

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 |