Analytical information

Name: Quality Controlled Standard of Mussel matrix with DSP toxins.

Code: Mussel-DSP-2 Batch: 14.001

General description:

Mussel-DSP-2 is homogenous mussel tissue with known concentrations of Okadaic acid, Dinophysistoxin-1 and Dinophysistoxin-3. Concentration of DSP toxins was established by implementation of EURLMB extraction SOP coupled to UPLC-MS/MS method. Extraction efficiency and matrix effect were performed in order to determine recovery value and best value of toxin content.

Product Specification	
MOLECULAR FORMULA:	$C_{44}H_{67}O_{13}Na$ (OA); $C_{45}H_{69}O_{13}Na$ (DTX1); DTX3 alkyl esters from OA
MOLECULAR WEIGHT:	826.985 $g \cdot mol^{-1}$ (OA); 841.011 $g \cdot mol^{-1}$ (DTX1)
MONOISOTOPIC MW	826.448 $g \cdot mol^{-1}$ (OA); 840.464 $g \cdot mol^{-1}$ (DTX1)
CAS NUMBER:	78111-17-8 (OA); 81720-10-7 (DTX-1); not applicable for DTX-3
SOURCE/HOST:	Natural OA and DTX-3 mussel (*Mytilus galloprovincialis*) tissued contaminated. DTX-1 from algae culture of *Prorocentrum lima*.
CONCENTRATION:	[OA] = (361 ± 34) $\mu g \cdot kg^{-1}$ [DTX-1] = (206± 19) $\mu g \cdot kg^{-1}$ [DTX-3] = (283 ± 61) $\mu g \cdot kg^{-1}$
PURITY:	not applicable
FORMULATION:	Homogenized slurry mussel tissue
SHIPPING:	SHIPPED ON ICE
LONG TERM STORAGE:	- 20 °C
HAZARD:	This mixture is not classified as hazardous. Harmful

Intended use:

Mussel-DSP-2 is suitable for a range of analytical DSP testing functions. The material is ideal for test the accuracy and repeatability of analytical methods, so can be a valuable tool for control charts or validation studies. Furthermore it would enable for development of new analytical method and instrument calibration.

Mussel-DSP-2 can be used as negative control for the analysis of next shellfish toxins: paralytic shellfish toxins (PSPs), domoic acid (DA) and its isomers, azaspiracids (AZAs), yessotoxins (YTXs), pentenotoxins (PTXs) and gymnodimine (GYM). This material may be used also in spiking experiments for the toxins indicated in Tables 1-3.

This material is not intended for human or animal use. It must be used only for testing and research purposes.

Source and Preparation of Material:

Mussel-DSP-2 has been prepared using harvested mussels (Mytilus galloprovincialis) collected from Galician coast, Northwestern of Spain (FAO capture zone number 27). It was prepared by blending whole mussel tissue with high level of DSPs (approximately 0.8 kg with 1250 µg·kg-1 of OA) with whole mussel with low level of DSP amount (approximately 2.5 kg with 65 µg·kg-1 of OA) and a small amount of the dinoflagellate Prorocentrum lima methanolic extract. Shell removal, ice - bath ultraturrax homogenization and storage in Teflon bags at – 20ºC were carried out for two fractions before mixture. After ice – bath ultraturrax mixture, five frost/thaw cycle were used to initial characterization, water addition (0.5 L distilled water was added to the slurry to bring the final homogenate up to 85% water), DTX-1 contamination (8 mL methanolic extract), antioxidant (0.6 g BHT - Butylated hydroxytoluene - in 6 mL of ethanol) / antibiotic (0.6 g of Oxitetracycline in 12 mL of water) addition, final mixture homogenization further before final toxicity, water content and pH checks and disposal.

The material was bottled in > 4 g units for distribution into amber glass vials in one single processing batch. Each vial was filled with N_2, closed with septa butyl stopper, sealed with a (tear of) crimp cap and individually labeled.

No gamma irradiation, neither thermal stabilization were performed in order to maintain this material similar to fresh tissue taken from live animals.

Vials have been stored at -80 ºC for long term storage until dispatch.

Instructions for proper handling use:

Mussel-DSP-2 is sent from Laboratorio CIFGA S.A in a frozen physical state inside packaging containing frozen ice blocks and depending on delivery time the samples may arrive in a semi-frozen or even thawed state although the samples will arrive cool.

It is not recommend that vials be refrozen between sub-sampling. The content of the vial is not determined accurately (> 4 g). Therefore, the entire contents of the vial should not simply be transferred to a volumetric flask and diluted to volume. Once tissues have been weighed accurately (4.0 g or 2 x 2.0 g of material could be accurately measured) they can be treated accordingly using proper extraction procedures. Before vial opening, it must be guaranteed that the content is a liquid (allowed to thaw to RT) and properly mixed to ensure homogeneity.

The Material Safety Data Sheet (MSDS) should be consulted before material usage. Use proper disposal methods.

Instructions for appropriate conditions of storage:

Once received, it is recommended that Mussel-DSP-2 is stored in a freezer (< -15 ºC) until purpose.

Analytical data of Mussel-DSP-2, 14.001 is valid for **12 months**[#] after purchase date within the measurement uncertainties specified, provided the material is handled and stored in accordance with the instructions given in this certificate.

[#]The material will be subjected to Laboratorio CIFGA inverse isochronous stability monitoring programme to control its further stability. If substantive changes occur that affect the analytical data before the expiration date, Laboratorio CIFGA will notify the purchaser.

Property values:

Date of analysis:	September 2014
Concentration[†] of:	**[OA] = (361 ± 34) µg·kg^{-1}**
	[DTX-1] = (206± 19) µg·kg^{-1}
	[DTX-3][‡] = (283 ± 61) µg·kg^{-1}

[†] Toxin concentrations are reported as µg/kg in the homogenate. The estimated uncertainties for values are based on judgment of $u = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2}$ and are intended to correspond to one standard deviation.

[‡] Qualitative mayor molecules content of DTX-3 are esters of Okadaic acid, see Figure 6. Equimolar recovery factor to Okadaic acid is assumed.

Moisture content and pH parameter were evaluated through studies involving characterization, homogeneity and stability. The mean moisture content was evaluated by oven-dry test until constant and pH parameter is the value of supernatant solution after centrifugation.

Moisture content	**(85.01 ± 0.69) %**
Care shall be taken to avoid change of the moisture content once the bottles are opened.	
pH	**5.17 ± 0.12**

All instrumentation and measurement equipment utilized in material study is regularly checked and calibrated. All analytical balances, pipettes and other measurement devices used during the characterization studies were also calibrated. Quantization of toxins was conducted against calibrations prepared using certified reference standards obtained from Laboratorio CIFGA S.A.

Analysis for UPLC MS/MS reveal one signal that elutes before DTX-1, with a very similar retention time to that of DTX2 (see Figure 1), but confirmatory MRM transition was not detected using typical fragmentation pattern of DSPs. Further work is required to further characterize this signal.

Some marine toxins are present at very low concentrations (Tables 1-3, Figures 3 and 4). Analytical HPLC test were performed to determine the presence of another marine toxins:

- EURLMB extraction SOP coupled to UPLC-MS/MS method was used to determine: DSPs, YTXs, AZAs, PTXs, SPXs and Gym.
- Pre-COX LC-FLD method (AOAC HPLC method: 2005.06) was performed to determine PSPs content and,
- DA determination was carried by developing EU Harmonised SOP ASP HPLC-UV Version 1.

Indicated concentration of minor toxin levels correspond to the limit of quantification (LOQ) of Laboratorio CIFGA S. A. methods according to a signal to noise ratio of 10:1. Non detected toxins, if present, the level of toxin in this homogenate is lower than the detection thresholds of our instrumentation.

Table 1. Summary of minor PSP toxin concentrations in Mussel-DSP-2 (14.001) from characterisation studies as determined by Pre-COX LC-FLD method (AOAC HPLC method: 2005.06) with external calibration. *NC: not calculated, nd: not detected.*

Toxin	Code	$LOQ_{instrumental}$ / µg·equiv STX diHCl·kg^{-1}	Concentration / µg·equiv STX diHCl·kg^{-1}
Decarbamoylsaxitoxin	dcSTX	20	nd
Saxitoxin	STX	15	(12 ± 8)
Neosaxitoxin	NEO	40	nd
Decarbamoylneosaxitoxin	dcNeo	15	nd
Decarbamoylgonyautoxin-3	dcGTX3	20	nd
Decarbamoylgonyautoxin-2	dcGTX2		
Gonyautoxin-1	GTX1	40	nd
Gonyautoxin-4	GTX4		
Gonyautoxin-2	GTX2	20	nd
Gonyautoxin-3	GTX3		
Gonyautoxin-5	GTX5	15	(5 ± 2)
Gonyautoxin-6	GTX6	nc	nd
N-sulfocarbamoyl-gonyautoxin-2	C1	20	nd
N-sulfocarbamoyl-gonyautoxin-3	C2		
N-sulfocarbamoyl-gonyautoxin-1	C3	nc	nd
N-sulfocarbamoyl-gonyautoxin-4	C4		

Table 2. Summary of minor Lypophylic marine toxin concentrations in Mussel-DSP-2 (14.001) from characterization studies as determined by EURLMB extraction SOP coupled to UPLC-MS/MS with external calibration.

Toxin	Code	$LOQ_{instrumental}$ / µg·kg^{-1}	Concentration / µg·kg^{-1}
Azaspiracid-1	AZA-1	1.8	(2.9 ± 1.1)
Azaspiracid-2	AZA-2	2.2	nd
Azaspiracid-3	AZA-3	2.4	nd
13 desmethyl spirolide C	13-desMeC	2.0	(9.1 ± 0.9)
13,19 didesmethyl spirolide C	13,19-didesMeC	2.6	nd
20 methyl spirolide G	20-SPXG	3.8	nd
Gymnodimine	GYM	NC	nd
Pectenotoxin-1	PTX-1	NC	nd
Pectenotoxin-2	PTX-2	1.9	nd
Yessotoxin	YTX	15.0	nd
1a-homoyessotoxin	hYTX	47.0	nd
Okadaic acid	OA	9.3	See property value
Dinophysistoxin-1	DTX-1	2.3	See property value
Dinophysistoxin-2	**DTX-2**	**6.3**	**(16.9 ± 6.3)**

Table 3. Summary of minor ASP concentrations in Mussel-DSP-2 (14.001) from characterization studies as determined by EU Harmonised SOP ASP HPLC-UV Version 1 with external calibration.

Toxin	Code	$LOQ_{instrumental}$ / µg·kg^{-1}	Concentration / µg·kg^{-1}
Domoic acid + 5'epiDomoic acid	DA + epiDA	1.8	nd

Figures:

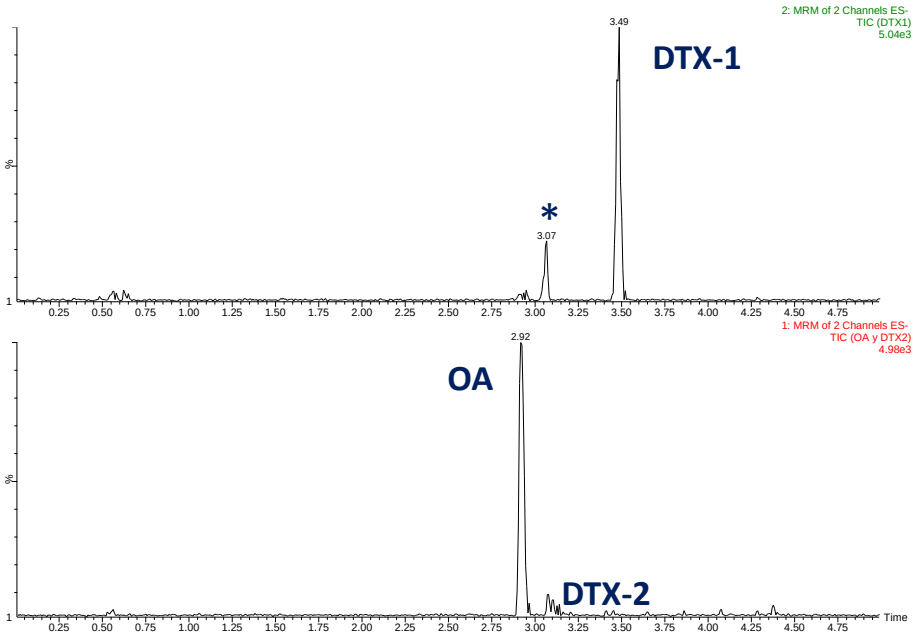

Figure 1. Analysis of the **Mussel-DSP-2** after EURLMB extraction SOP by UPLC-MS/MS. Conditions: ACQUITY UPLC BEH C18, 1.7 µm, 100x2.1 mm, UPLC column. Elution conditions, gradient mode with mobile phases: (A) Water and (B) Aqueous 95% acetonitrile, both with 2mM ammonium formate and 50mM formic acid; Gradient: 0 min 30% B; 3.0 min 90% B; 3.6 min 90% B; 3.7 min 30% B; 5.0 min 30% B; flow rate: 0.40 mL/min. Temperature: 30°C. MS detector: Waters Quattro Premier XEVO using the typical MRM transitions of Okadaic acid and Dinophysistoxin-1.

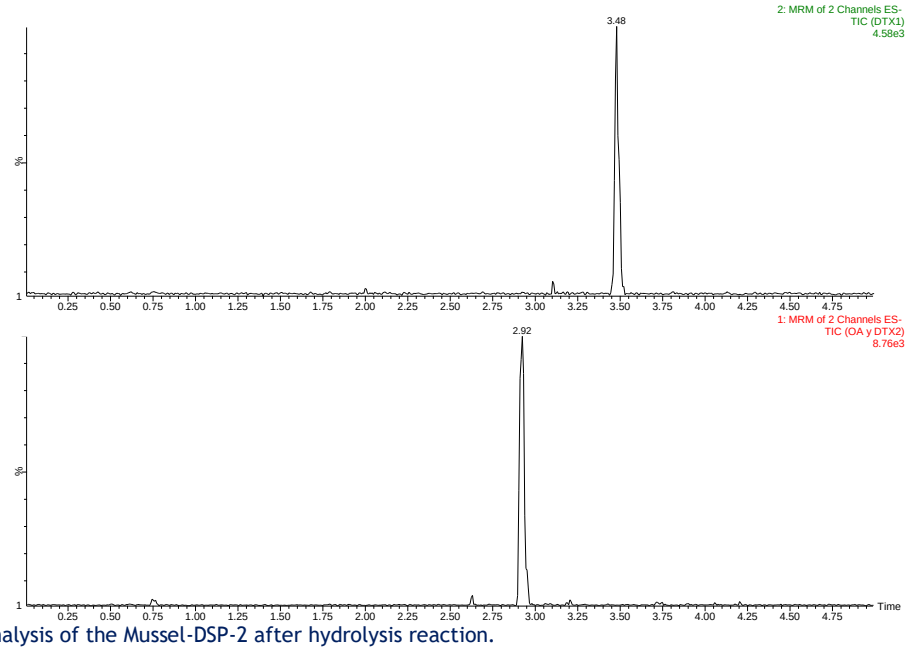

Figure 2. Analysis of the Mussel-DSP-2 after hydrolysis reaction.

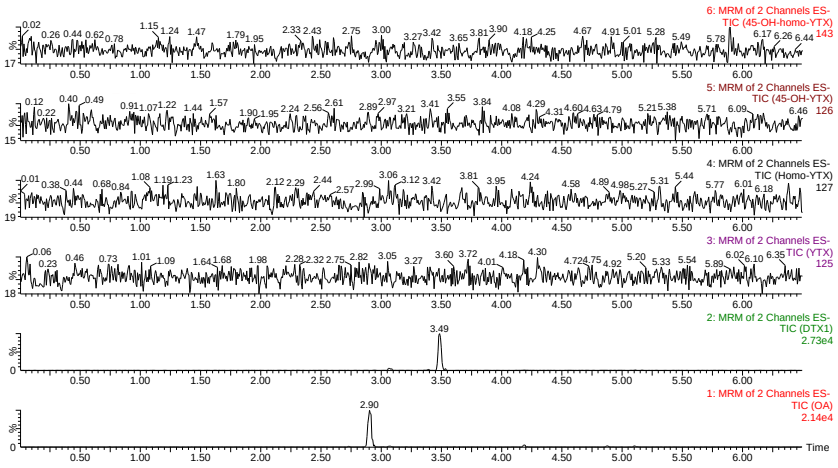

Figure 3. Analysis of the **Mussel-DSP-2** after EURLMB extraction SOP by UPLC-MS/MS. toxins detected by ESI-. Typical MRM transitions of Okadaic acid and Dinophysistoxin-1, YTX, hYTX, 45-OH-YTX and 45-OH-hYTX.

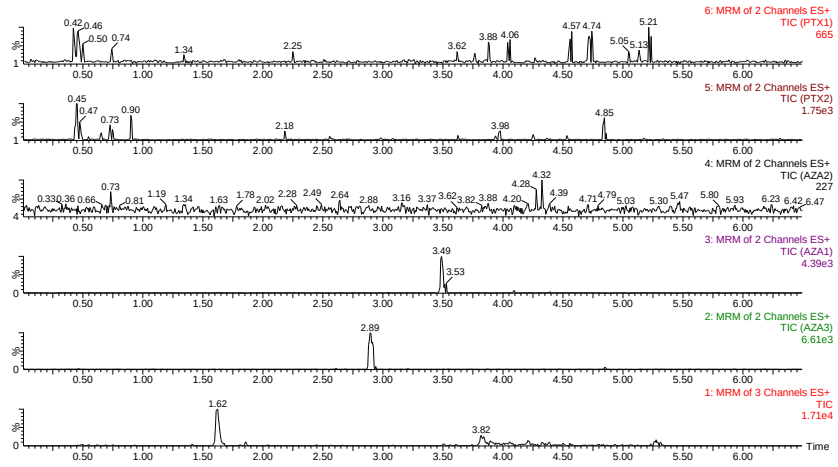

Figure 4. Analysis of the **Mussel-DSP-2** after EURLMB extraction SOP by UPLC-MS/MS, toxins detected by ESI+. Typical MRM transitions of 13 desmethyl spirolide C, 13,19 didesmethyl spirolide C, 20 methyl spirolide G, Azaspiracid-1, Azaspiracid-2, Azaspiracid-3, Pectenotoxin-1 and Pectenotoxin-2

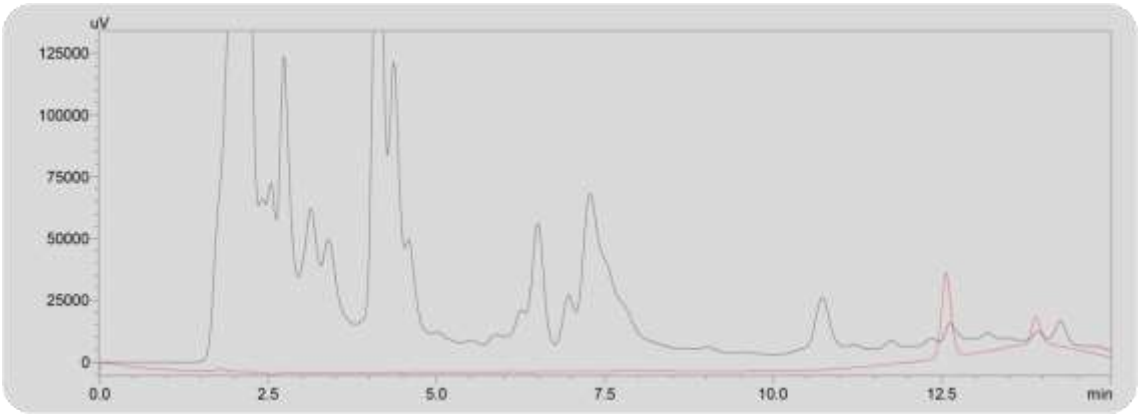

Figure 5. Analysis of the **Mussel-DSP-2** after AOAC HPLC method: 2005.06 by Pre-COX LC-FLD. Conditions: SUPELCOSIL™ LC-18, 5 µm, 150 x 4.6 mm, HPLC column. Elution conditions, gradient mode with mobile phases: (A) Water and (B) Aqueous 5% acetonitrile, both with 0.1 M ammonium formate, pH = 6 (regulated with AcOH 0.1 M); Gradient: 0 min 100 % A; 5.0 min 95 % A; 9.0 min 30 % A; 11.0 min 100 % A; 15.0 min 100 % A; flow rate: 1.0 mL/min. Temperature: 35 °C. Black line, spectrum after extraction and C18 clean-up; red line, spectrum from GTX5 (12.5 min) and STX (14.0 min) standard mixture for comparative purpose.

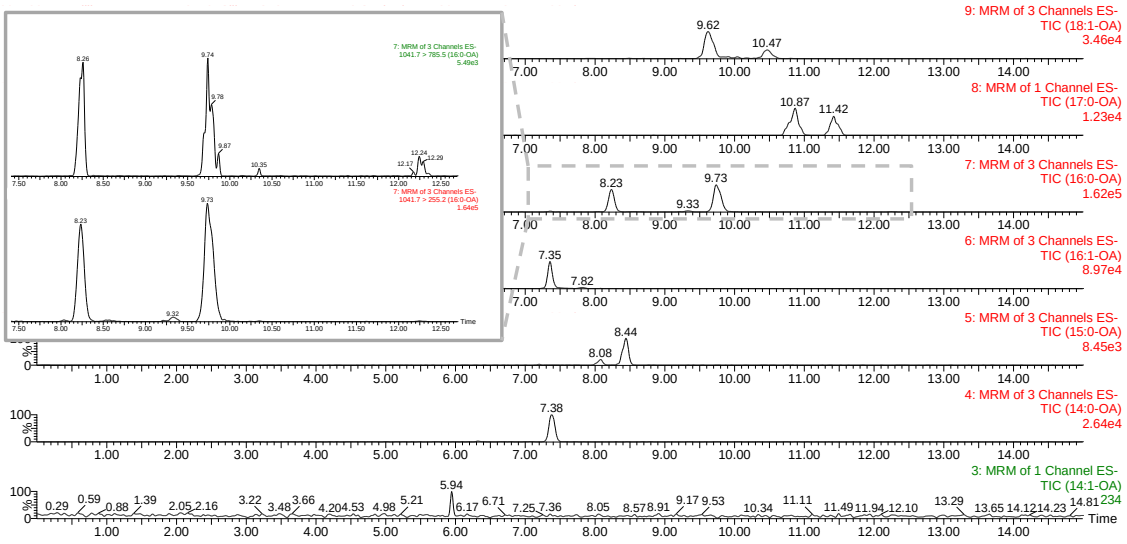

Figure 6. Analysis of the **Mussel-DSP-2** after hexane partition and concentration. Conditions: ACQUITY UPLC BEH C18, 1.7 µm, 100x2.1 mm, UPLC column. Elution conditions, gradient mode with mobile phases: (A) Water and (B) Aqueous 95% acetonitrile, both with 2mM ammonium formate and 50mM formic acid; Gradient: 0 min 60% B; 1.0 min 60% B; 3.0 min 100% B; 13.0 min 100% B; 13.1 min 60% B; 15.0 min 60% B flow rate: 0.40 mL/min. Temperature: 30°C. MS detector: Waters Quattro Premier XEVO using the MRM transitions for 1011.7, 1013.7, 1027.7, 1039.7, 1041.7 and 1067.7 to 785.5 and characteristic lipid ion fragment. At Rt = 8.3 min signal is tentatively identified as isoprenoid fatty acid (TMTD) ester of OA, *OA 7-O-(4,8,12-trimethyltridecanoyl) ester* (isopC16:0-OA) while at Rt = 9.7 min this compound is tentatively identified *OA 7-O-Palmitoyl ester* (C16:0-OA).

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This Analytical Information is only valid if the product was obtained directly from Laboratorio CIFGA S.A. or one of our authorized distributors.

Certificate Revision History: *November, 18th 2014 (Original certificate issue date, revision 1).*

August, 16th 2021. Certificate expiration date, uncertainty statement, Figure 6 update, editorial changes *(certificate rev. 2).*