Dermatoxins Report

Project: Florida Department of Environmental Protection

Submitted to:	FDEP Laboratory
Organization:	Florida Department of Environmental Protection
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Sample Receipt Date:	03 August 2021
Sample Condition:	3.8 ºC upon arrival
Report#	210729_FDEP_amended
Date Prepared:	09 August 2021
Prepared by:	Amanda Foss
Amended:	8/12/2021 to add holding time exceedance and corrected sample receipt date

Table 1: Samples analyzed

Sample ID	Site/Description	Collection Date	Matrix
2266193	G3NW0215	29 July 2021	Surface Fresh Water

Analytes: Lyngbyatoxin-a (LA), Debromoaplysiatoxin (DAT), Aplysiatoxin (AT)

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification

Sample Preparation

Water Sample Sonication

LA/DAT/AT (Dermatoxins)

The sample was received and thawed upon receipt, inverted to mix, and an aliquot (0.5 mL) was diluted in MeOH (0.5 mL) with duplicate subsets prepared as LFSM and LDSMD (Table 2). The samples were lysed by water bath sonication for 10 min followed by filtration (0.2 µm PVDF). A method blank (Blank) and spiked method blank (laboratory fortified blank - LFB) were prepared in the same manner and analyzed.

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

Dermatoxins (LA/AT/DAT)

High performance chromatography coupled with tandem mass spectrometry was used for a targeted lyngbyatoxin-a, debromoaplysiatoxin, and aplysiatoxin analysis. The $[M+Na]^+$ ion for DAT (*m/z* 615) was fragmented and the product ions (*m/z* 597, 583, 481, and 393) were monitored. The $[M-H]^-$ ion for AT (*m/z* 670) was fragmented and product ions (*m/z* 509, 626) were monitored. The $[M+H]^+$ ion for LA (*m/z* 438) was fragmented and product ions (*m/z* 410, 395, 302, 271) were monitored. A S:N ratio of 3 was used to determine detection limits for DAT (0.02 ng/mL), LA (0.08 ng/mL), and AT (0.07 ng/mL). External standard curves were used to determine LFSM returns.

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Quality Control

Table 2: LFSM QC samples prepared for analyses pre-extraction. LFSM QC samples prepared for analyses pre-extraction (unless otherwise noted). Additional Quality Control/Quality Assurance checks included method blanks, continued calibration checks, LFBs, and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
DAT	5	2266193	LFSM	130%
LA	5	2266193	LFSM	90%
AT	5	2266193	LFSM	76%
DAT	5	2266193	LFSM	91%
LA	5	2266193	LFSM	80%
AT	5	2266193	LFSM	68%^{N}
DAT	5	LFB	LFSM	143%^{N}
LA	5	LFB	LFSM	45%^{N}
AT	5	LFB	LFSM	47%^{N}

**Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%*

N Qualifier: The N qualifier was used for the laboratory fortified blank (LFB) sample for exaggerated (DAT) and lower (LA, AT) recoveries outside control limits. This observation is likely related to the use of deionized water coupled with sonication, which is not fully representative of a field sample matrix. A lower recovery for AT was also observed (just below control limits) for one of the matrix spikes (LFSM).

Table 3: The percent reproducibility (%RPD) between lab duplicates or LFSM/LFSMDs prepared and analyzed

QC Type	Sample ID	Analyte	Value 1	Value 2	% RPD	Pass/Fail (<40%)
LFSMs	2266193	DAT	130%	91%	35%	Pass
LFSMs	2266193	LA	90%	80%	11%	Pass
LFSMs	2266193	AT	76%	68%	11%	Pass

%RPD is NA when values are below the method reporting/detection limit

Summary of Results

Table 5: Summary of results in ng/mL for debromoaplysiatoxin (DAT), lyngbyatoxin-A (LA), and aplysiatoxin (AT).

Sample ID	DAT (ng/mL)	LA (ng/mL)	AT (ng/mL)
2266193	ND[PT]	ND[PT]	ND[PT]
MRL (ng/mL):	*1.0*	*1.0*	*1.0*
Analyst Initials:	*AF*	*AF*	*AF*
Date Analyzed:	*8/9/2021*	*8/9/2021*	*8/9/2021*
Analysis Time:	*1441*	*1441*	*1441*
Extraction Date:	*8/9/2021*	*8/9/2021*	*8/9/2021*
Extraction time:	*1129*	*1129*	*1129*

Interpretations:

Dermatoxins (lyngbyatoxin-a, debromoaplysiatoxin aplysiatoxin) were not detected in the submitted samples above the method reporting limits.

The holding time (not experimentally determined) for the dermatoxins (10-days from sample collection) was exceeded by 1-day (qualifier PT used). However, it is currently unknown what the impacts on holding time for freshwater or marine water sample collections for dermatoxins such as lyngbyatoxin or the aplysiatoxins due to a lack of published studies addressing hold times.

Submitted by: Mark T. Aubel, Ph.D.
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Date: August 9, 2021

The results in this report relate only to the samples listed above.
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