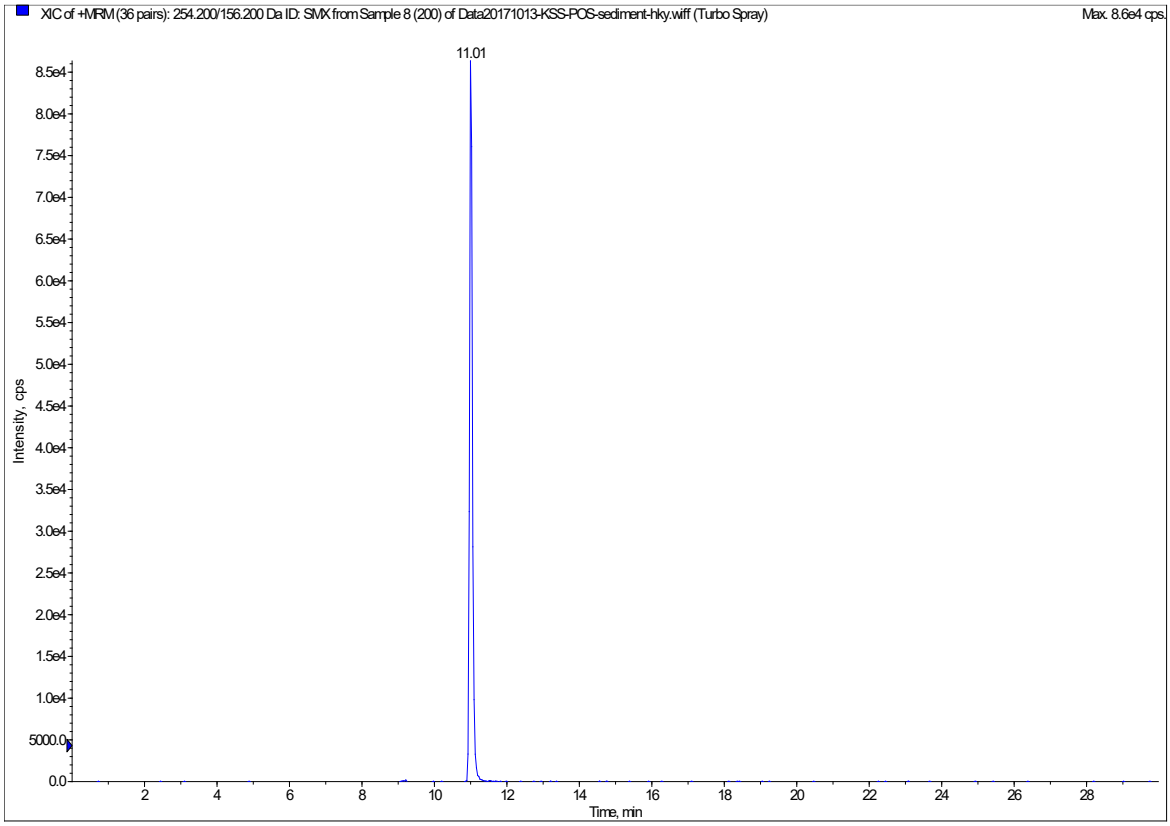
Table S2 MS/MS parameters for antibiotics

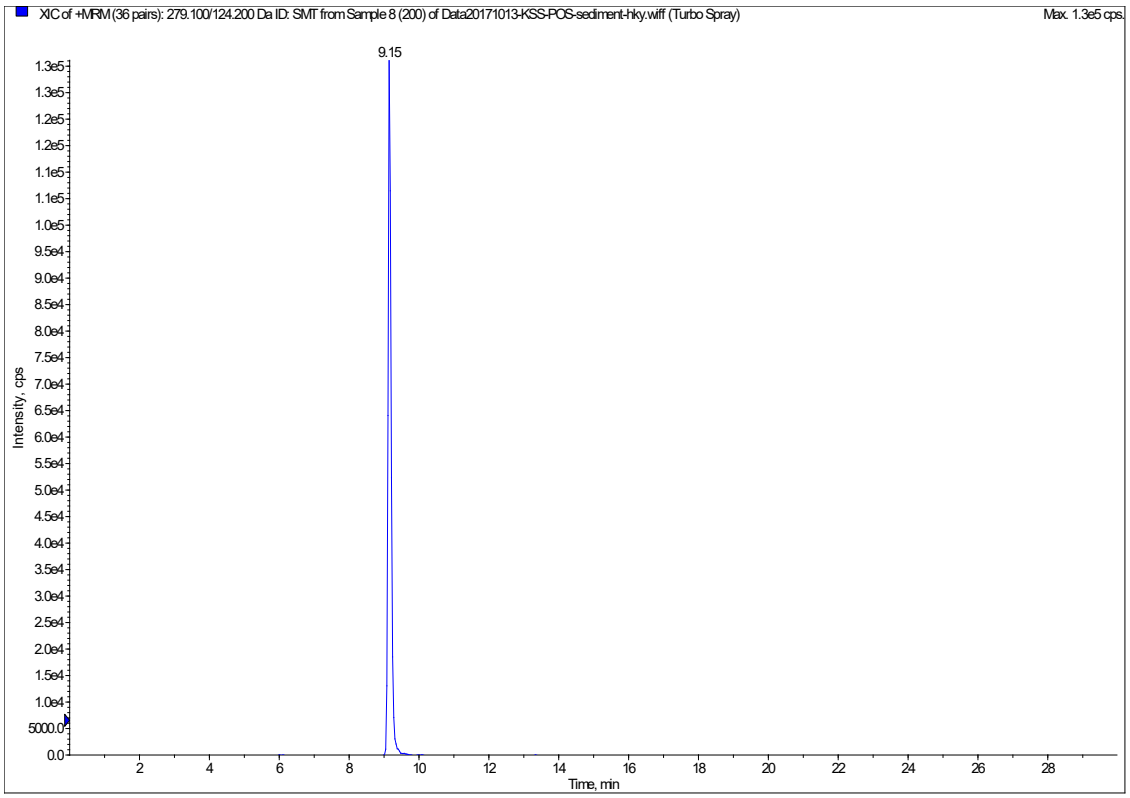
Analytes	Precursor ion (m z^{-1})	Production (m z-1)	DP（V）	EP（V）	CEP（V）	CE（V）	CXP（V）

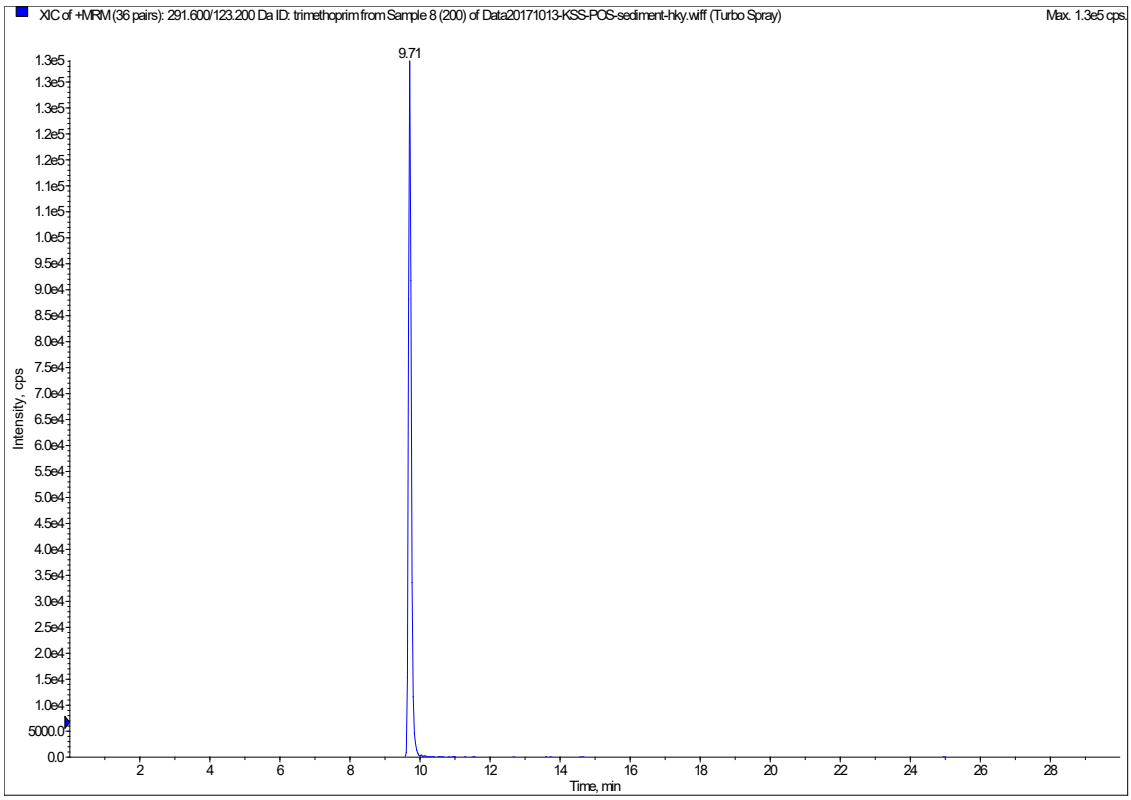
SD	251.1	156.2*	46	6	12	19	4
		92.1	46	6	12	33	4
SMX	254.2	156.2*	46	6	14	19	4
		92	46	6	14	35	4
SMT	279.1	124.2*	51	6	16	31	4
		186.3	51	6	16	21	4
TMP	291.6	123.2*	56	5	16	31	4
		230.2	56	5	16	21	4
NOR	320.1	302.2*	53	4	9	27	3
		276.3	53	4	27	22	3
CIP	332.3	314.5*	52	5	10	26	3
		231.3	52	5	17	44	3
ENR	360.2	342.3*	80	5	11	27	3
		316.3	80	5	11	27	3
OFLO	362.1	318.1*	57	4	13	27	3
		261.3	57	4	13	35	3
SFLO	385.9	368.3*	60	3	20	25	5
		299.3	60	3	20	33	5
TC	445.3	410.2*	51	5	16	21	6
		154.1	51	5	16	33	4
OTC	461.3	426.2*	52	5.6	25	31	5.8
		201.2	52	5.6	25	48	5.8
CTC	479.2	462.2*	56	7	18	21	6
		444.3	56	7	18	23	6
ERM-H_2O	716.4	158.2*	66	9	24	37	4
		116.1	66	9	24	55	4
ROM	837.6	158.3*	61	8	34	43	4
		116.2	61	8	34	65	4
PHE-^{13}C	181.2	110.1*	56	5	12	29	4
		139.1	56	5	12	17	4
CIP-D_8	340.2	322.3*	53	4	20	27	3.4
		235.2	53	4	20	45	3.4
SMZ-$^{13}C_6$	285.1	124.1*	45	5	16	33	4
		186.2	45	5	16	23	4
ERM-^{13}C	718.4	160.3*	61	9	26	37	4
		83	61	9	26	71	4
ATR-$^{13}C_3$	219.3	177.2*	51	5	14	19	4
		106.1	80	10	16	50	3

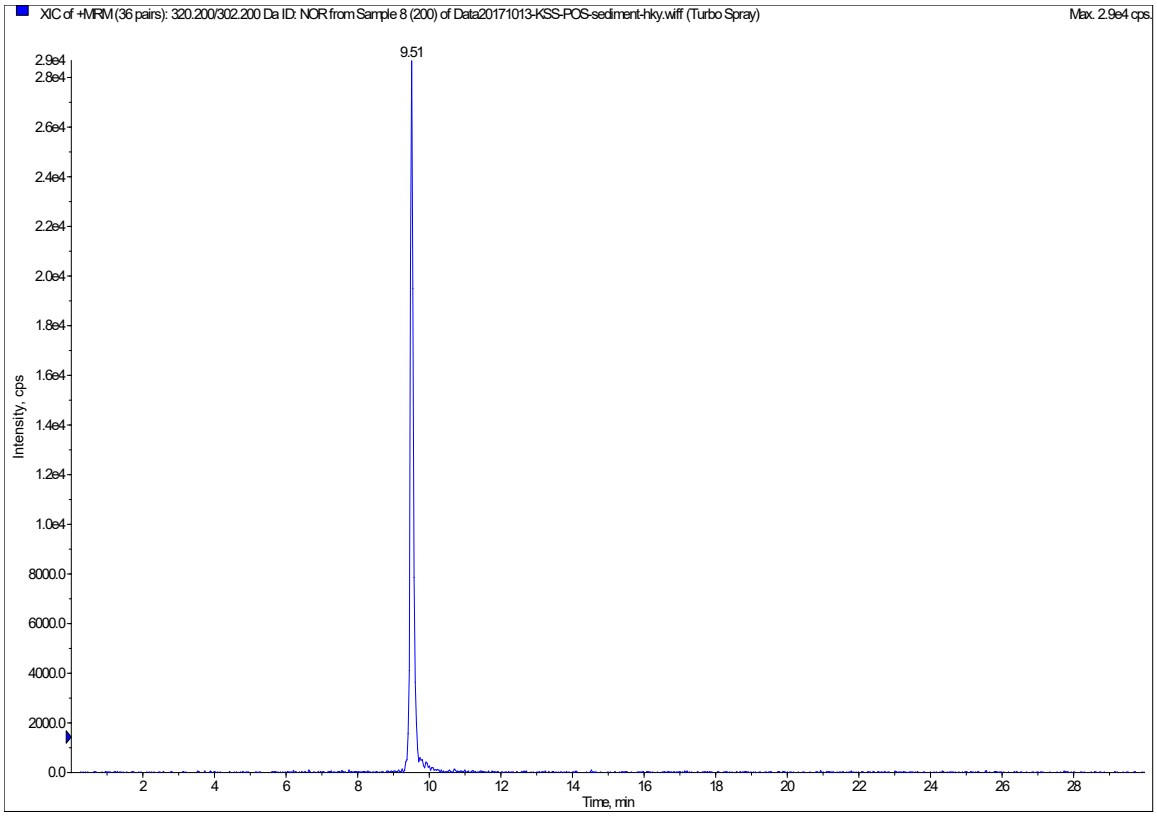
*：quantitative ion

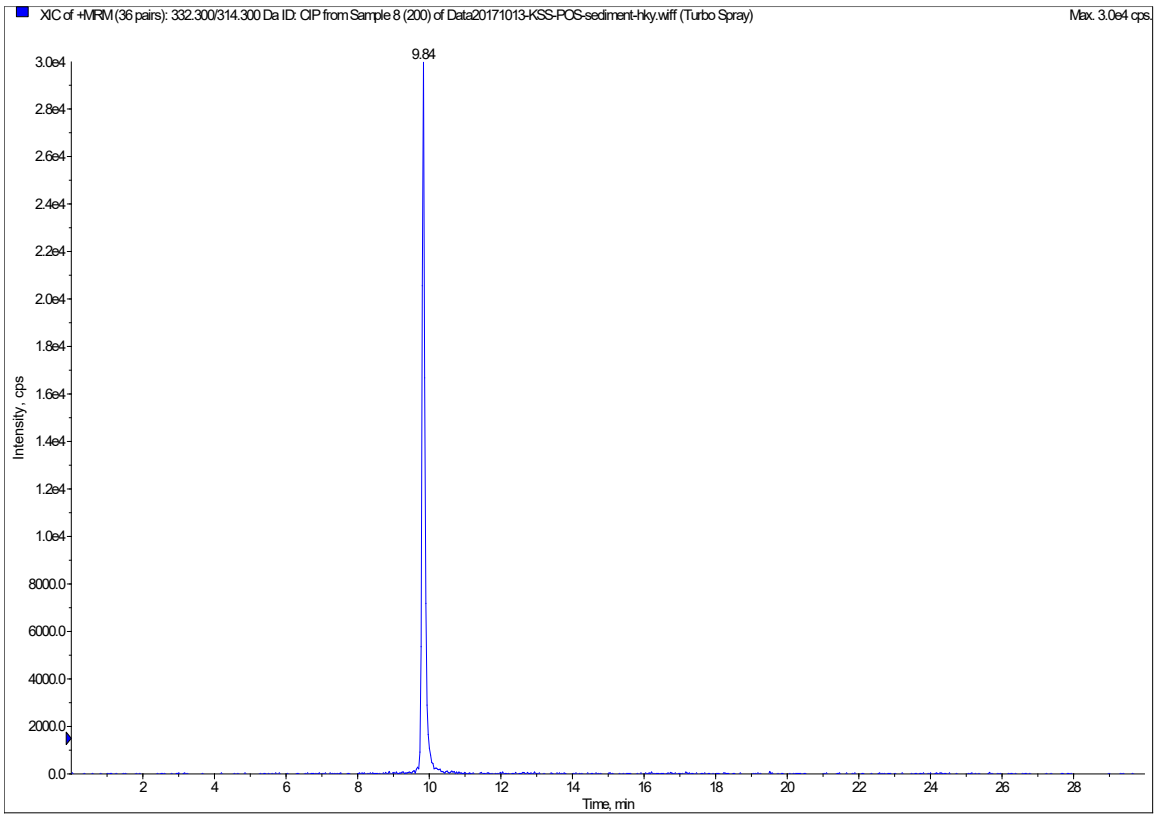

XIC of +MRM (36 pairs): 251.100/156.200 Da ID: SD from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 5.8e4 cps.
6.19
Intensity, cps
Time, min

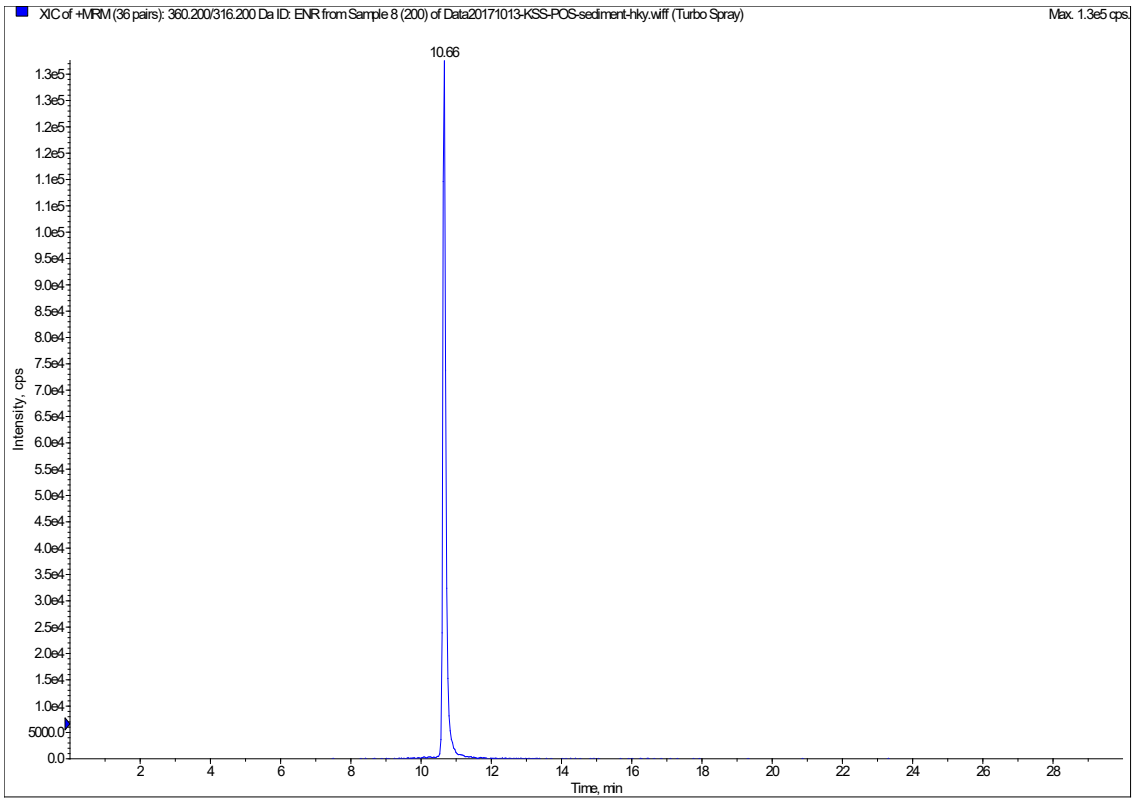

XIC of +MRM (36 pairs): 254.200/156.200 Da ID: SMX from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 8.6e4 cps.
11.01
Intensity, cps
Time, min

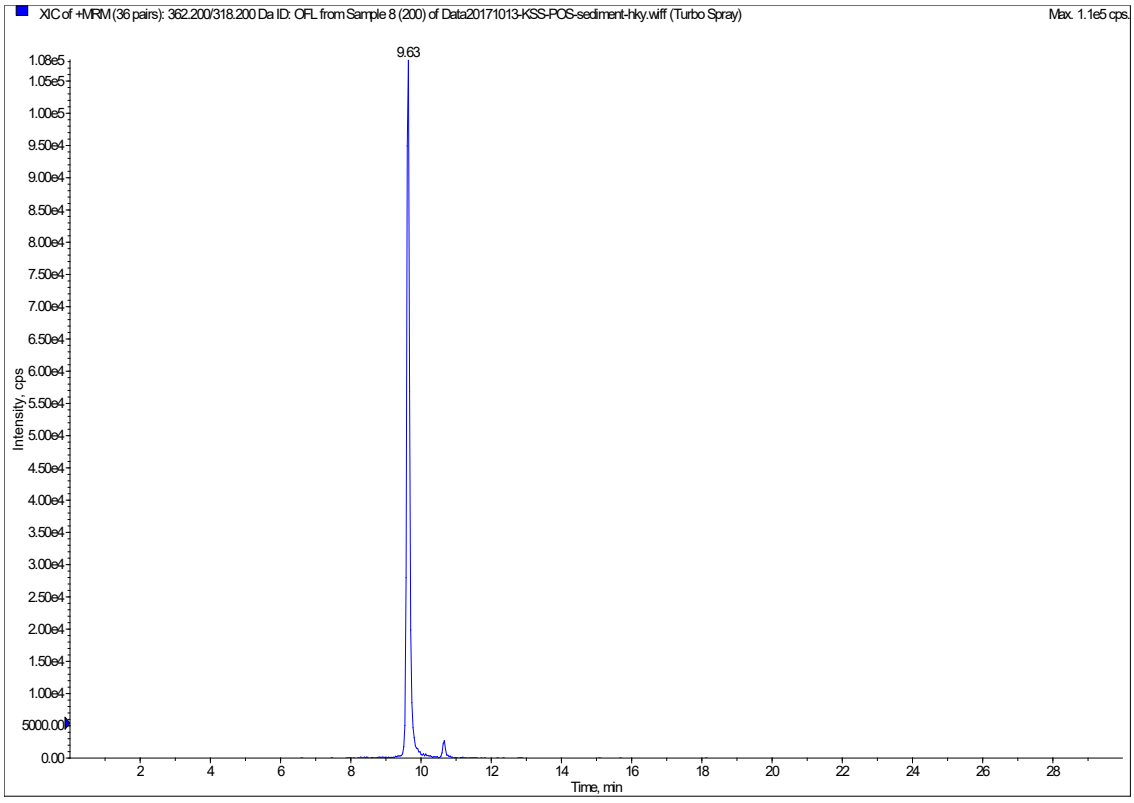

XIC of +MRM (36 pairs): 279.100/124.200 Da ID: SMT from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 1.3e5 cps.
9.15
Intensity, cps
Time, min

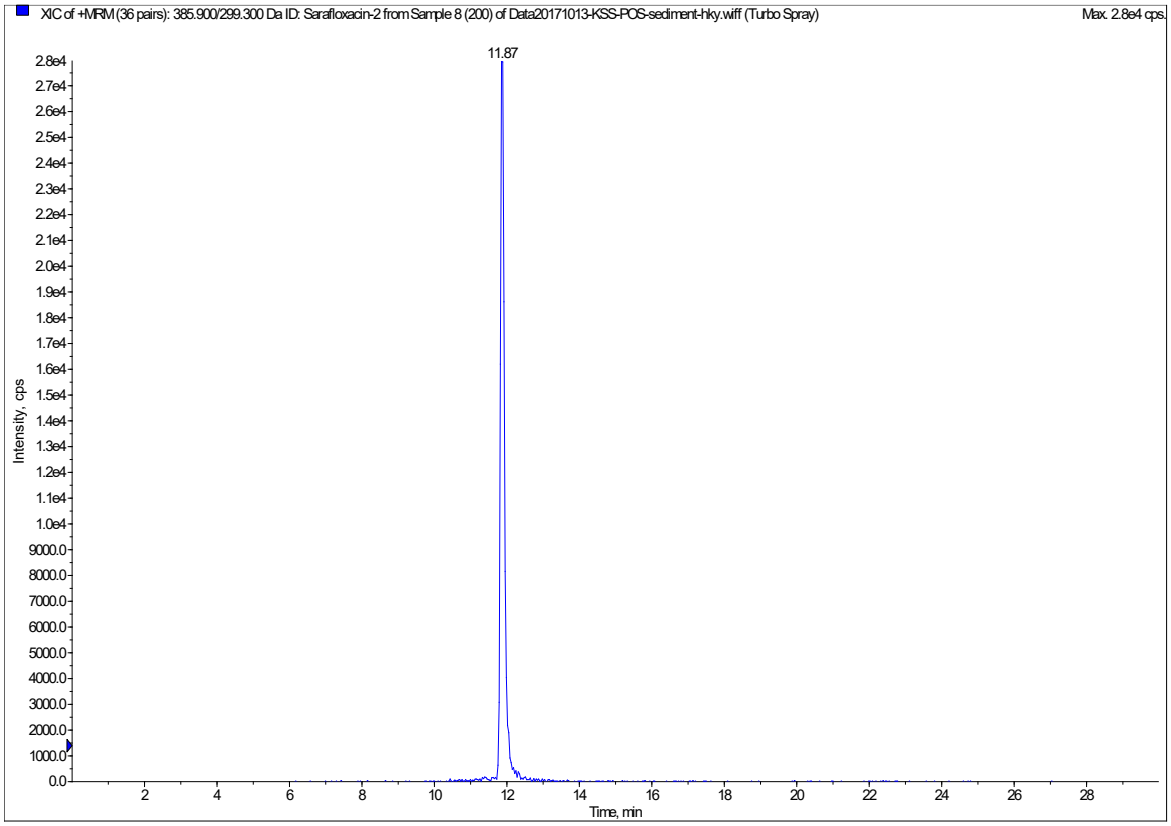

XIC of +MRM (36 pairs): 291.600/123.200 Da ID: trimethoprim from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 1.3e5 cps.
9.71
Intensity, cps
Time, min

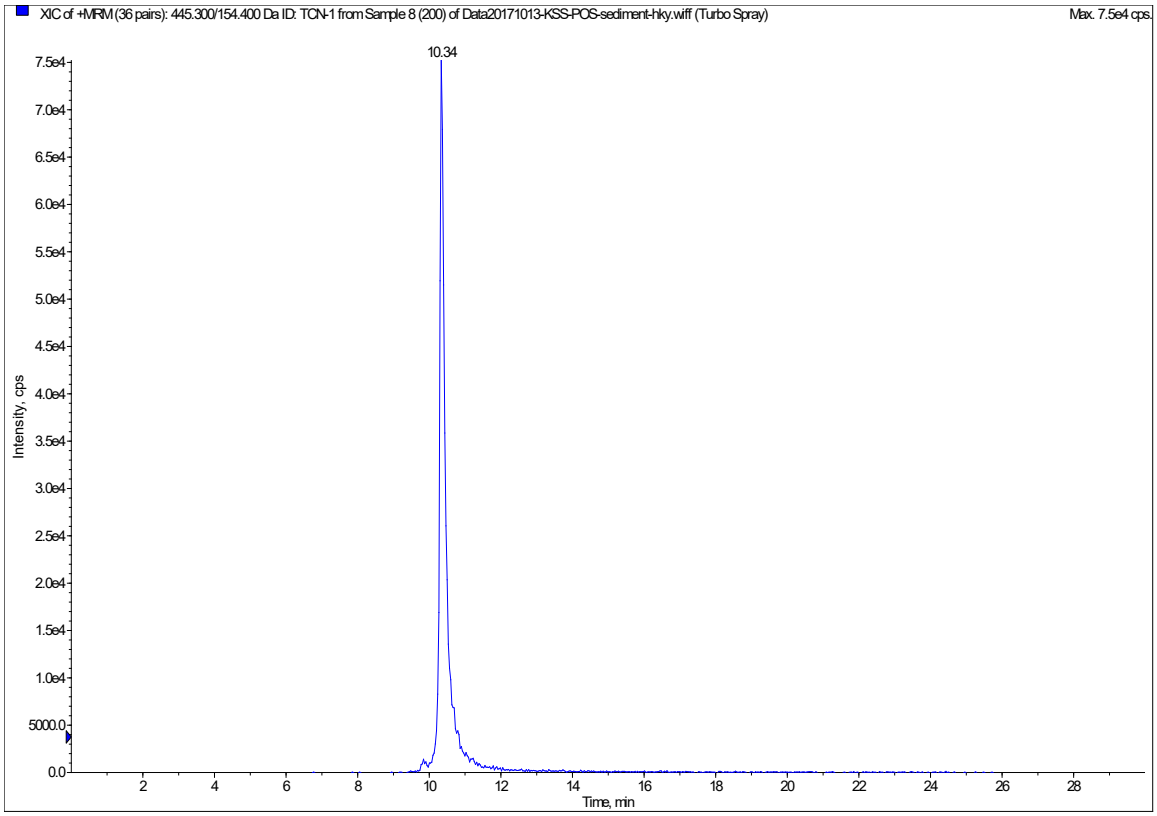

XIC of +MRM (36 pairs): 320.200/302.200 Da ID: NOR from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 2.9e4 cps.
9.51
Intensity, cps
Time, min

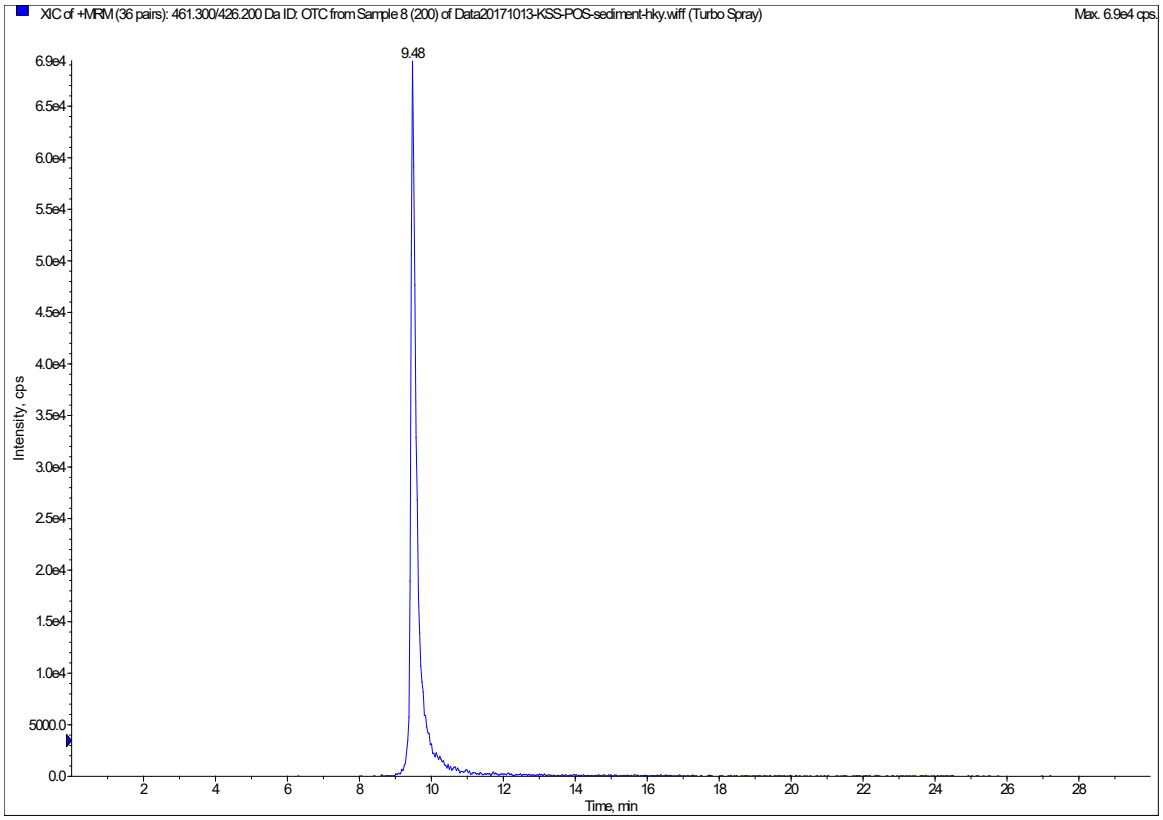

XIC of +MRM (36 pairs): 332.300/314.300 Da ID: CIP from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 3.0e4 cps.
9.84
Intensity, cps
Time, min

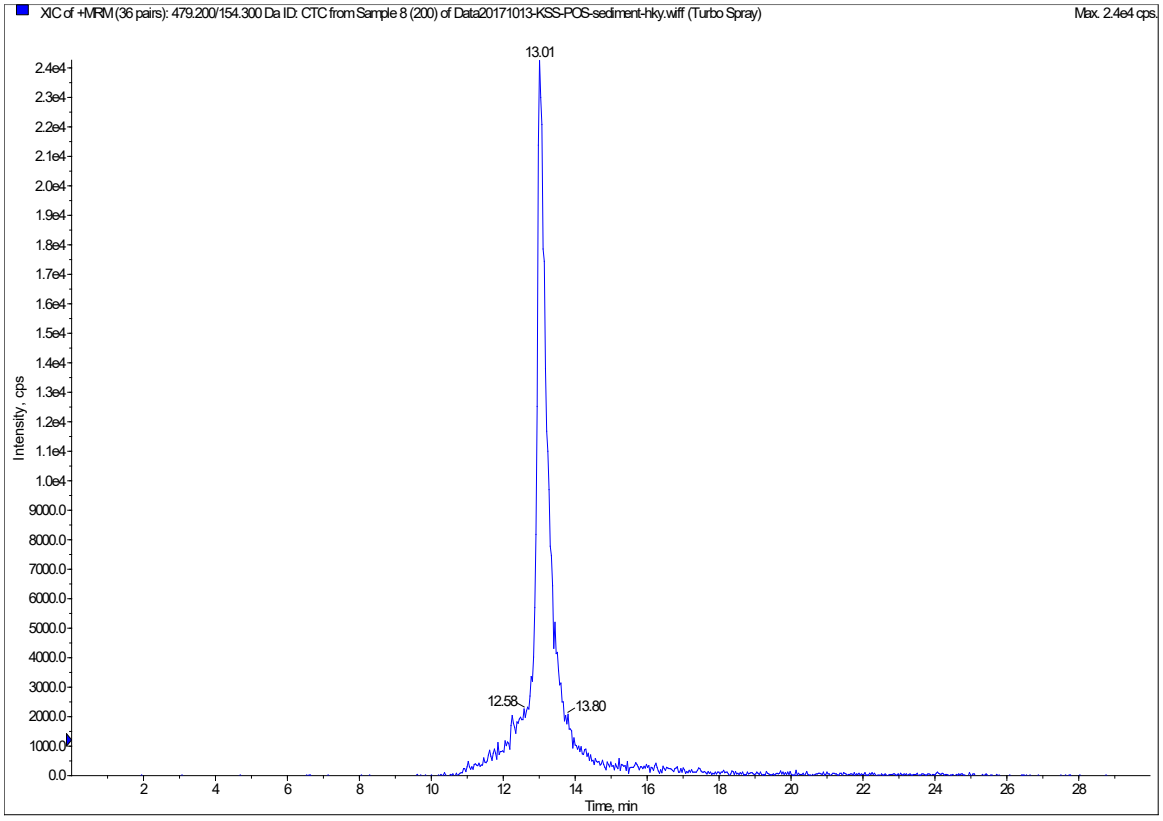

XIC of +MRM (36 pairs): 360.200/316.200 Da ID: ENR from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 1.3e5 cps.
10.66
Intensity, cps
Time, min


XIC of +MRM (36 pairs): 362.200/318.200 Da ID: OFL from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 1.1e5 cps.
9.63
Intensity, cps
Time, min


XIC of +MRM (36 pairs): 385.900/299.300 Da ID: Sarafloxacin-2 from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 2.8e4 cps.
11.87
Intensity, cps
Time, min


XIC of +MRM (36 pairs): 445.300/154.400 Da ID: TCN-1 from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 7.5e4 cps.
10.34
Intensity, cps
Time, min


XIC of +MRM (36 pairs): 461.300/426.200 Da ID: OTC from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 6.9e4 cps.
9.48
Intensity, cps
Time, min


XIC of +MRM (36 pairs): 479.200/154.300 Da ID: CTC from Sample 8 (200) of Data20171013-KSS-POS-sediment-hky.wiff (Turbo Spray)
Max. 2.4e4 cps.
13.01
12.58
13.80
Intensity, cps
Time, min

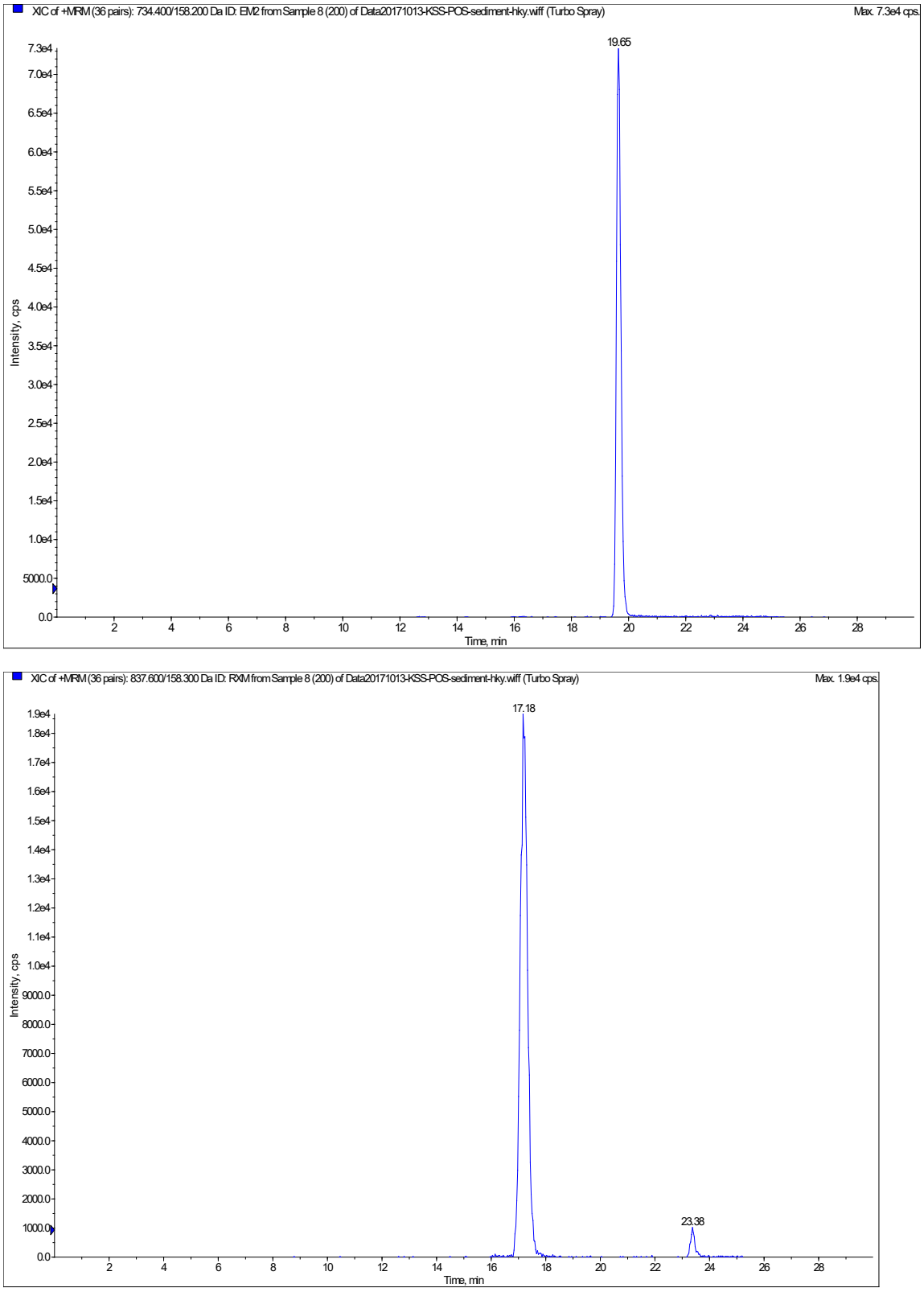


Fig.S1 Base peak-extracted ion chromatograms for SD, SMX, SMT, TMP, NOR, CIP, ENR, OFLO, SFLO, TC, OTC, CTC, ERM- H_2O, ROM

Supplemental Information for Quality assurance and quality control

Table S3 Antibiotic recoveries of solid-phase extraction, relative standard deviation (RSD), limits of detection (LOD) and limits of quantification (LOQ)

Antibiotics	Average recoveries	RSD (%)	LOD(ng L^{-1})	LOQ (ng L^{-1})
SDZ	93.10	3.90	0.43	2.21
SMZ	91.07	4.02	0.39	1.11
SMX	89.61	2.98	0.27	1.01
TMP	89.66	4.33	1.06	3.15
NOR	83.91	5.21	0.67	1.20
CIP	90.11	2.01	1.01	2.20
ENR	85.35	5.06	2.1	5.31
OFLO	101.07	6.77	1.11	3.50
SFLO	88.77	4.24	3.21	7.92
TC	83.00	3.28	2.16	4.10
OTC	117.01	5.86	2.82	6.04
CTC	84.64	4.11	0.09	3.50
ERM	91.99	2.09	0.12	0.66
ROM	87.63	2.51	0.33	1.05

DNA extraction. The genomic DNA of homogenized rhizome sample (5g) were extracted using DNA extraction kit(MEGA Bio-Tek, USA) according to the manufacturer's introductions. Nano Drop ND-1000 (Nano Drop Technologies, Willmington, DE) and gel electrophoresis were used to quantify and qualify DNA products, respectively. The extracted DNAs dissolved in 50μL of TE buffer were stored at -20℃.

Table S4 Primer pairs used in qPCR assays for specific detection and quantification of ARGs

Primer pair	Sequence	PCR annealing andextension temp (°C)	Amplicon size (bp)	References
sul1-F	CACCGGAAACATCGCTGCA	65	158	Luo et al., 2010
sul1-R	AAGTTCCGCCGCAAGGCT			
sul2-F	CTCCGATGGAGGCCGGTAT	62	190	Luo et al., 2010
sul2-R	GGGAATGCCATCTGCCTTGA			
tetA-F	GCTACATCCTGCTTGCCTTC	60	210	Tamminen et al., 2010
tetA-R	CATAGATCGCCGTGAAGAGG			
tetB-F	CGAAGTAGGGGTTGAGACGC	60	192	Luo et al., 2010
tetB-R	AGACCAAGACCCGCTAATGAA			
tetC-F	TGCGTTGATGCAATTTCTATGC	60	335	Tamminen et al., 2010
tetC-R	GGAATGGTGCATGCAAGGAG			
tet(W)-FX	GAGAGCCTGCTATATGCCAGC	62	168	Luo et al., 2010
tet(W)-FV	GGGCGTATCCACAATGTTAAC			
tetM-F	GCAATTCTACTGATTTCTGC	60	186	Tamminen et al., 2010
tetM-R	CTGTTTGATTACAATTTCCGC			
qnrS-F	GTGAGTAATCGTATGTACTTTTGC	62	169	Guillard et al., 2011
qnrS-R	AAACACCTCGACTTAAGTCT			
erm(B)-91f	GATACCGTTTACGAAATTGG	58	364	Chen et al., 2007
erm(B)-454r	GAATCGAGACTTGAGTGTGC			
int1-F	GGCTTCGTGATGCCTGCTT	57	146	Luo et al., 2010
int1-R	CATTCCTGGCCGTGGTTCT			
16S-F	TCCTACGGGAGGCAGCAGT	60	466	Nadkarni et al., 2002
16S-R	GGACTACCAGGGTATCTAATCCTGTT			

References

Chen J, Yu Z, Michel FC, Wittum T, Morrison M.,2007.Development and application of real-time PCR assays for quantification of erm genes conferring resistance to macrolide-lincosamidesstreptogramin B in livestock manure and manure management systems. Appl Environ Microbiol, 14, 4407–4416.

Guillard, T., Moret, H., Brasme, L., Carlier, A., Vernet-Garnier, V., Cambau, E., de Champs, C., 2011. Rapid detection of qnr and qepA plasmid-mediated quinolone resistance genes using real-time PCR. Diagno. Micr. Infec. Dis. 70, 253-259.

Luo, Y., Mao, D., Rysz, M., Zhou, Q., Zhang, H., Xu, L., JJ Alvarez, P., 2010. Trends in antibiotic resistance genes occurrence in the Haihe River, China. Environ. Sci. Technol. 44, 7220-7225.

Nadkarni, M. A., Martin, F. E., Jacques, N. A., Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148, 257-266.

Tamminen, M., Karkman, A., Lõhmus, A., Muziasari, W. I., Takasu, H., Wada, S., et al., 2010. Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environ. Sci. Technol. 45, 386-391.

Table S6 Environmental factors in multipal water bodies of Beijing

Sampling sites	NH3-N	COD	TP	TN	NO3-N	TOC
WYH1	1.57	17.03	0.42	0.04	10.76	10.86
WYH2	9.18	17.83	2.42	4.15	3.67	79.82
WYH3	1.85	17.11	0.73	16.63	9.58	14.34
WYH4	1.83	16.73	0.67	13.23	8.96	17.30
WYH5	1.83	16.65	0.44	12.60	9.18	15.40
WYH6	1.82	17.24	0.46	11.44	8.95	17.74
WYH7	1.88	16.48	1.42	8.62	8.94	15.82

G1	0.62	15.67	0.09	1.30	0.28	1.17
G2	0.01	14.23	0.51	1.26	0.96	1.47
G3H	0.32	15.80	0.10	1.18	0.26	2.04
G3M	1.03	13.76	0.15	1.48	0.83	22.55
G3L	1.98	14.44	0.10	21.64	16.18	4.46
G4	0.35	13.59	0.13	0.44	0.15	5.38
G5	1.02	14.31	0.13	0.42	0.15	17.17
CW1	13.45	668.81	1.53	54.98	-0.60	135.48
CW2	1.05	15.97	0.31	5.80	5.67	7.97
HW1	21.66	675.18	3.99	59.54	11.15	52.31
HW2	0.20	15.50	0.58	15.47	13.81	3.79
HW3	0.20	15.75	0.30	12.02	13.50	3.73
MW1	32.11	698.53	23.66	47.05	26.37	393.20
MW2	9.18	17.20	1.24	16.59	0.67	118.70
MW3	0.19	16.31	0.01	23.30	13.60	80.49
YY1	24.65	724.01	5.22	91.47	22.08	1140.70
YY2	20.32	700.66	3.99	68.54	7.41	1040.10