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Beach, Daniel G.; Walsh, Callee M.; Rourke, Wade A.; Reeves, Kelley; Cantrell, Pamela; O'Brien, Sinead; McCarron, Pearse

High-Throughput Screening of Domoic Acid in Shellfish by Laser Ablation Electrospray Ionization (LAESI)-MS

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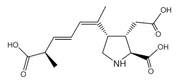
Measurement Science and Standards

Pearse McCarron, Kelley Reeves, Daniel G. Beach National Research Council Canada, Halifax, NS
Callee M. Walsh, Pamela Cantrell Protea Biosciences Inc., Morgantown, WV, USA
Wade A. Rourke Canadian Food Inspection Agency, Dartmouth, NS
Sinead O'Brien Marine Institute, Galway, Ireland

Abstract

We recently showed that Laser Ablation Electrospray Ionization (LAESI)-MS/MS could detect and quantify Domoic Acid (DA) directly from mussel tissue homogenates without sample extraction, cleanup or chromatographic separation [1]. The decrease in run time from ~ 20 min for LC methods to ~ 10 sec/sample for LAESI-MS is of interest to regulatory labs carrying out shellfish safety testing. Here, in collaboration with international regulatory partners, we assess the suitability of LAESI-MS as a high-throughput screening or quantitation tool for DA in a variety of shellfish matrices. The method was first optimized for use with high resolution MS detection. Samples analyzed included 190 shellfish samples previously analyzed by regulatory labs and DA certified reference materials. LAESI-MS shows promise as a screening tool capable of differentiating samples above and below 5 mg/kg, compatible with the action level of 20 mg/kg set for DA in edible shellfish tissue.

Introduction

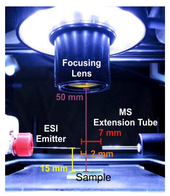


Domoic Acid

Domoic Acid (DA) is a potent neurotoxin that is produced by marine diatoms and accumulates in shellfish. DA was first identified as the causative agent of amnesic shellfish poisoning (ASP) after a serious outbreak in 1987 in Prince Edward Island, Canada, that left 3 people dead from consuming contaminated mussels. Regulatory analysis of DA is typically carried out by LC-UV using a 20-30 min run after extraction with aqueous methanol. The scope of routine DA analysis worldwide is large enough that increases in sample throughput would lead to significant cost/time savings for regulatory labs. For example, the Canadian Food Inspection Agency (CFIA) currently runs about 10,000 shellfish samples annually testing for DA, the vast majority of which are negative.

Laser Ablation Electrospray Ionization (LAESI) is an ambient ionization technique for mass spectrometry that uses a mid-IR laser to produce a fine mist of neutral droplets of sample liquid. Ionization is then carried out by charge transfer from charged droplets in an electrospray plume of solvent. This results in ionization specificity that is comparable to ESI rather than laser ablation ionization techniques. Most studies have focused on the use of LAESI for qualitative analysis and in particular high resolution MS imaging, but the quantitative capabilities of the technique have rarely been considered.

Commercial LAESI System



LAESI – MS Source interface

Experimental

Samples – 190 shellfish samples analyzed by the CFIA (Canada) and the Marine Institute (Ireland) as part of routine monitoring.

Standards – NRC Certified Reference Materials (CRMs) for DA included calibration solution (CRM-DA-f) and mussel matrices (CRM-ASP-Mus, CRM-PSP-Mus, CRM-FDMT, CRM-DSP-Mus, NRC-Zero-Mus). Matrix matched standards were prepared for each matrix by blending control tissue with ≤ 5% highly contaminated mussel tissue (> 600 mg/kg) and were quantitated by LC-UV.

Sample Preparation – Regulatory samples were diluted 1:1 with H_2O and further homogenized using a polytron blender to facilitate reproducible transfer of 20 µL aliquots to low volume 96-well plates.

LAESI Ionization – A Protea LAESI DP-1000 direct ionization system was used to ablate samples with 50 pulses of a mid-IR (λ = 2940 nm) laser at 10 Hz with 700 µJ of energy.

Mass Spectrometry – A Thermo QExactive+ was operated in tSIM mode at a mass resolution of 140k for all quantitative analysis. Average MS peak height at *m/z* 312.144 across the laser pulse was used to quantify DA.

LAESI-MS Method Optimization

Product ion scan of *m/z* 312 precursor showed excellent selectivity when mussel tissue homogenates were analyzed directly by LAESI-MS/MS.

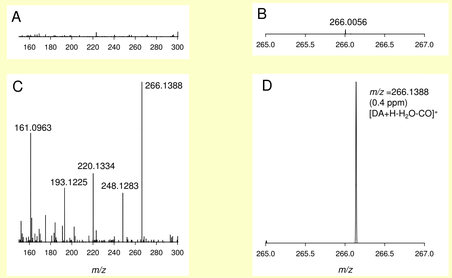


Figure 1: MS/MS of control mussel tissue homogenate un-spiked (A + B) and spiked with 20 mg/kg DA (C + D).

Targeted SIM mode showed improved sensitivity and LOD, compared to MS/MS. DA spiked at 1 mg/kg could only be detected by tSIM mode.

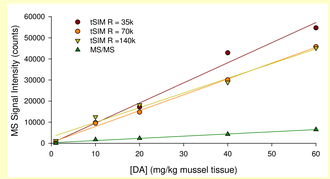


Figure 2: Comparison of sensitivity of different Orbitrap resolutions and scan modes for DA spiked mussel tissue homogenates.

Orbitrap resolution setting of 140k was required to resolve interfering mussel matrix peaks and allow for selective analysis of DA by LAESI-HRMS

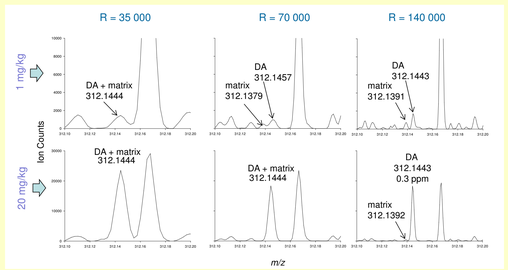


Figure 3: Separation of DA from mussel matrix interference by HRMS

Sample Preparation and Matrix Matched Calibration

- Additional homogenization and 1:1 dilution with H_2O allowed for reproducible dispensing of homogenates onto low-volume 96-well plates.
- Extraction with aqueous methanol followed by strong anion exchange SPE cleanup was effective but incompatible with a high throughput workflow.

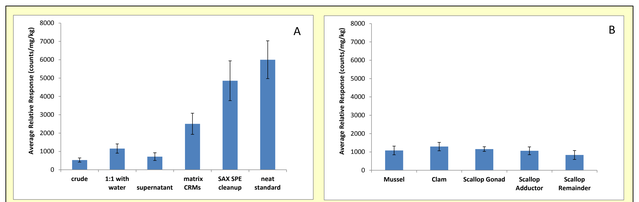


Figure 4: Relative response of sample preparation approaches for DA in mussel tissue homogenate (A) and sensitivity of matrix matched curves for different shellfish tissues blended 1:1 with water (B).

Different calibration approaches were considered:

- use of mussel tissue homogenate CRMs
- matrix matched calibration curves for quantitation
- one point matrix matched check standards for screening

DA response was similar between shellfish matrices (Fig. 4B) but lower than the more highly processed mussel tissue homogenate CRMs.

Tissue	LOD (mg/kg)	%RSD of Matrix Standard	R^2 of Matrix Matched Curve
Scallop Adductor	0.25	27 (N = 13)	0.994
Scallop Gonad	0.79	38 (N = 18)	0.98
Scallop Remainder	0.31	38 (N = 12)	0.98
Clam	0.12	44 (N = 13)	0.9992
Mussel	0.55	36 (N = 22)	0.9991

Regulatory Sample Screening

- 190 real shellfish samples obtained from the CFIA and the MI, which had previously been tested for DA by LC-MS or LC-UV.
- LAESI-MS was evaluated as a screening method with the goal of identifying samples with > 5 mg/kg DA, which could then be quantitated by LC-MS or LC-UV to determine their toxicity relative to the 20 mg/kg action level.
- Samples were analyzed in triplicate and 5 mg/kg matrix matched check standards were run about every 20 samples and used for single point calibration.

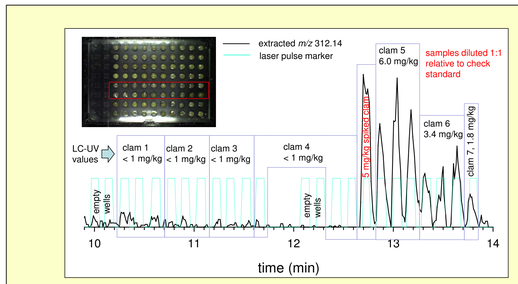


Figure 6: Sample data from LAESI-MS shellfish screening for DA

- All samples (n = 18) above the regulatory limit of 20 mg/kg were identified.
- Eight samples (~ 4%) were incorrectly identified as containing DA above 5 mg/kg.
- One sample (0.5%) with above 5 mg/kg was missed by LAESI-MS. This sample gave a value of 9.8 mg/kg by LC-UV, still under half the regulatory limit.

LAESI-HRMS screening results agreed well with quantitation by LC-MS and LC-UV and all toxic samples were successfully identified.

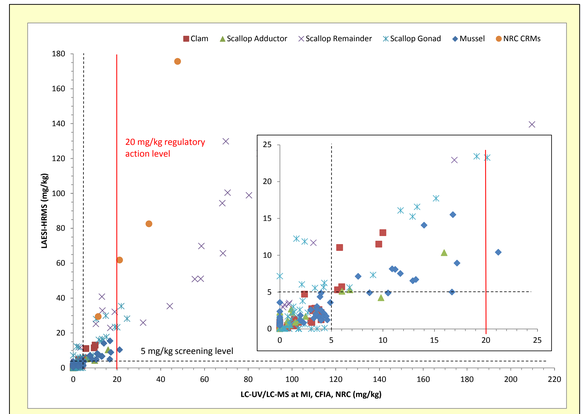


Figure 7: Comparison of LAESI-HRMS screening to quantitation by validated routine LC-MS and/or LC-UV methods.

Conclusions and Future Work

- LAESI-HRMS performed well as a high-throughput screening method for DA in a variety of shellfish matrices.
- No sample extraction or cleanup was required after tissue was homogenized. Analysis time was ~ 12 sec.
- Use of this technique could result in significant cost and time savings for regulatory testing labs and expand their capacity during periods of unusually high sample volume, such as the 2015 *Pseudo-nitzschia* bloom on the west coast of North America.
- Variable matrix effects between samples limited the utility of the technique for direct quantitation. Confirmatory analysis by LC-UV is currently required to quantitate DA in positive samples.
- The LAESI-MS system was very robust. Over 2000 analyses were done in 2 days. MS extension tube required cleaning after approximately 500 samples, which greatly exceeds sample volumes of routine use.
- Remaining challenges include how to store and aliquot shellfish homogenate standards required for LAESI-MS calibration. Supernatants showed similar response to homogenates and could be used as matrix matched standards.
- High-throughput quantitation by LAESI should be equally viable for other analytes with excellent ESI sensitivity, little to no matrix effects in LC-ESI-MS and relatively high action level.

Reference

1. DG Beach, CM Walsh, P McCarron. High-Throughput Quantitative Analysis of Domoic Acid Directly From Mussel Tissue Using Laser Ablation Electrospray Ionization - Tandem Mass Spectrometry. *Toxicon* **2014**, 92, 75-80.

Acknowledgements

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