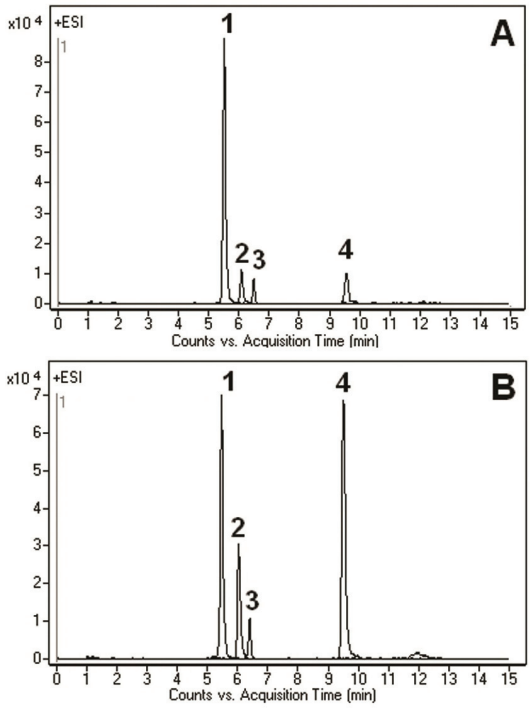
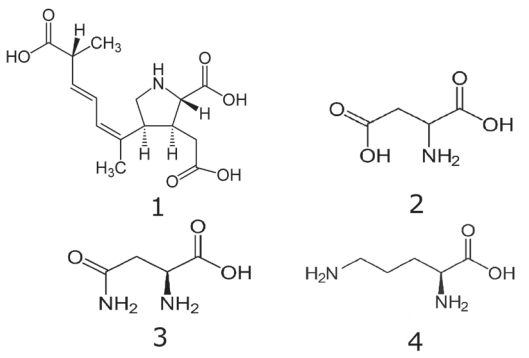
Domoic Acid, Amino Acids and Ornithine in Cell Extracts

LC-MS analysis without analyte derivatization





Note: Domoic acid, a compound known for some 30 years, was found to have neurological effects when ingested by humans. Upon ingestion, it causes diarrhea, dizziness, seizures, permanent loss of short-term memory and sometimes death.

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

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

Gradient:

time (min.)	%B
0	95
7	20
10	20
11	95

Post Time: 5 min

Injection vol.: 1µL

Flow rate: 0.4 mL/min

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

Sample: Methanolic extracts of proprietary cell cultures A and B, isolated by filtration

Peaks: 1. Domoic acid 312.1442 m/z $(M+H)^+$
2. L-aspartic acid 134.0453 m/z $(M+H)^+$
3. L-asparagine 133.0608 m/z $(M+H)^+$
4. Ornithine 133.0972 m/z $(M+H)^+$

t_0: 0.9 min

Discussion

The potent toxin domoic acid (DA) is a highly polar compound which is poorly retained on most reversed phase columns. When a Cogent Diamond Hydride column was used, not only DA was retained but also two isobaric compounds (L-asparagine and ornithine) were retained and separated. In addition, the peak obtained for aspartic acid was very symmetrical. From these chromatograms, it is clear that the two cell extracts show differences in the ratios between the analyzed compounds (Figure A vs. B). The presented method can be used in studies of cell extracts. In addition, it is robust and reproducible. Cogent columns require a very short equilibration time between gradient runs.The developed method based on ANPHPLC and MS detection does not require derivatization of DA, amino acids or ornithine.

MANUFACTURED BY:
MICROSOLV TECHNOLOGY CORPORATION
9158 Industrial Blvd NE
Leland, NC 28451
p: 1.732.380.8900
f: 1.910.769.9435
customers@mtc-usa.com
www.Cogent-HPLC.com