

Supporting Information

Optimization of the determination method for dissolved cyanobacterial toxin BMAA in natural water

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1. The details of the optimization of the SPE process.

Five kinds of cation-exchange sorbents, Oasis-MCX (6cc, 500mg, Waters, USA), Strata-X-C (6cc, 500mg, Phenomenex, USA), Bond ElutPlexa PCX (6cc, 500mg, Agilent, USA), DSC-MCAX (3cc, 500mg, Supelco, USA) and LC-SCX (3cc, 300mg, Supelco, USA) SPE cartridges, were used to concentrate the target compounds and the recoveries of spiked BMAA samples treated by these five cartridges were compared. The cartridges were activated with 10mL of methanol followed by 10mL of 2% formic acid. Then, 100mL of sample containing 20mM HCl was loaded onto the cartridge. After the percolation of sample, the cartridge was rinsed with 10mL of 2% formic acid and 10mL of methanol. Finally, the targets were eluted by 10mL of 5% of $\text{NH}_3 \cdot \text{H}_2\text{O}$. The filtrate during the sample loading and the washing processes was collected and analyzed, and the results could reveal the loss mechanism of BMAA during the SPE process.

The performances of SPE with different combinations of sample loading rate and elution rate were compared. This experiment was performed with Oasis-MCX cartridge at different levels of sample loading and elution rates, 1 mL/min, 1.85 mL/min and 3 mL/min for sample loading rates and 1 mL/min and 3 mL/min for eluting rates. The other conditions were the same as that in the experiment of SPE cartridge selection.

The adjustment of elution conditions was conducted by changing the constitute (2%, 3% and 5% of $\text{NH}_3 \cdot \text{H}_2\text{O}$ in methanol) and the volume (1mL, 3mL, 5mL, 7.5mL and 10mL) of eluents. The method that provided the best recovery of BMAA with the lowest consumption of solvent was adopted in the following experiments.

After SPE concentration, the eluents should be evaporated under N_2 . Four kinds of conditions (room temperature, 55°C, 80°C and vacuum) were used to compare with the recoveries of spiked BMAA samples. For the underivatized LC/MS/MS methods, the drying fraction was reconstituted by 1mL of a mixture of acetonitrile containing 0.1% formic acid and water containing 0.1% formic acid (85/15, v/v), and D_5DAB was added as the internal standard at a final concentration of 350 $\mu\text{g/L}$. For the

derivatized LC-MS/MS analysis, the samples were treated according to AQC standard derivatized procedure as described by Cohen,¹ and D₅DAB was added before the derivatization at a final concentration of 200 µg/L.

All reconstituted samples were filtered through 0.22 µm membrane and transferred to an auto-sampler vial, and then 10 µL were injected into the LC-MS/MS system. In order to reduce the losses of filtration, the rejections of BMAA by five kinds of membranes, PTFE, PVDF, PES, NY and GF, were compared.

REFERENCE

1. Cohen, S. A., Amino Acid Analysis Using Precolumn Derivatization with 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate. *Amino Acid Analysis Protocols* **2000**, 159, 39-47.

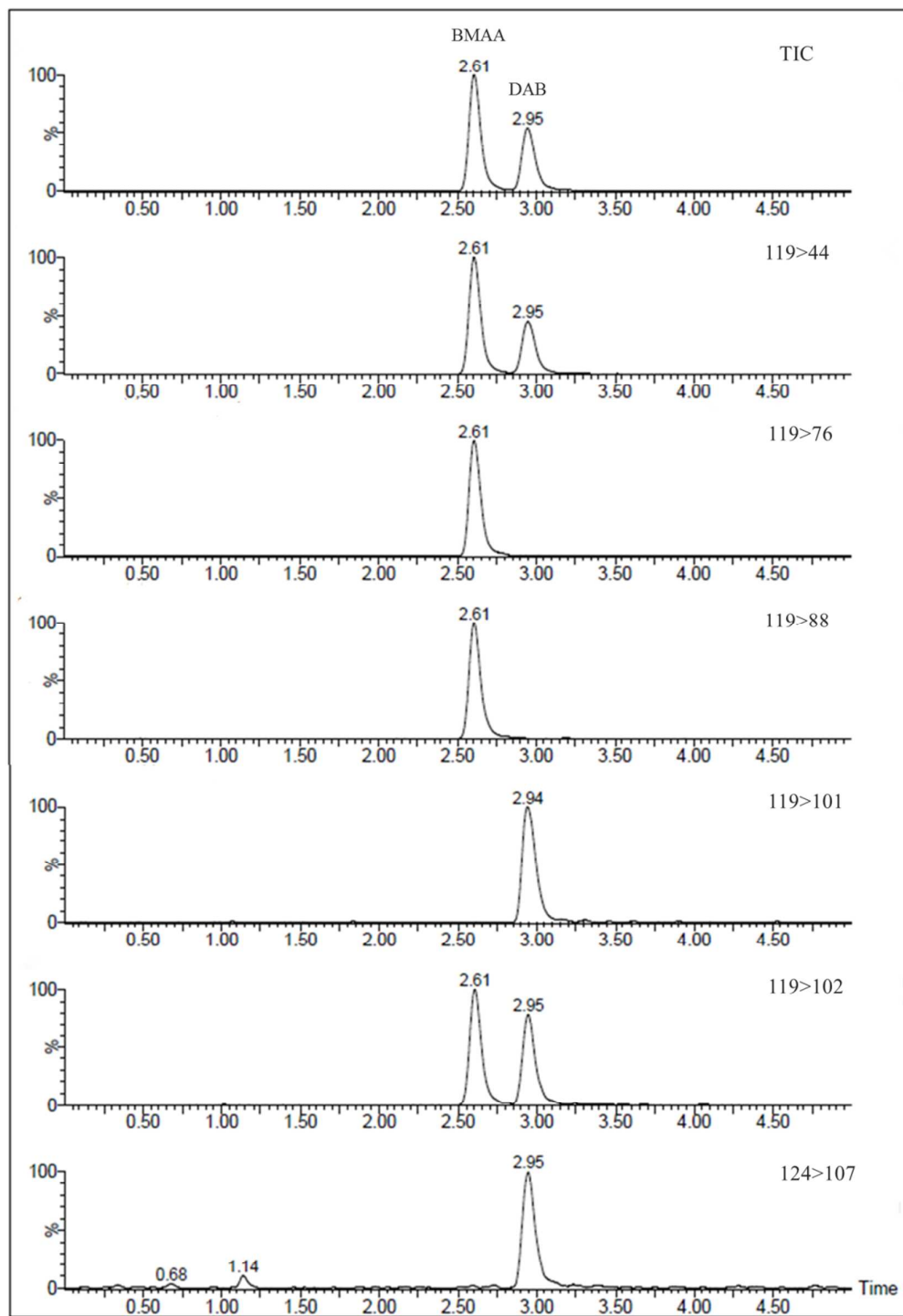


Fig S1. LC-MS/MS chromatograms for BMAA and DAB in a mixed standard
(100µg/L)

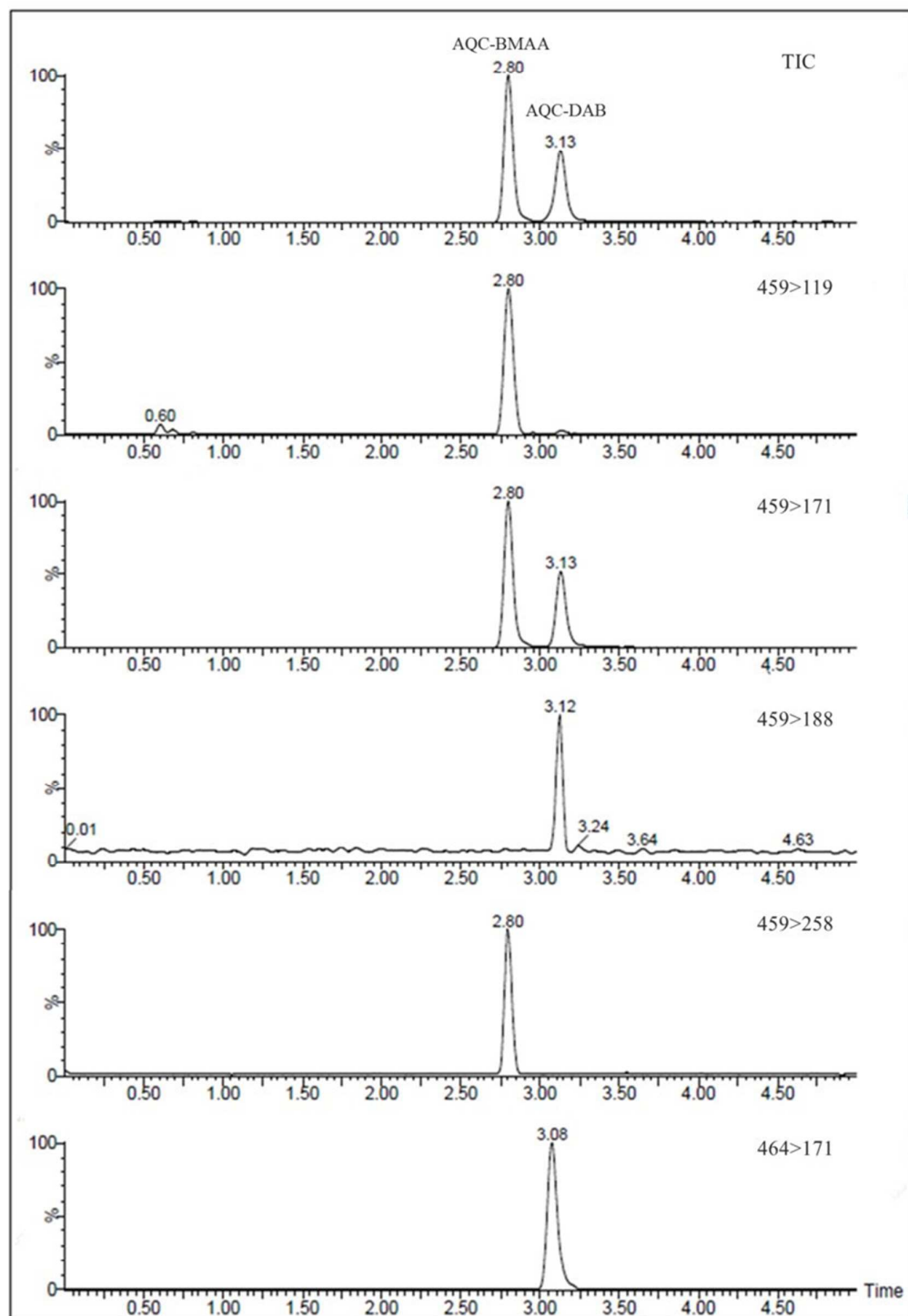


Fig S2. LC-MS/MS chromatograms for AQC-BMAA and AQC-DAB in a mixed standard (50µg/L)

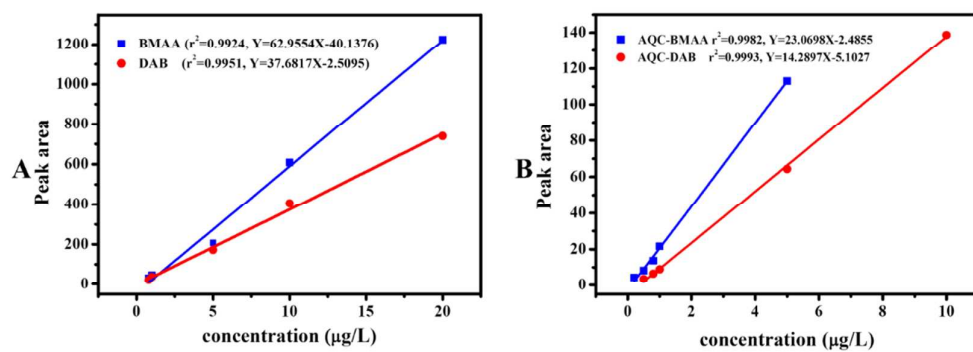


Fig. S3 Standard working curves of BMAA and DAB created with the underderivatization method (A) and derivatization method (B).

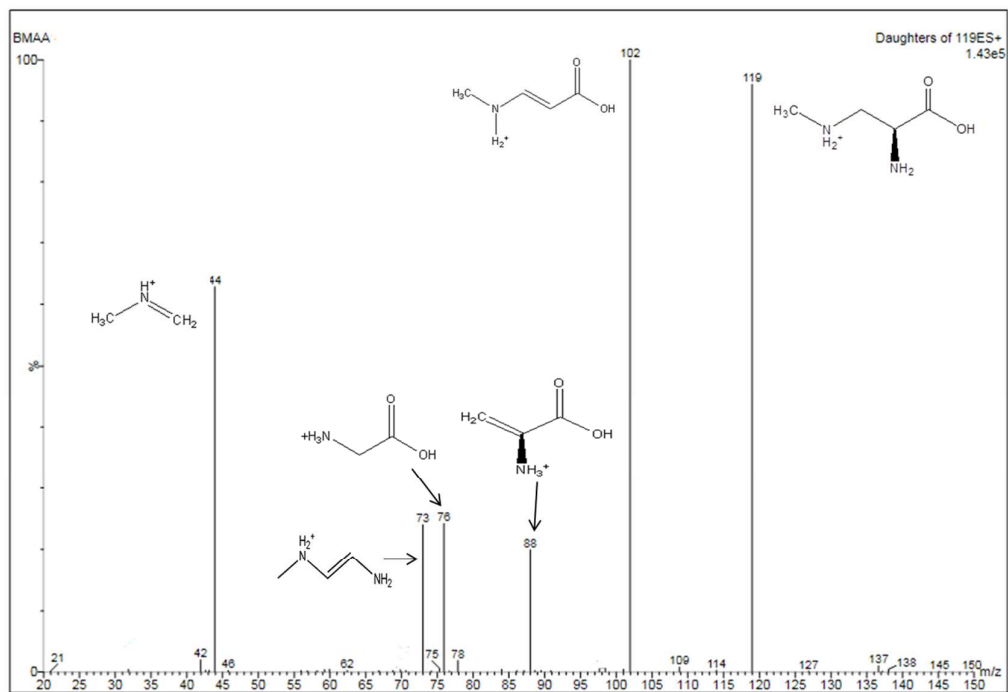


Fig S4. Spectrogram and daughter ions of BMAA

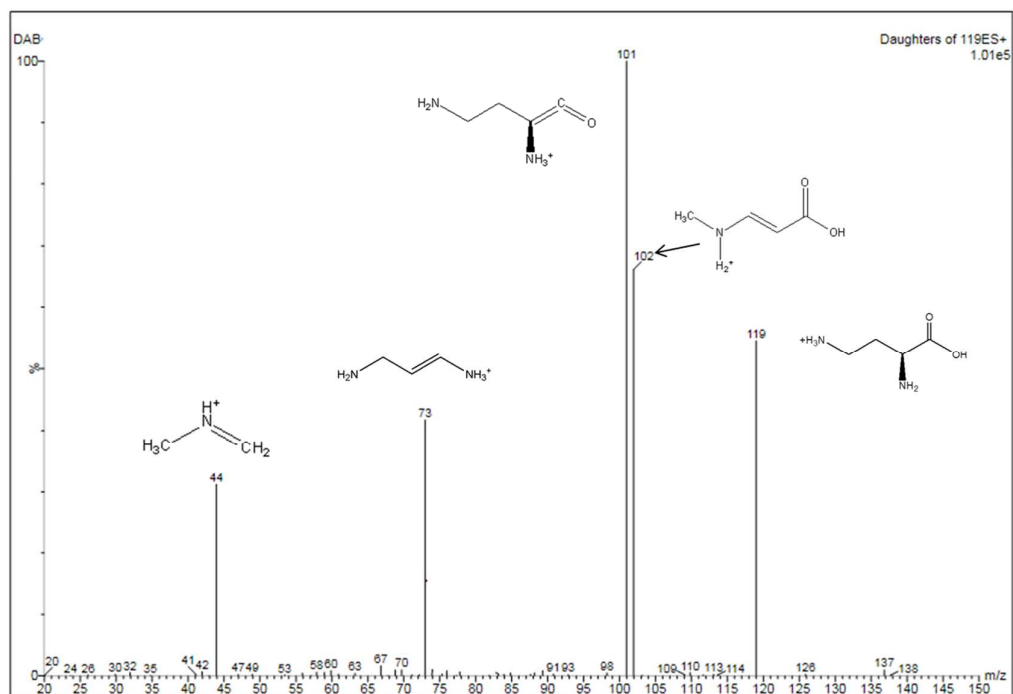


Fig S5. Spectrogram and daughter ions of DAB