Supplementary Information

Simultaneous Determination of Seven Antibiotics and Five of Their Metabolites in Municipal Wastewater and Evaluation of Their Stability under Laboratory Conditions

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Table S1. The selected analytes in this study.

Groups	Parent Antibiotics	Deuterated analogs	Main Human Metabolite	Deuterated analogs	Reference
Sulfonamides	Sulfapyridine	Sulfapyridine-d_4	N^4-Acetyl Sulfapyridine	N^4-Acetyl Sulfapyridine-d_4	[1]
	Sulfamethoxazole	Sulfamethoxazole-d_4	N^4-Acetyl Sulfamethoxazole	N^4-Acetyl Sulfamethoxazole-d_4	[1]
Macrolides	Roxithromycin	Roxithromycin-d_7	N-Demethyl Roxithromycin	Roxithromycin-d_7	[2]
	Azithromycin	Azithromycin-d_3	Descladinose Azithromycin	Azithromycin-d_3	[3]
	Clarithromycin	Clarithromycin-N-d_3	—	—	—
Others	Trimethoprim	Trimethoprim-d_3	4-Hydroxy Trimethoprim	4-Hydroxy Trimethoprim-d_9	[1]
	Lincomycin	Roxithromycin-d_7	—	—	—

[1] Petrie, B., Barden, R., Kasprzyk-Hordern, B., A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res. 2015, 72, 3-27.

[2] Li, X. Q., Zhong, D. F., Huang, H. H., Wu, S. D., Demethylation metabolism of roxithromycin in humans and rats. Acta Pharmacologica Sinica. 2001, 22, 469-474.

[3] Luke, D. R., Foulds, G., Disposition of oral azithromycin in humans. Clinical Pharmacology & Therapeutics. 1997, 61, 641.

Table S2. The chemical characteristics of SPE cartridges used in this study.

SPE cartridge	Abbreviation	Sorbent material	Surface area (m^2/g)	Pore size (A^o)	Particle size (μm)
Cleanert ® PEP (3 mL, 60 mg)	PEP	Polystyrene and divinylbenzene	700	80	30
Oasis® MCX (3 mL, 60 mg)	MCX	N-vinylpyrrolidone and divinylbenzene bonding with sulfonate group	786	84	31.9
Oasis® HLB (3 mL, 60 mg)	HLB	Hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene	810	83	29.8

Table S3. Optimized instrument and MRM conditions of target analytes.

Antibiotics	Dwell time (s)	Parent ion (m/z)	Cone voltage (V)	Product ion (m/z)	Collision energy (eV)	Retention time (min)	Internal standard	IDL (ng/mL)	IQL (ng/mL)
SPY	0.004	250.1	35	92.0*	25	1.58	SPY-d_4	0.004	0.01
				156.0	15				
N-SPY	0.004	292.0	16	133.5	22	1.79	N-SPY-d_4	0.02	0.06
				197.7*	14				
SMX	0.004	254.1	30	92.0*	25	2.03	SMX-d_4	0.007	0.02
				156.0	15				
N-SMX	0.003	296.0	25	133.6*	20	2.33	N-SMX-d_4	0.02	0.06
				198.0	20				
CTM	0.016	748.5	30	158.0*	30	3.43	CTM-N-d_3	0.002	0.008
				590.2	20				
RTM	0.016	837.6	40	158.1*	35	3.47	RTM-d_7	0.004	0.01
				679.5	20				
N-RTM	0.016	823.5	14	143.7*	38	3.46	RTM-d_7	0.003	0.009
				665.5	22				
ATM	0.003	749.5	30	158.2	40	2.61	ATM-d_3	0.01	0.03
				591.5*	30				
Des-ATM	0.004	591.5	62	115.5	34	1.77	ATM-d_3	0.07	0.2
				157.7*	30				
LIN	0.005	407.2	40	126.1*	25	1.83	RTM-d_7	0.02	0.06
				359.3	20				
TMP	0.004	291.3	30	123.0*	30	1.78	TMP-d_3	0.009	0.03
				230.0	30				
4-H-TMP	0.004	307.0	26	138.6*	16	1.89	4-H-TMP-d_9	0.003	0.01
				180.7	18				
SPY-d_4	0.004	253.9	30	112.0	22	1.58	-	-	-
SMX-d_4	0.004	257.9	36	159.7	14	2.03	-	-	-
N-SPY-d_4	0.004	296.0	8	137.6	22	1.79	-	-	-
N-SMX-d_4	0.003	300.2	45	137.0	25	2.33	-	-	-
CTM-N-d_3	0.005	751.6	6	160.7	30	3.43	-	-	-
RTM-d_7	0.016	844.7	52	157.7	34	3.47	-	-	-
ATM-d_3	0.003	752.7	46	594.6	28	2.61	-	-	-
TMP-d_3	0.004	294.1	4	229.9	24	1.78	-	-	-
4-H-TMP-d_9	0.004	316.1	2	138.6	20	1.89	-	-	-

* Product ion was used for quantification

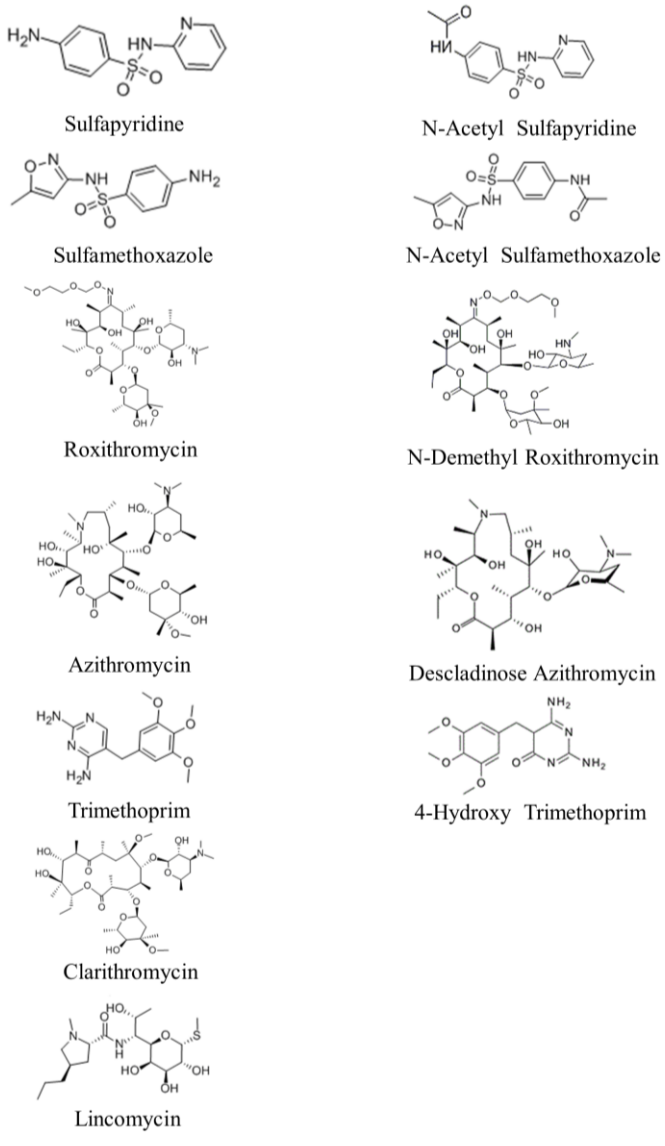

Figure S1. The structures of the selected analytes in this study.

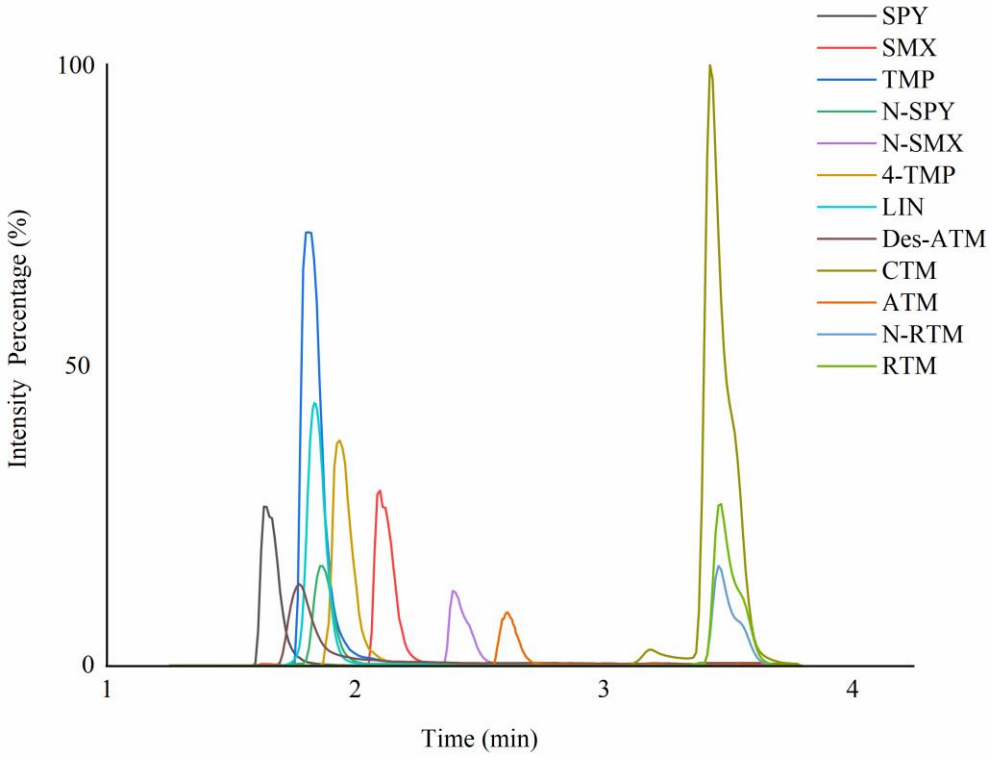

Figure S2. Total-ion chromatogram of the selected analytes prepared in methanol/ultrapure water (1:1) under mobile phase: 0.2% formic acid with 2 mM ammonium acetate in ultrapure water and acetonitrile (Conbination 1). The concentration of the analytes was 50 μg/L.

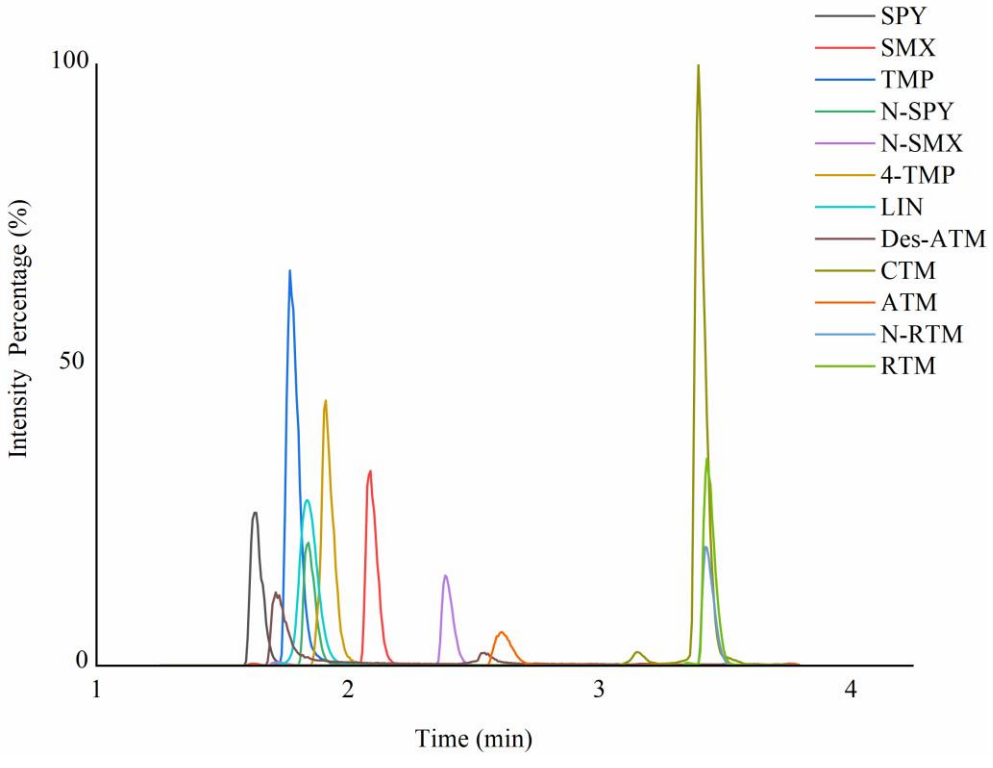

Figure S3. Total-ion chromatogram of the selected analytes prepared in methanol/ultrapure water (1:1) under mobile phase：ultrapure water containing 0.1% formic acid and 0.1% formic acid in methanol and acetonitrile (1:1,v/v) (Conbination 2). The concentration of the analytes was 50 μg/L.

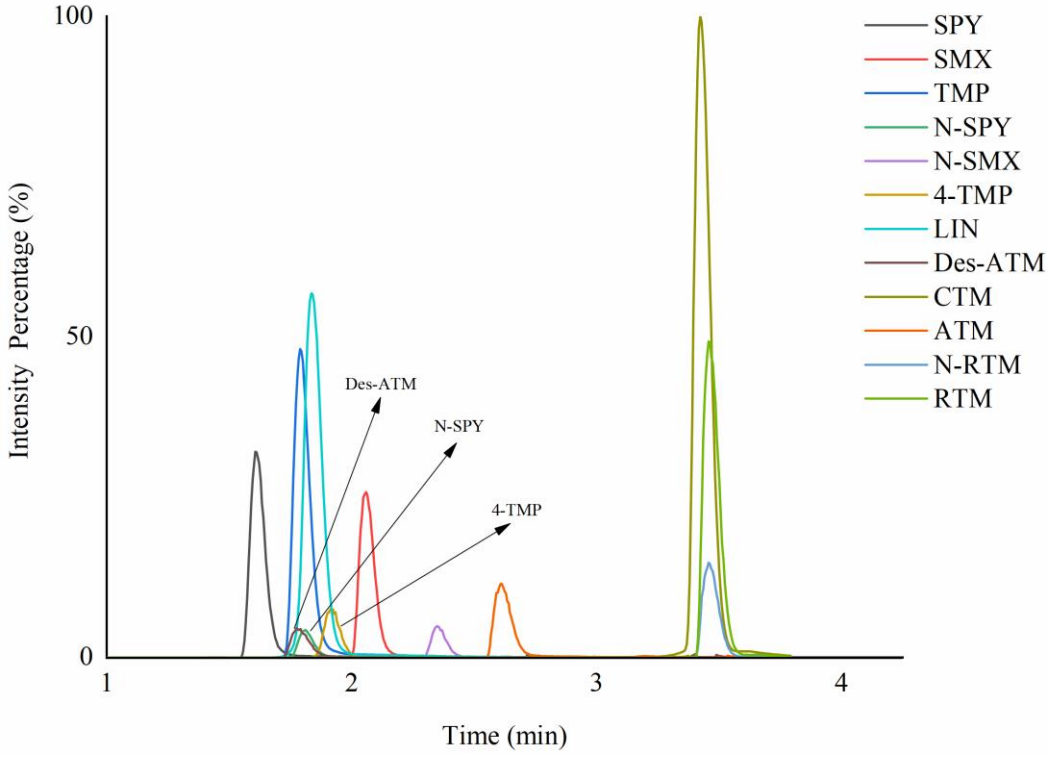

Figure S4. Total-ion chromatogram of the selected analytes prepared in methanol/ultrapure water (1:1) under mobile phase: 0.1% formic acid with 10 mM ammonium acetate in ultrapure water and 0.1% formic acid in methanol (Conbination 3). The concentration of the analytes was 50 μg/L.