

Supplementary material for

Combined effects of sulfamethoxazole and erythromycin on a freshwater

microalga, *Raphidocelis subcapitata*: toxicity and oxidative stress

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SPE methods and processes

The collected samples were diluted to 50 mL with culture medium, and each sample was spiked with 30 ng of erythromycin-13C-d3 and Sulfamethazine-d4, as the internal surrogate standard. Samples were extracted by pressurized liquid extraction using solid phase extraction unit (Shanghai Nai Precision Instrument Co., Ltd.) Placed the Oasis® HLB solid phase extraction cartridge (3cc, 60 mg, Waters, USA) on the extraction unit and then activated with 3 mL of methanol and 3 mL of ultrapure water. The sample solution was passed through the cartridge at flow rate of 5–10 mL/min, rinsed the beakers by 10 mL ultra-pure water after samples were all passed through the cartridges, and then evacuated for one hour. After that, the analyte was eluted into the test tube four times with methanol, 3 mL each time. Under a 37°C water bath, dry under a nitrogen stream. The samples of different concentration were reconstituted with Different amounts of methanol. Then Added different volume of Atrazine-d5 internal standards (IS) to these groups, and made the concentration of Atrazine-d5 in each sample is 20 ng/mL. Mixed well and filtered with 0.22 µm micron nylon membrane. The sample solution was then transferred to an autosampler vial (with insert) for LC/MS/MS analysis.

Supplementary Table 1. HPLC program on the condition of positive electrospray ionization.

Column	Agilent ZORBAX Eclipse Plus C18 HPLC column (3×100 mm, 1.8 μm)		
Mobile phase	A: ultrapure water with 0.1% formic acid (v/v)		
	B: Acetonitrile		
Column Temperature	24°C		
Injection volume	10 μL		
Flow rate	0.3 mL/min		
Gradient	Time (min)	A (%)	B1(%)
	0.00	92.5	7.5
	1.00	92.5	7.5
	3.00	88.0	12.0
	4.50	80.0	20.0
	6.00	40.0	60.0
	9.00	10.0	90.0

Supplementary Material

10.00

10.0

7.5

11.00

92.5

7.5

Supplementary Table 2. Optimized retention time, ion transitions, ion transitions, collision cell exit potential for MS/MS determination of target antibiotic.

Compound	Retention time (min)	Ion transitions (m/z)	Collision energy (eV)	Collision cell exit potential (V)
Sulfamethoxazole	7.06	254 > 155.9	80	15
		254 > 108		25
Sulfamethazine-d4	7.073	283.1 > 124.2	124	30
		283.1 > 186.1		20
Atrazine-d5	8.579	221.0 > 101.0	113	30
		221.0 > 137.0		25
		221.0 > 179.0		20
Erythromycin	7.400	734.5 > 158.2	140	30
		734.5 > 576.4		20
Erythromycin-13C-d3	7.151	738.6 > 162.0	146	34
		738.6 > 582.5		19

Supplementary Table 3. Actual concentration of SMX and ERY in algal medium (mg/L).

SMX		ERY		Mixture (SMX: ERY=10:1)	
Nominal concentration	Actual concentration	Nominal concentration	Actual concentration	Nominal concentration	Nominal concentration
0	0.001	0	0	0	0
0.1	0.098	0.01	0.011	0.01+0.001	0.011+0.0012
0.3	0.292	0.03	0.027	0.03+0.003	0.029+0.0029
0.5	0.511	0.05	0.049	0.05+0.005	0.049+0.0052
0.7	0.681	0.07	0.072	0.07+0.007	0.069+0.0071
0.9	0.934	0.09	0.091	0.09+0.009	0.088+0.0101