Unique Method Development Strategy for Polar Compounds - AppNote

Anatoxin-a Analyzed with LCMS

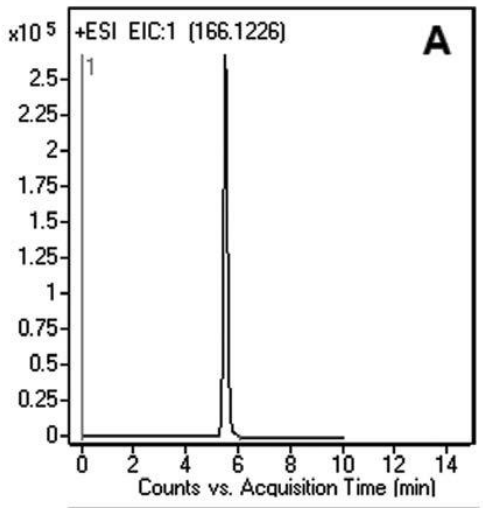
Figures below illustrate an example of the work flow in developing a Method for the Analysis of a Polar Compound using Cogent Diamond Hydride™ Columns and the impact of Aqueous Normal Phase HPLC.

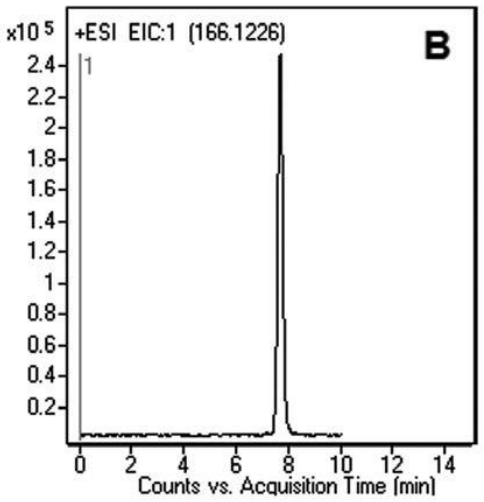
The simplified steps of Method Development are as follows:

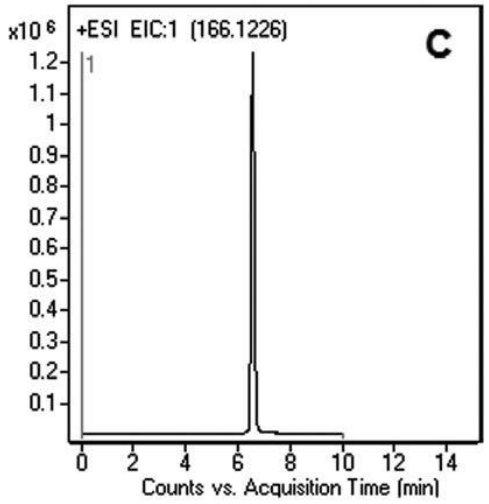
A. Injection of the Sample at 50:50 Solvent A / Solvent B Isocratic Mobile Phase Composition results in *Figure A*. In this case, Anatoxin-a has considerable retention.

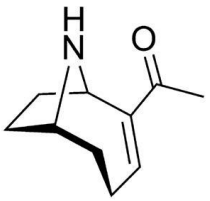
B. Injection of the Sample at 40:60 Solvent A / Solvent B Isocratic Mobile Phase Composition results in *Figure B*. As expected, the Retention of Anatoxin-a is longer and the Peak Shape is broader with higher organic content.

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)+

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_0 : 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.*

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