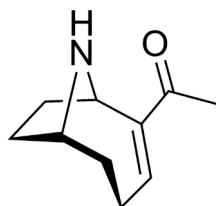


Method Development Strategy for a Polar Compound Using Anatoxin-a

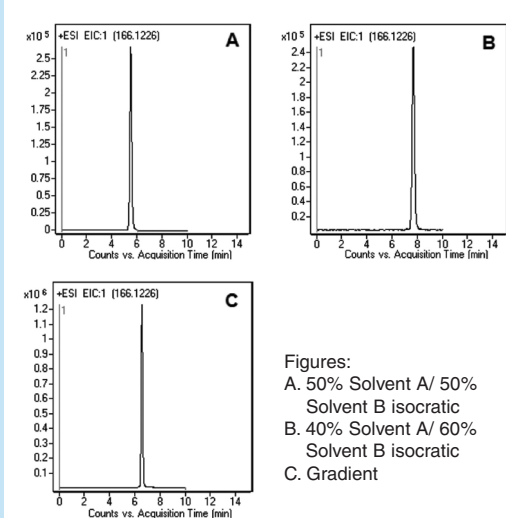


Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100A
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150 mm
Solvents: A: 50% MeOH/ 50% DI H₂O/ 0.1% formic acid
 B: Acetonitrile/ 0.1% formic acid
Gradient:

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

Post Time: 5 min
Injection Vol.: 1 µL
Flow Rate: 0.4 mL/min
Temperature: 25 °C
Detection: ESI – POS - Agilent 6210 MSD TOF MS
Peak: Anatoxin-a, 166.1226 m/z (M + H)⁺
t₀: 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).

For more information visit www.MTC-USA.com

Notes:
 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.

| Cat. No. | Description |
|-------------|---|
| 70000-15P-2 | Diamond Hydride™ HPLC Column, 100A, 4µm, 2.1 x 150 mm |