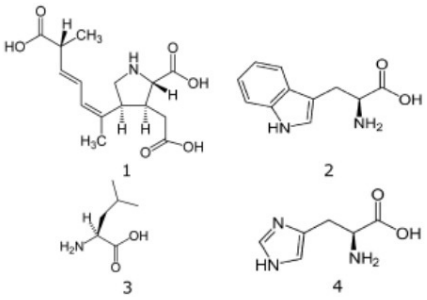
COGENT Diamond Hydride™

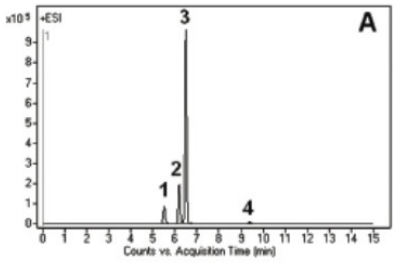
HPLC APPLICATION NOTE

ANP

Domoic Acid (DA) & Amino Acids in Algae Extracts

Low level analysis without analyte derivatization





Note: Toxic pseudo-nitzschia australis produces the potent neurotoxin domoic acid. In 1998, a widespread bloom of these algal species affected the central California coastline and resulted in the mass death of over 400 sea lions. The animals died by the ingestion of shellfish (e.g. mussels) that have fed upon diatom blooms that include toxic species of pseudo-nitzschia.

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.: 1 µL

Flow rate: 0.4 mL/min

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

Sample: Methanolic extracts of *pseudo-nitzschia australis* diatom cultures 1 and 2, isolated by filtration.

Peaks: 1. Domoic acid 312.1442 m/z $(M+H)^+$
2. L-Tryptophan 205.0972 m/z $(M+H)^+$
3. L Leucine 132.1025 m/z $(M+H)^+$
4. L-Histidine 156.0773 m/z $(M+H)^+$

t_0: 0.9 min

Discussion

This LC-MS method can achieve very low detection levels of DA and amino acids without the need for analyte derivatization. Figures A and B show the chromatograms of both algae extracts. Figure C shows a zoom-in overlay of both cultures 1 and 2 for the histidine peak.

The data show how the tryptophan levels are significantly different between the two cultures whereas the other levels are comparable. The method also produces sufficient separation of DA from tryptophan, which is often an interferent in DA quantitation.

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