

## Unique Method Development Strategy for Polar Compounds - AppNote

### Anatoxin-a Analyzed with LCMS

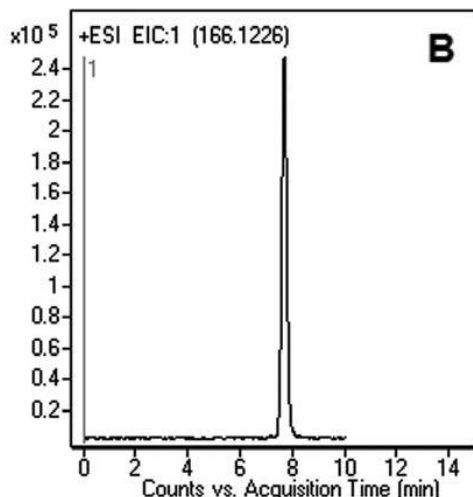
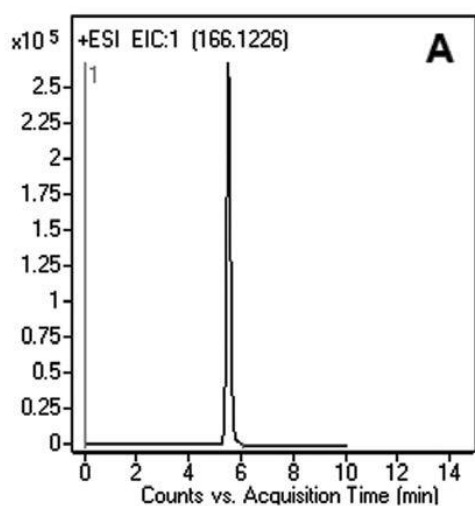
Figures below illustrate an example of the work flow in developing a Method for the Analysis of a Polar Compound using Cogent Diamond Hydride™ Columns and the impact of Aqueous Normal Phase HPLC.

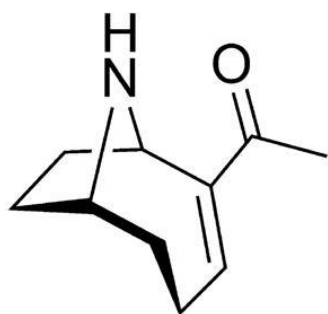
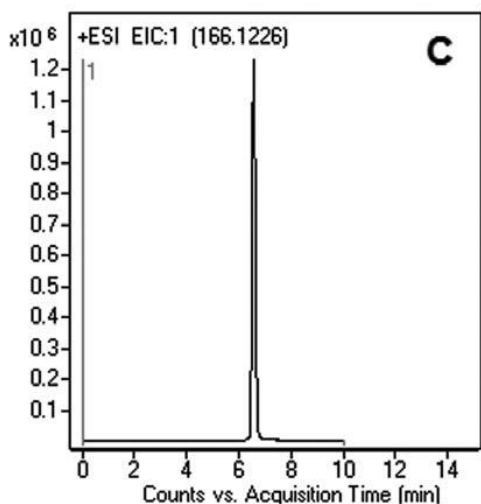
*The simplified steps of Method Development are as follows:*

A. Injection of the Sample at 50:50 Solvent A / Solvent B Mobile Phase Composition results in *Figure A*. In this case, Anatoxin-a has considerable retention.

B. Injection of the Sample at 40:60 Solvent A / Solvent B Mobile Phase Composition results in *Figure B*. As expected, the Retention of Anatoxin-a is longer and the Peak Shape is broader with higher organic content.

C. Based on the above results, a simple Linear Gradient is designed to achieve the desired Retention of the Compound and excellent Peak Shape (*Figure C*). If shorter Retention time is desired it can be accomplished by changing the starting concentration of Solvent B to 60%, designing a steeper gradient, or using a shorter Column such as 2.1 x 50mm.





### Peak:

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

### Method Conditions

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

**Catalog No.:** [70000-15P-2](#)

**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

**t<sub>0</sub>:** 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

**No 142 Method Development Strategy for Polar Compounds pdf** 0.3 Mb [Download File](#)

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