Certificate of Analysis

NRC·CNRC Certified Reference Material

CRM-Zero-Mus (Lot# 200604)

Control Mussel Tissue Certified Reference Material for Marine Toxins

Intended Use

CRM-Zero-Mus is a mussel tissue (*Mytilus edulis*) matrix CRM intended for use as a negative control for the analysis of shellfish toxins. It is free of the following toxins: paralytic shellfish toxins (PSTs), domoic acid (DA) and its isomers, okadaic acid (OA) and dinophysistoxins (DTXs), azaspiracids (AZAs), yessotoxins (YTXs), pectenotoxins (PTXs) and gymnodimine (GYM). It is suited for use in testing analytical procedures for selectivity, spike recovery and matrix matched calibration. CRM-Zero-Mus is not suitable as a negative control for spirolides (SPXs) and pinnatoxins (PnTXs) because these toxins are present, albeit at very low levels.

Preparation of the CRM

Since most shellfish toxin monitoring is performed on whole mussel tissues, a thermally-stabilized homogenized liquid slurry of whole mussels and water was selected as a representative control tissue. Such an approach has previously been proven successful by Biotoxin Metrology in the production of other mussel tissue reference materials.

The mussel tissue used in CRM-Zero-Mus was provided by the Canadian Food Inspection Agency. The mussels were harvested from New London Bay, Prince Edward Island during the last week of April 2005.

A large scale preparation of homogenate was made using approximately 13.5 kg of pre-cooked mussels. The homogenate was prepared by multiple passes through a Comitrol food cutter. Double distilled water was added to bring the final homogenate up to approximately 85% water, similar to fresh tissue taken from live animals. An antioxidant (ethoxyquin) was also added to the homogenate. The homogenate was de-aerated under vacuum after bubbling with nitrogen prior to packaging. During the bottle filling process, the mussel homogenate was continuously mixed and flushed with nitrogen. Approximately 8 g aliquots of the homogenate were transferred into nitrogen-flushed 8 mL polypropylene vials. Immediately after filling, the vials were flushed with nitrogen, heat sealed with trilaminate polypropylene film closures, and numbered in order of filling. The sealed samples were placed in racks and thermally processed at +118 °C for 20 min in a steam retort. After cooling, the seals were trimmed, inspected and bottle caps were attached. The bottles were then individually heat sealed in 100 × 165 mm trilaminate pouches.

Homogeneity

Since there are no significant toxins present in this tissue, there were no stringent homogeneity studies carried out on CRM-Zero-Mus. Identical preparation and processing procedures have been used in the production of other mussel matrix CRMs by Biotoxin Metrology. Based on the fact that these tissues proved to have acceptable homogeneity throughout the production run, homogeneity of CRM-Zero-Mus is assumed.

Stability Studies

This material was prepared by Biotoxin Metrology in the same manner as previous mussel tissue homogenates. Long-term stability of such mussel matrices has been historically established with storage in a freezer (-12 °C or below).

Certified Value

The toxins investigated are listed below (Table 1) along with the primary instrumental analysis method. The certified levels indicated correspond to the limit of detection (LOD) of our methods based on a signal to noise ratio of 3. If present, the level of toxin in this homogenate is lower than the detection thresholds of our instrumentation. This CRM may be used as a negative control or in spiking experiments for the toxins indicated in Tables 1a-c. Some cyclic imine toxins are present at very low concentrations (Table 2) and these values should be taken into account if used for applications involving these toxins.

Uncertainty

Values identified are for information purposes only. There are no certified concentrations for CRM-Zero-Mus and therefore no associated uncertainties were derived.

Storage Instructions

CRM-Zero-Mus should be stored in a freezer (-12 °C or below).

Expiry

If stored unopened at the recommended conditions (see Section Storage Instructions), since no appreciable levels of toxins are present, CRM-Zero-Mus is valid for 3 years from the date of sale.

Instructions for use

Each bottle should be allowed to warm to room temperature prior to opening and the contents thoroughly mixed by vortexing for a minimum of 2 min. Each bottle contains approximately 8 g (± 0.5 g) of tissue. The mass of homogenate is not certified. Remove hermetic seal carefully using a knife or a blade, mix contents thoroughly with a spatula and measure the required sample recording the weight. It is not recommend that bottles be refrozen between sub-sampling, so all sub-samples should be taken following the initial defrosting. A minimum sub-sample size of 2 g is recommended. Once tissues have been weighed accurately into an extraction vessel they can be treated accordingly using prescribed extraction procedures.

Safety Instructions

Care should be taken when cutting the seal on a bottle of CRM-Zero-Mus. The usual laboratory safety precautions apply.

Table 1a: Concentration values for paralytic shellfish toxins in CRM-Zero-Mus were determined following an acidic extraction and analysis by LC-pcox-FLD [1]. Quantitation was performed relative to NRC certified calibration solutions or in-house prepared standards (nd = not detected).

Toxin name	Code	Concentration (µmol/kg)	Detection Limit (µmol/kg)
Saxitoxin	STX	nd	0.1
Neosaxitoxin	NEO	nd	0.3
Decarbamoylsaxitoxin	dcSTX	nd	0.2
Gonyautoxin-4	GTX4	nd	0.1
Gonyautoxin-1	GTX1	nd	0.1
Gonyautoxin-5	GTX5	nd	0.3
Decarbamoylgonyautoxin-3	dcGTX3	nd	0.04
Decarbamoylgonyautoxin-2	dcGTX2	nd	0.06
Gonyautoxin-2	GTX2	nd	0.03
Gonyautoxin-3	GTX3	nd	0.02
N-Sulfocarbamoylgonyautoxin-2	C1	nd	0.06
N-Sulfocarbamoylgonyautoxin-3	C2	nd	0.04
N-Sulfocarbamoylgonyautoxin-1	C3	nd	0.3
N-Sulfocarbamoylgonyautoxin-4	C4	nd	0.2
Decarbamoylgonyautoxin-4	dcGTX4	nd	0.06
Decarbamoylgonyautoxin-1	dcGTX1	nd	0.1
Decarbamoylneosaxitoxin	dcNEO	nd	0.3
Gonyautoxin-6	GTX6	nd	0.5

Table 1b: Domoic acid concentration values for CRM-Zero-Mus were determined following an exhaustive, 3-step volumetric extraction with 50% methanol and analysis by LC-UV [2]. Quantitation was performed relative to NRC certified calibration solutions or in-house prepared standards (nd = not detected).

Toxin name	Code	Concentration (µg/kg)	Detection Limit (µg/kg)
Domoic acid	DA	nd	0.05
C5'-epidomoic acid	epiDA	nd	0.05
Isodomoic acids A-H		nd	0.05

Table 1c: Regulated lipophilic toxin concentration values for CRM-Zero-Mus were determined following an exhaustive, 4 step volumetric extraction with 90% (PTX2, YTX) or 100% (AZA, OA) methanol and analysis by LC-MS/MS [3]. Quantitation was performed relative to NRC certified calibration solutions or in-house prepared standards (nd = not detected).

Toxin name	Code	Concentration (µg/kg)	Detection Limit (µg/kg)
Okadaic acid	OA	nd	1
Dinophysistoxin-1	DTX1	nd	3
Dinophysistoxin-2	DTX2	nd	2
Azaspiracid-1	AZA1	nd	5
Azaspiracid-2	AZA2	nd	2
Azaspiracid-3	AZA3	nd	0.6
Yessotoxin	YTX	nd	8
Pectenotoxin-2	PTX2	nd	4

Table 2: Cyclic imine toxin concentration values for CRM-Zero-Mus were determined following an exhaustive volumetric extraction with methanol followed by LC-MS/MS analysis. Quantitation was performed relative to NRC certified calibration solutions or in-house prepared standards. These concentrations are for information purposes only and are not certified.

Toxin name	Molecular Weight	Code	Peak Number[a]	Concentration (µg/kg)
Gymnodimine	507.3	GYM	n/a	nd (<0.5)
Unknown spirolide	691.5	-	1	1.5
13-Desmethyl-spirolide C	691.5	13-desMe-SPX C	2	1.9
13-Desmethyl-spirolide D	693.5	13-desMe-SPX D	3	3.1
Pinnatoxin G	693.5	PnTX G	4	2.0
Unknown spirolide	705.5	-	5	1.1
Spirolide C	705.5	SPX C	6	1.7
Unknown spirolide	705.5	-	7	1.5
Unknown spirolide	705.5	-	8	1.3
Unknown spirolide	705.5	-	9	0.7
Spirolide D	707.5	SPX D	10	4.1
Unknown spirolide	707.5	-	11	1.4

[a] Peak numbers in Figure 1.

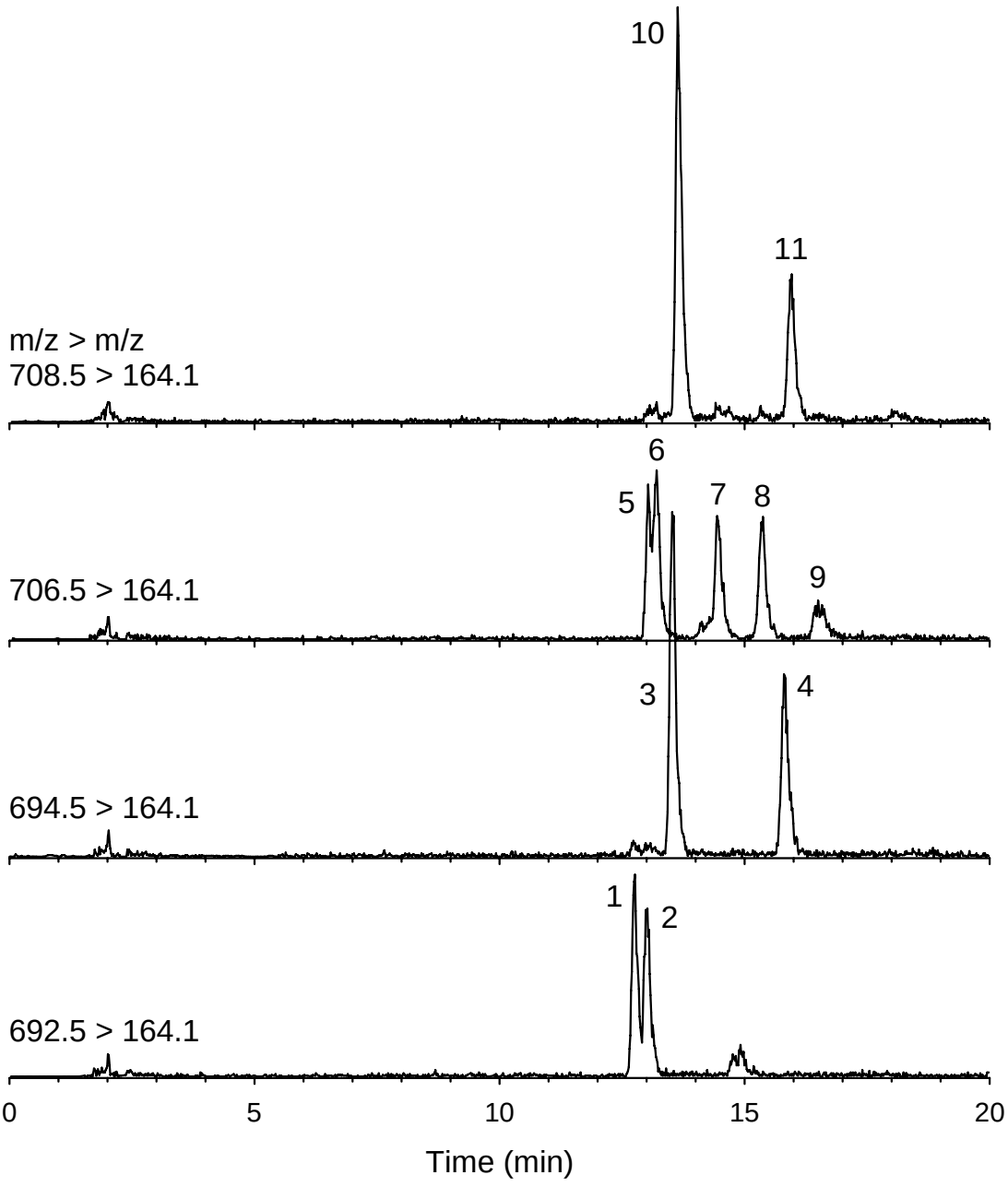

Figure 1: Extracted mass chromatograms from the LC-MS/MS analysis of CRM-Zero-Mus for spirolides and pinnatoxin G. Conditions: Agilent 1200 HPLC interfaced to a QTRAP5500 MS; 3 μm BDS Hypersil-C8 column (2 × 150 mm; Keystone) held at +20 °C; 0.2 mL/min flow with a gradient from 25% to 55% B over 10 min and hold at 55% B for 10 min; mobile phase, A is water and B is acetonitrile/water (95:5) both containing 50 mM formic acid and 2 mM ammonium formate; a total of 10 transitions were monitored, each with a 90 msec dwell (only 4 transitions showed cyclic imine toxins, which were confirmed by EPI product ion scans).

References

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Signed: 

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