Ultra-fast analysis of anatoxin-A using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry: validation and resolution from phenylalanine

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Table of contents

Page S-3, Figure S-1: Variability of the corona discharge current (µA) at constant corona discharge voltage (V).

Page S-3, Figure S-2: Effect of solvent pH on ANA-a analysis by LDTD-APCI-MSMS. (n = 3, SRM m/z 166.1 → 149.1)

Page S-4, Figure S-3: Degradation assessment of ANA-a under experimental conditions. (pH 11.5, n = 3, SRM m/z 166.1 → 149.1)

Page S-5, Figure S-4: Effect on ANA-a signal intensity/variability of analyte deposition solvent. (n = 8, SRM m/z 166.1 → 149.1)

Page S-5: Explanation of the 500 µg/L PHE concentration associated with a dense cyanobacterial bloom.

Page S-7, Figure S-5: Atrazine, caffeine, 17α-ethinylestradiol and CLO structures and corresponding M.W.s.

Page S-8, Figure S-6: Desorption profile of bloom matrix blank and Ana-a 1 µg/L in bloom matrix for SRM m/z 166.1 → 131.1 (a,b) and SRM m/z 166.1 → 149.1 (c,d)

Page S-9, Figure S-7: Matrix effect assessment for an external calibration with optimal conditions for Ana-a analysis (SRM m/z 166.1 → 149.1, n = 3, pH 11.5, 50 % MeOH with laser power at 20 %). Vertical error bars represent standard deviations from the mean.

Page S-10, Table S-1: Evaluation of CLO (10 µg/L) as an IS (n = 8, SRM m/z 406.1 → 101.1 and 166.1 → 149.1).
Figure S-1. Variability of the corona discharge current (µA) at constant corona discharge voltage (V).

Figure S-2. Effect of solvent pH on ANA-a analysis by LDTD-APCI-MSMS. (n = 3, laser power 20%, SRM m/z 166.1 → 149.1)
Figure S-3. Degradation assessment of ANA-a under experimental conditions. (pH 11.5, n = 3, laser power 20%, SRM m/z 166.1 → 149.1)
Figure S-4. Effect on ANA-a signal intensity/variability of analyte deposition solvent. (n = 8, SRM m/z 166.1 → 149.1)

Explanation of the 500 µg/L PHE concentration associated with a dense cyanobacteria bloom.

A qualitative measurement of phytoplankton abundance in a water body can be achieved by the Total Carbon : Chlorophyll $a$ ratio, (C (g/L) : chl $a$ (g/L)). This ratio evolves with changing conditions like sunlight, temperature and nutrient content. A ratio value of 30 is associated with eutrophic conditions, commonly associated with cyanobacterial blooms. The Total Carbon, and the protein content, are estimated to be about 50 % of the total phytoplankton biomass. This protein mass can be converted into a total amino acids concentration, with an average amino acid molar mass (135 g/mol). Generally, the PHE content in proteins (%) is around 3.5 %. The World Health Organization set a chl $a$ concentration > 50 µg/L, related to a toxic biomass of cyanobacteria in drinking water, as a very high risk level causing adverse health effects, and noted that
an average concentration of 300 $\mu$g/L chl $a$ is representative of cyanobacterial blooms in eutrophic conditions. With a starting value of 400 $\mu$g/L chl $a$, we can approximate a high PHE concentration generated in a bloom:

$$\text{Eq.1} \quad \frac{C(\mu g/L)}{\text{chl } a \ (\mu g/L)} = 30 = \frac{C(\mu g/L)}{400 \ \mu g/L} \quad \Rightarrow \quad C (\mu g/L) = 12000 \ \mu g/L$$

$$\text{Eq.2} \quad C (\mu g/L) = \text{proteins content (\mu g/L)} = 12000 \ \mu g/L$$

$$\text{Eq.3} \quad \frac{\text{proteins content (\mu g/L)}}{\text{average amino acid molar mass (g/mol)}} = \text{total amino acids concentration (\mu M)}$$

$$\frac{12000 \ \mu g/L}{135 \ \text{g/mol}} = 88.9 \ \mu M$$

$$\text{Eq.4} \quad \text{total amino acids concentration (\mu M)} \times \text{PHE relative abundance} = \text{PHE concentration (\mu M)}$$

$$88.9 \ \mu M \times 0.035 \text{ PHE} = 3.1 \ \mu M$$

$$\text{Eq.5} \quad \text{PHE concentration (\mu mol/L)} \times \text{PHE molar mass (g/mol)} = \text{PHE concentration (\mu g/L)}$$

$$3.1 \ \mu \text{mol/L} \times 165 \ \text{g/mol} = 513 \ \mu g/L$$
Figure S-5: Atrazine, caffeine, 17α-ethynylestradiol and CLO structures and corresponding M.W.
Figure S-6. Desorption profile of bloom matrix blank and Ana-a 1 μg/L in bloom matrix for SRM $m/z$ 166.1 → 131.1 (a,b) and SRM $m/z$ 166.1 → 149.1 (c,d)
Comparison of the ratio of bloom:solvent signal

<table>
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<tr>
<th></th>
<th>5 ppb</th>
<th>10 ppb</th>
<th>25 ppb</th>
<th>50 ppb</th>
<th>100 ppb</th>
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<tbody>
<tr>
<td>Peak area SRM m/z 166.1 → 149.1</td>
<td>0.600</td>
<td>0.616</td>
<td>0.614</td>
<td>0.537</td>
<td>0.518</td>
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Figure S-7. Matrix effect assessment for an external calibration with optimal conditions for Ana-a analysis (SRM m/z 166.1 → 149.1, n = 3, pH 11.5, 50 % MeOH with laser power at 20 %). Vertical error bars represent standard deviations from the mean.
Table S-1. Evaluation of CLO (10 µg/L) as an IS. (n = 8, SRM m/z 406.1 → 101.1 (CLO) and 166.1 → 149.1(ANA-a)).

<table>
<thead>
<tr>
<th>Replicate</th>
<th>ANA-a 25 µg/L Peak Area</th>
<th>CLO 10 µg/L Peak Area</th>
<th>Ratio (ANA-a/CLO)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1008096</td>
<td>1091913</td>
<td>0.92</td>
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<tr>
<td>2</td>
<td>1222030</td>
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<td>3</td>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<td>1.05</td>
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<td>Average</td>
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<td>1162412</td>
<td>0.99</td>
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<tr>
<td>Standard Deviation</td>
<td>100976</td>
<td>78808</td>
<td>0.048</td>
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<tr>
<td>% RSD</td>
<td>9</td>
<td>7</td>
<td>5</td>
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References


