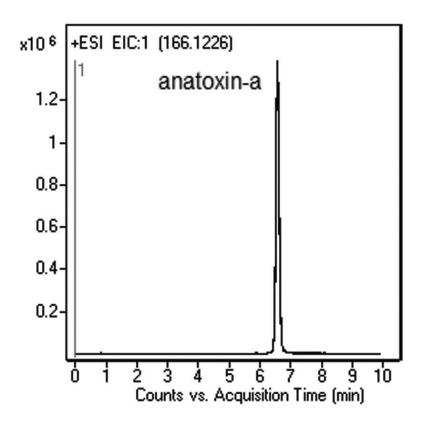


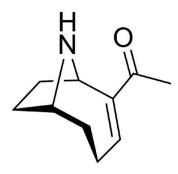
Anatoxin-a, ANTX-A Analyzed with LCMS - AppNote

Potent Neurotoxin Analyzed without Derivatization

Anatoxin-a or ANTX-A is a low molecular weight compound with a secondary amine which can be difficult to retain with most Reversed Phase HPLC Columns. This Application Note illustrates a very simple Method for Retention of Anatoxin-a which does not require pre-Column or post Column Derivatization as with other Methods.

The Retention achieved with this Method is good, and the Repeatability is demonstrated by the overlay of Five Chromatograms, shown in the *Figure* below. If desired, a lower retention time of Anatoxin-a can be obtained by changing the starting concentration of Solvent B in the gradient to 60%, designing a steeper gradient, or using a shorter Column length such as 2.1 x 50mm.





Peak:

Anatoxin-a, 166.1226 m/z (M+H)+

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: <u>70000-15P-2</u> **Dimensions**: 2.1 x 150mm

Mobile Phase:

A: 50% Methanol / 50% DI Water / 0.1% Formic Acid

B: Acetonitrile / 0.1% Formic Acid

Gradient:

Time (minutes)	%B
0	70
5	30
6	30
7	70

Temperature: 25°C
Post time: 5 minutes
Injection vol.: 1µL

Flow rate: 0.4mL / minute

Detection: ESI – POS - Agilent 6210 MSD TOF Mass Spectrometer

Sample Preparation:

Stock Solution: 0.5mg / mL of Anatoxin-a Fumarate was prepared in DI Water and stored at –20°C. Working Solution: Sample for injection was diluted 1:100 using 50:50 Solvent A / Solvent B mixture. It was stored in the dark at 4°C.

to: 0.9 minutes

Note: Anatoxin-a (ANTX-A) is a Cyanobacterial neurotoxin, implicated in many animal and human poisoning incidents. ANTX-A blocks neurotransmission causing death by respiratory arrest. The presence of this toxin in freshwater has to be monitored in order to prevent fatalities.



Attachment

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