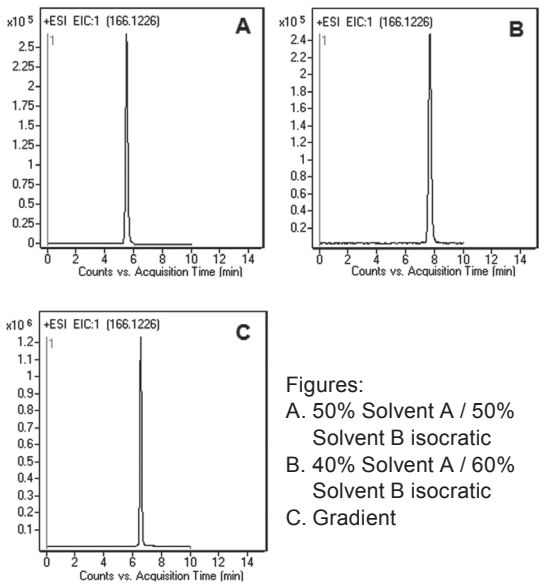
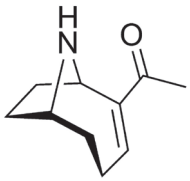
Method Development Strategy for a Polar Compound

Using Anatoxin-a



Figures:
A. 50% Solvent A / 50% Solvent B isocratic
B. 40% Solvent A / 60% Solvent B isocratic
C. Gradient



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.

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvents: A: 50% MeOH / 50% DI H_2O / 0.1% formic acid
B: Acetonitrile / 0.1% formic acid

Gradient:

time (min.)	%B
0	70
5	30
6	30
7	70

Temperature: 25˚C

Post time: 5 min

Injection vol.: 1µL

Flow rate: 0.4 mL/min

Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer

Peak: Anatoxin-a, 166.1226 m/z $(M+H)^+$

t_0: 0.9 min

Discussion

Figures A, B, and C illustrate the work flow in developing a method for analysis of polar compounds using Cogent TYPE-C™ columns. The steps of method development are as follows:

A. Injection at 50%A/50%B mobile phase composition (Figure A). In the case of Anatoxin-a, considerable retention is observed.
B. Injection at 60%B (Figure B). As expected, the retention of Anatoxin-a is longer and the peak shape is broader.
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 (2.1 x50 mm).

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