

Food and Drug Laboratory Branch

FDLB Method: 17 (Rev.8) (02/12/2014)
Commodity: Seafood
Sample: Crabs, mussels, clams, fish
Analyte: Domoic acid
Reference: J.S. Dhoot, A.R. Del Rosario, B.R. Appel and B.R. Tamplin, "An Improved HPLC Procedure for Domoic Acid Analysis in Seafood," Intern. J. Environ. Anal. Chem., Vol.53, pp.261-268 (1993).

1.0 Principle:

Samples are extracted with aqueous methanol. The extracts are diluted with acetonitrile/water and then analyzed by HPLC with an on-line cyano pre-column cartridge and diode array detection.

2.0 Apparatus and Materials:

- 2.1 Agilent LC equipment consisting of the following (an equivalent system maybe substituted):
 - 2.1.1 Agilent 1100 Quaternary Pump (G1311A)
 - 2.1.2 Agilent 1100 Diode Array Detector (G1315A)
 - 2.1.3 Agilent 1100 Autosampler (G1313A)
 - 2.1.4 Agilent 1100 Column Compartment (G1316A)
 - 2.1.5 Agilent 1100 Degasser (G1322A)
- 2.2 Data System: A computerized data collection and processing system is highly recommended. Ex. Agilent Chemstation
- 2.3 Analytical balance capable of weighing to the nearest 0.1 mg
- 2.4 Top Loading balance capable of weighing to the nearest 0.1 g
- 2.5 Brinkman Polytron Model PT 3000 Homogenizer or equivalent
- 2.6 Centrifuge
- 2.7 Heat source
- 2.8 Cooking pot
- 2.9 Blender
- 2.10 Spatula
- 2.11 Timer (stopwatch)

- 2.12 Trays (stainless steel or plastic)
- 2.13 Tube racks
- 2.14 Centrifuge tubes, graduated
- 2.15 Volumetric flasks, assorted sizes
- 2.16 Graduated cylinders
- 2.17 Beakers
- 2.18 Wash bottles
- 2.19 Pasteur pipettes
- 2.20 Micropipettors with tips
- 2.21 Volumetric pipettes, assorted sizes
- 2.22 Autosampler vials
- 2.23 0.45 um filters
- 2.24 Plastic containers with caps
- 2.25 pH meter

3.0 Reagents:

- 3.1 Domoic acid standard (National Research Council of Canada)
- 3.2 Methanol (HPLC Grade)
- 3.3 Acetonitrile (HPLC Grade)
- 3.4 Orthophosphoric acid (85%)
- 3.5 Deionized water

4.0 Calibration Standard:

- 4.1 Stock standard solution: The concentration of the domoic acid standard obtained from National Research Council of Canada varies from lot to lot [~ 100 ug/mL in acetonitrile/deionized water (1:19)].
- 4.2 Working calibration standard solutions: Prepare 0.25, 0.5, 1, 2.5, 5, 10, 20, and 50 ug/mL calibration standard solutions as follows:
 - 4.2.1 Prepare 2.5, 5, 10, 20, and 50 ug/mL by appropriate dilution of the stock standard from Section 4.1 using acetonitrile/deionized water (1:9) as diluent.
 - 4.2.2 Prepare 0.25, 0.5, and 1 ug/mL by appropriate dilution of the 5 ug/mL solution from Section 4.2.1 using

acetonitrile/deionized water (1:9) as diluent.

5.0 Procedure:

5.1 Sample Preparation:

Notes: Group samples by type (e.g., crabs, mussels, clams, anchovies) and sampling site.

Crabs are analyzed as individual samples; all other sample types are analyzed as pooled samples from individual sites.

Crabs should be cooked before analysis. As many batches of crab samples as is feasible should be cooked on one day. The samples should be refrigerated until ready for evisceration.

5.1.1 Crab Samples

5.1.1.1 Cooking of Crab Samples

- 5.1.1.1.1 Weigh individual crabs and record weight
- 5.1.1.1.2 Use a kitchen knife to remove crab legs and claws
- 5.1.1.1.3 Wrap each specimen (crab body without legs or claws) individually in aluminum foil to prevent leakage during steaming and label samples
- 5.1.1.1.4 Place crab body upside-down in steamer so that fluids will collect inside the shell
- 5.1.1.1.5 Fill tank with tap water up to point beneath baskets containing specimens so that specimens will be steamed and not immersed in water
- 5.1.1.1.6 Place wrapped crab specimens into steam baskets, then place in tank after the water is boiling
- 5.1.1.1.7 Close lid to tank and steam for 15-20 minutes for fresh samples or 20-30 minutes if crab is frozen
- 5.1.1.1.8 After steaming, remove specimens and unwrap aluminum foil carefully after cooling
- 5.1.1.1.9 Remove viscera using metal spatula and place into 150 mL or 250 mL labeled plastic container and record weight
Note: If not ready for extraction, keep the viscera in the

refrigerator.

5.1.1.1.10 Homogenize viscera by mixing with spatula in container. Weigh $4.0 \pm 0.1g$ for the extraction. If the viscera are not homogenized well, use a blender (Cuisinart Mini-Prep Processor Model DLC-1SS or equivalent).

5.1.1.1.11 Archive body and leg meat and remaining viscera in $-20^{\circ}C$ freezer, or prepare body meat and leg meat as below

Note: If the viscera contain high domoic acid, then the homogenized crabmeat maybe analyzed.

5.1.1.2 Crab Body Meat

5.1.1.2.1 Rinse cooked body with tap water to remove any remaining viscera

5.1.1.2.2 Drain water from the body and use paper towels to absorb excess water

5.1.1.2.3 Remove body meat from shell into a 150 mL labeled plastic container

5.1.1.2.4 Homogenize body meat by mixing with a blender (Cuisinart Mini-Prep Processor Model DLC-1SS or equivalent). Weigh $4.0 \pm 0.1g$ for the extraction.

5.1.1.3 Crab Leg

5.1.1.3.1 Wrap each specimen (legs and/or claws) individually in aluminum foil to prevent leakage during steaming, label samples

5.1.1.3.2 Fill tank with tap water up to point beneath baskets containing specimens so that specimens will be steamed and not immersed in water

5.1.1.3.3 Place wrapped crab specimens into steam baskets, then place in tank after the water is boiling

5.1.1.3.4 Close lid to tank and steam for 10-15 minutes for fresh samples or 15-20 minutes if crab is frozen

5.1.1.3.5 After steaming, remove specimens and unwrap aluminum foil carefully after

cooling

- 5.1.1.3.6 Remove leg/claw meat from shell into a 150 mL labeled plastic container
- 5.1.1.3.7 Homogenize body meat by mixing with a blender (Cuisinart Mini-Prep Processor Model DLC-1SS or equivalent). Weigh $4.0 \pm 0.1g$ for the extraction.

5.1.2 Anchovy (or Sardine) Samples

5.1.2.1 Samples < 3 Inches

- 5.1.2.1.1 Select 20 fish samples if possible, at random from throughout the lot.
- 5.1.2.1.2 Weigh the selected fish samples as a group.
- 5.1.2.1.3 Determine the average weight of a fish sample by dividing the weight obtained in Section 5.1.2.1.2 by 20 or the number of fish samples weighed from Section 5.1.2.1.1.
- 5.1.2.1.4 Homogenize the fish samples (heads, tails, and viscera) in a blender. The sample is now ready for extraction.

5.1.2.2 Samples > 3 Inches

- 5.1.2.2.1 Select 6 or more fish samples at random from throughout the lot.
- 5.1.2.2.2 Weigh the selected fish sample as a group.
- 5.1.2.2.3 Determine the average weight of a fish sample by dividing the weight obtained in Section 5.1.2.2.2 by the number of fish samples weighed from Section 5.1.2.2.1.
- 5.1.2.2.4 Homogenize the fish samples (heads, tails, and viscera) in a blender. The sample is now ready for extraction.

5.2 Sample Extraction:

- 5.2.1 From the homogenized composite or single (crab) sample (viscera, body), weigh accurately $4.0 \pm 0.1g$ grams tissue into a labeled centrifuge tube.

5.2.2 Fill up to 20 mL volume with methanol/deionized water (1:1).

5.2.3 Homogenize the sample using the Polytron for 3 minutes.

Caution: Do not start the Polytron unless the probe is immersed in the sample or water.

5.2.3.1 Start the Polytron homogenizer at low speed and gradually build up the speed to 10,000 rpm (or 70% of full scale voltage, if rpm unknown). Homogenize for 3 minutes, with the probe positioned close to, but not touching the bottom of the tube.

5.2.3.2 Do not try to recover tissue from the probe, but wash it thoroughly afterwards to prevent cross-contamination with the next sample. A jet of water is useful for cleaning.

5.2.4 Cap the centrifuge tube and centrifuge at 4000 rpm for 10 minutes.

5.2.5 Make 1:1 dilution of the extract with acetonitrile/deionized water (1:9).

5.2.6 Pass through a 0.45 um filter. The extract (filtrate) is ready for LC analysis (DF=10).

5.3 Instrumental Parameters:

5.3.1 HPLC Parameters:

Detector: Diode Array
Monitor at 240 nm

Spectrum: Start: 220 nm End: 320 nm

Pre-column: Spheri-5 Cyano
10 cm x 4.6 mm ID (5 um)

Column: Vydac C18
25 cm x 4.6 mm ID (5um)
Column jacket heater set at 40°C

Column Switching Valve: Pre + Analytical Col.

<u>Time (min.)</u>	<u>Column</u>
0.0	Pre-column
3.0	Pre + Analytical

Mobil Phase: Solvent A: Deionized water [(adjusted pH to 2.5 with 1:1 DI water: orthophosphoric acid (85%)]
Solvent B: Acetonitrile

Flow Rate: 1.0 mL/minute

Solvent Delivery System Program:

<u>Time(min.)</u>	<u>%A</u>	<u>%B</u>
0	100	0
15	88	12
25	88	12
27	100	0
30	100	0

Injection Volume: 20 uL

Run time: 30 minutes

5.4 External Calibration:

5.4.1 Initial Calibration of the HPLC System:

5.4.1.1 Analyze a solvent blank to demonstrate that the system is clean and interference free.

5.4.1.2 Analyze the working calibration standard solutions from Section 4.2 starting with the lowest concentration and ending with the highest. Run a linear regression. If the correlation coefficient(r) is at least 0.995, the system is calibrated and the analysis of the samples may proceed.

5.4.2 Continuing Calibration:

5.4.2.1 Using the established calibration from Section 5.4.1, check the calibration of the instrument at the beginning of each analysis day by analyzing at least the midrange calibration standard from Section 4.2.

5.4.2.2 If the observed concentration falls within +/- 10% of the expected value, the instrument is within the calibration specifications and the analysis may proceed. If the observed value of the standard differs by >10% of the expected value, the instrument should be recalibrated as in Section 5.4.1.

5.4.3 After every 10 samples or less, the midrange calibration standard from Section 4.2 must be analyzed to show that the responses are still within acceptable limits. Significant variations (i.e., observed concentrations which differ from the true concentrations by >10%) requires sample reanalysis and/or recalibration.

Note: Analysis of a midrange calibration standard is required at the completion of the entire analysis sequence, regardless of the total number of samples in the sequence.

5.5 Sample Analysis:

5.5.1 After establishing the calibration of the instrument, analyze the extracts from Section 5.2.

5.6 Calculations:

5.6.1 Calculate the concentration of domoic acid in the sample (ug/g) as follows:

$$\text{ug/g} = (\text{ug/mL})(\text{DF})$$

where: ug/mL = Concentration of the injected extract from Section 5.5 from the linear regression

DF = Dilution Factor (factor to convert the concentration in the injected extract to concentration in the sample, taking into consideration the dilution and conversion of units)

6.0 Quality Control:

6.1 A sample batch is defined as a group of 10 samples or less that is processed together and is comprised of samples with similar matrix composition. A sample batch must be accompanied by the following (See Section 6.1.3):

6.1.1 Method Blank and reagent blank --- To determine that all glassware and reagents are interference free.

6.1.2 One sample in duplicate --- To determine the reproducibility of the analytical method.

6.2 Calculate the retention time (RT) window for the analyte using the results of three or more calibration check standards analyzed during the course of the analytical sequence. The RT window is defined as +/- three times the standard deviation of the mean retention time for the analyte.

6.3 Compare the sample spectrum with the standard spectrum. For samples that domoic acid is detected, the sample spectrum should match well with the reference spectrum.

7.0 Comments:

gp/WP/FDL17 (08/26/97)
gp/msword/FDL17 (Converted to Msword 10/28/98)
gp/msword/FDL17 (Rev.1)(7/17/01)
gp/msword/FDL17 (Rev.2)(2/05/02)
gp/msword/FDL17 (Rev.3)(6/23/03)
gp/msword/FDL17 (Rev.4)(12/01/04)
gp/msword/FDL17 (Rev.5)(7/11/06)
gp/msword/FDL17 (Rev.6)(3/5/2007)
ws/msword/FDLB17(Rev.7)(11/7/2013)

gv/msword/FDLB17(Rev.8)(02/12/2014)