Quantitative high-throughput analysis of domoic acid in mussel tissue homogenates using laser ablation electrospray ionization – MS/MS

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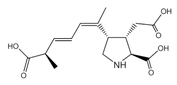
Abstract

We investigated the use of Laser Ablation Electrospray Ionization (LAESI) with MS/MS detection for Domoic Acid (DA) analysis directly from mussel tissue homogenates without sample extraction, cleanup or chromatographic separation. DA could be selectively detected directly from mussel tissue homogenates using LAESI-MS/MS in selected reaction monitoring scan mode. A matrix matched calibration curve was used to quantify mussel tissue reference materials and results were in good agreement with two established methods, LC-UV and LC-MS/MS, within the linear range. Most notable is the decrease in run time from about 20 minutes for a typical chromatographic method to about 10 seconds per sample for LAESI-MS/MS.

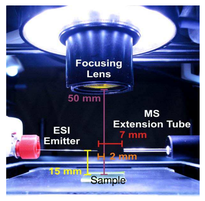
Introduction



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 after consuming contaminated blue mussels. Regulatory analysis of domoic acid is typically carried out by LC-UV using a 20 min run time 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 currently runs about 10, 000 shellfish samples annually testing for DA.

Laser Ablation Electrospray Ionization (LAESI) is an ambient ionization technique for mass spectrometry that uses a laser to produce a fine mist of neutral droplets of sample liquid followed by ionization using an electrospray plume. During application of the laser pulse, water functions as a matrix absorbing the mid-infrared laser energy which is then transferred to electrospray ionized droplets. This results in ionization specificity that is comparable to ESI rather than laser ablation techniques.



(A) Commercial LAESI System (B) LAESI – MS Source interface

Experimental

Sample Preparation – Mussel tissue samples were homogenized with an Omniprep homogenizer and aliquots (20 µL) were transferred to a low volume 96-well plate.

LAESI Ionization – A Protea LAESI DP-1000 direct ionization system was used to ablate samples with 30 pulses of a mid-IR (λ = 2940 nm) laser at 10 Hz with 700 µJ of energy.

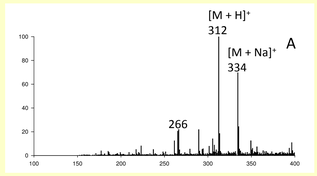
Mass Spectrometry – Thermo LTQ Velos linear ion trap operated in selected reaction monitoring (SRM) mode for all quantitative analysis monitoring the transition *m/z* 312 > 266. Additional product ions *m/z* 248 and *m/z* 175 were monitored for qualitative confirmation.

NRC Certified Reference Materials

National Research Council Reference Materials Used:

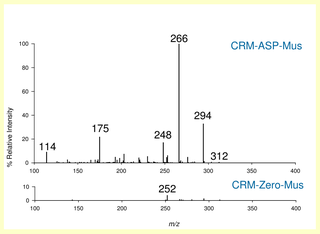
- *NRC CRM-DA-f*: certified reference material (CRM) calibration solution of 101.8 ± 2.1 mg/L DA
- *NRC CRM-ASP-Mus-d*: mussel tissue matrix CRM containing 49 ± 3 mg/kg DA
- *NRC CRM-Zero-Mus*: mussel tissue matrix CRM certified not to contain DA.
- *NRC CRM-DSP-Mus-c*: A mussel tissue matrix CRM certified for diarrheic shellfish toxins and also contains DA at 11.9 ± 1.2 mg/kg
- *NRC CRM-PSP-Mus*: A mussel tissue matrix CRM certified for paralytic shellfish toxins and also contains DA at 33 ± 2 mg/kg
- *NRC CRM-FDMT* – a freeze dried mussel tissue CRM certified for multiple lipophilic toxins and DA at 21.9 ± 0.9 mg/kg (wet)

DA Detection By LESI – MS/(MS)

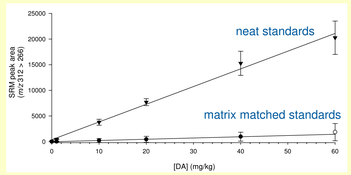


LAESI of DA standard shows comparable selectivity to electrospray ionization in full scan MS

Surprisingly good selectivity of MS/MS analysis of DA from tissue:



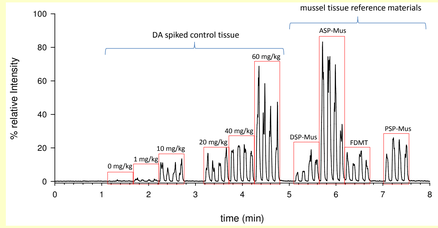
Product ion scan of [M + H]+ of DA at *m/z* 312 in mussel tissue homogenate. (A) DA containing CRM and (B) mussel tissue certified not to contain DA.



- Significant (94 %) ionization suppression observed in direct analysis of DA in tissue homogenates
- Linear range of matrix matched calibration curve was 1 mg/kg to 40 mg/kg (R^2 = 0.999)
- Method limit of detection for DA in tissue matrix was 1 mg/kg (S/N = 3)
- Well suited for analysis around the regulatory limit of 20 mg/kg

Extremely Rapid Analysis

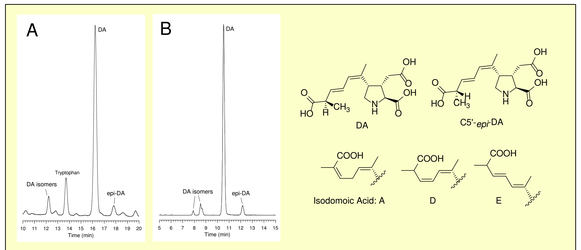
- LAESI-MS/MS analysis took just 10 sec/sample compared with ~ 20 min run time of chromatographic methods.
- No sample extraction or cleanup was required for samples in the linear range.



SRM plot of *m/z* 312 > 266 for DA in mussel tissue including quadruplicated samples of a matrix matched calibration curve and four mussel samples.

Quantitative Method Verification

Analysis of four mussel tissue reference materials was carried out with LAESI-MS and verified using two validated analytical methods under ISO 17025 guidelines.



Analysis of CRM-ASP-Mus by validated LC-UV (A) and LC-MS (B).

Reference Material	LC-MS/MS (mg/kg ± SD)	LC-UV (mg/kg ± SD)	LAESI-MS/MS (mg/kg ± SD)
CRM-DSP-Mus-c	11.9 ± 0.3	11.8 ± 0.7	15 ± 7
CRM-ASP-Mus-d (tissue)	49 ± 1	48.9 ± 0.4	80 ± 10
(extract)			50 ± 3[b]
CRM-FDMT [a]	21.7 ± 0.4	22.1 ± 0.4	22 ± 3
CRM-PSP-Mus	33 ± 1	32 ± 1	39 ± 10

[a] Tabulated values for FDMT represent mg/kg wet weight in reconstituted freeze dried tissue.
[b] DA concentration in ASP-Mus was beyond the linear range of the LAESI-MS/MS method, extraction and cleanup required.

- For CRM-ASP-Mus, with a DA concentration beyond the linear range of the matrix matched curve (49 mg/kg), a highly selective sample extraction (50 % MeOH) and SPE cleanup (strong anion exchange) was used to obtain good recoveries (102 %) *(Quilliam et. al. 1995).*
- Overestimation of values well above the regulatory limit (20 mg/kg) is not a significant limitation to the technique.

Conclusions

- Domoic acid could be quantified directly from mussel tissue homogenates by LAESI-MS/MS.
- Results showed good agreement with established methodology
- Analysis times of about 10 sec were a significant improvement over any established method.
- LAESI – MS/MS shows promise as a potential future regulatory method, either as a high throughput screening method or for direct quantitative analysis.

Future Work

- Further optimization of LAESI method to improve precision
- High resolution MS/MS detection for improved selectivity
- Analysis of a large set of shellfish samples in collaboration with regulatory agencies
- Expand study to include other important classes of algal toxins

References

DG Beach, CM Walsh, P McCarron. *Toxicon* **2014**, 92, 75-80.
MA Quilliam, M Xie, WR Hardstaff. *J. AOAC Int.* **1995**, 78, 543-554

Acknowledgements

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