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Rapid screening and quantitation of domoic acid in shellfish homogenates using laser ablation electrospray ionization mass spectrometry (LAESI-MS)

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Overview:

Developed LAESI-MS Orbitrap rapid screening method to quantify domoic acid directly from shellfish homogenates with minimal sample preparation, comparing results to traditional LC-UV/LC-MS methods.

Introduction:

Domoic acid is a neurotoxin produced by algal diatoms that accumulates in shellfish.¹ Due to the risk to human health, regulatory agencies worldwide are tasked with routine testing upwards of 10,000 shellfish samples per year with a regulatory limit of 20 mg/kg². Currently, sample preparation and LC-UV/LC-MS analysis methods often exceed 30 mins per sample^{3,4}. A rapid, quantitative screening process would be beneficial for routine testing during shellfish harvesting season, especially during an outbreak. Previously reported, domoic acid was directly analyzed by LAESI-MS/MS in blue mussel homogenates.⁵

Here, we report the use of laser ablation electro spray ionization (LAESI) interfaced to a high resolution/accurate mass instrument. The goal was to directly analyze shellfish homogenates and evaluate LAESI-MS as a screening method to identify samples with greater than 5 mg/kg domoic acid. In LAESI-MS, tissue homogenates were ablated with a mid-infrared laser (2.94 μm) at ambient pressure to form a plume of neutrals that are intercepted by electro spray to ionize the sample prior to introduction into the mass spectrometer (Figure 1). The LAESI-MS quantitative results were compared to traditional LC-UV/LC-MS.

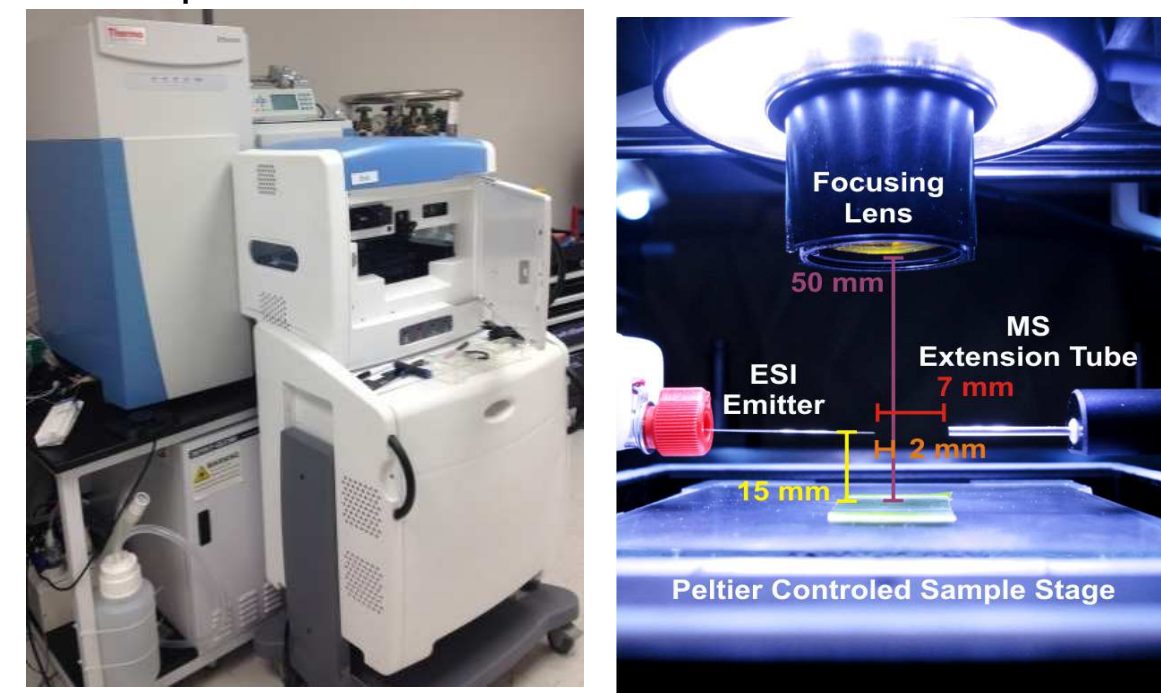


Figure 1: LAESI DP-1000 Direct Ionization System on Q Exactive mass spectrometer. The annotated image depicts the critical distances for optimal LAESI-MS results. The electro spray emitter is a 5 cm stainless steel tip with 320 μm OD and 100 μm ID (New Objective, Woburn, MA).

Methods:

A sample set consisted of 189 shellfish homogenates (clam, mussel, scallop adductor, scallop gonad, and scallop remainder), mussel homogenate certified reference standards, and a six point matrix calibration curve, 1-40 mg/kg of domoic acid per tissue type. High resolution accurate mass spectrometric methods were developed for targeted select ion monitoring (SIM) and MS/MS using matrix reference materials. Method development and rapid quantitative screening were performed using LAESI DP-1000 Direct Ionization System connected to a Q Exactive (Thermo Scientific) mass spectrometer. A total of 50 laser pulses (mid-IR 2940 nm) at 10-20 Hz and 700 μJ laser energy ablated the 20 μL tissue homogenate directly in a shallow 96-well plate. The LAESI quantitative results were compared to LC-UV/LC-MS methods. Domoic acid (m/z 312.1440) average peak height was used to quantitate domoic acid.

LAESI-MS Method Optimization:

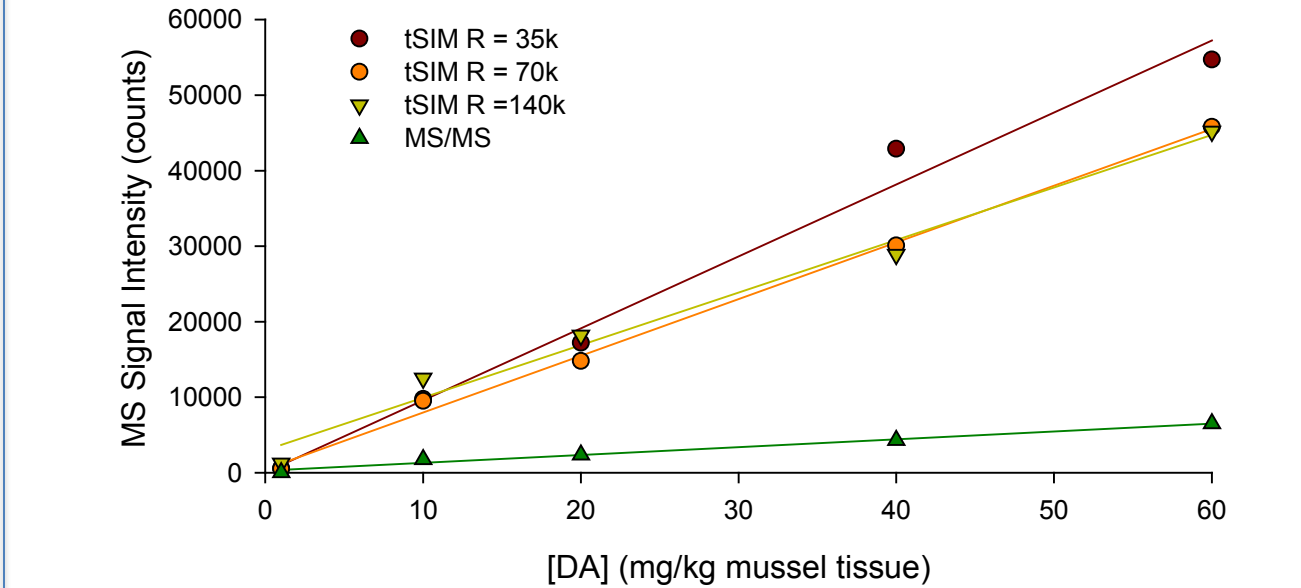
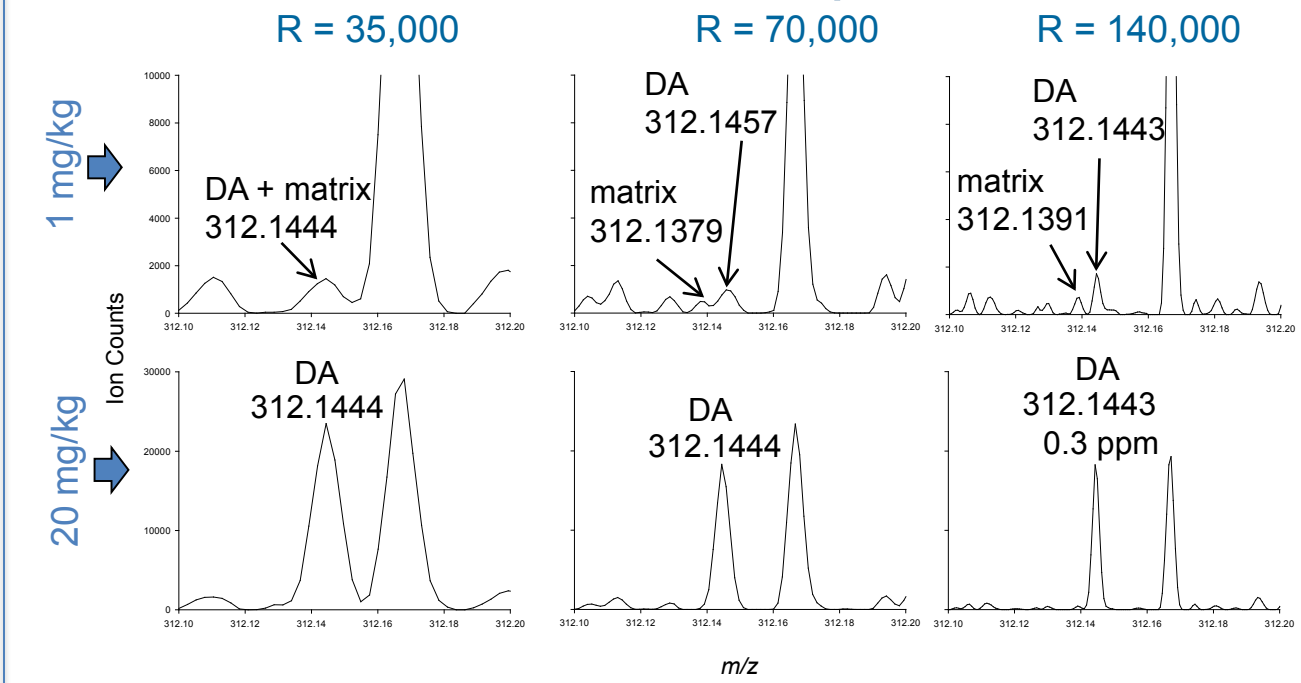
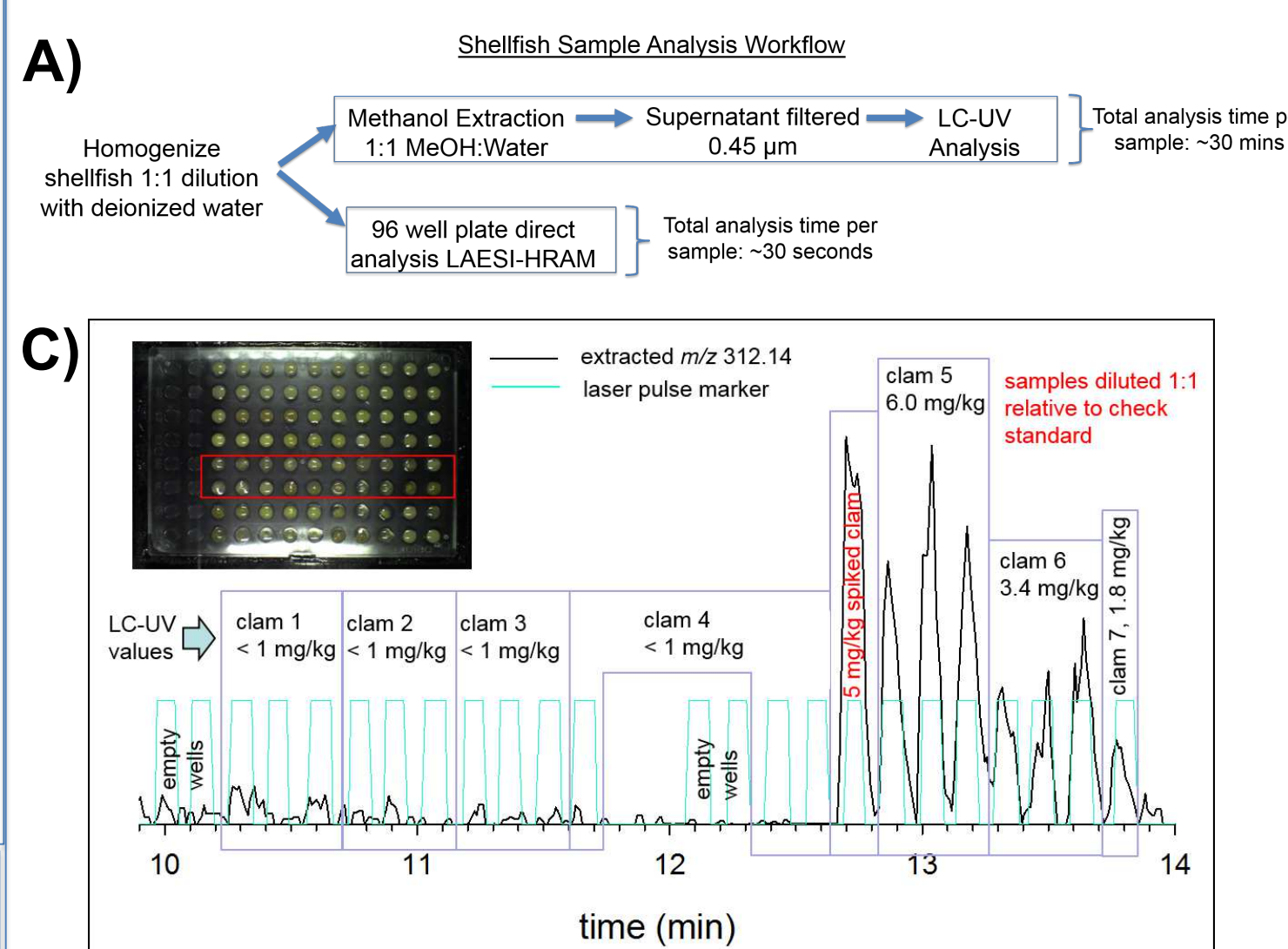


Figure 2: LAESI-MS method optimization on mussel homogenate. High mass resolution was required to resolve an interfering peak using the SIM scan mode (above). Targeted-SIM provided the greatest sensitivity with LOD of 1 mg/kg with an Orbitrap mass resolution setting of 140,000 to resolve a mussel homogenate matrix ion.

Domoic Acid Rapid Analysis:



Matrix Matched Calibration Curves:

Tissue Matrix	LOD ^a (mg/kg)	Precision of Matrix Standard %RSD	R ² of Matrix Matched Calibration Curve
Scallop Adductor	0.50	27 (N = 13)	0.994
Scallop Gonad	1.6	38 (N = 18)	0.980
Scallop Remainder	0.62	38 (N = 12)	0.980
Clam	0.24	44 (N = 13)	0.9992
Mussel	1.1	36 (N = 22)	0.9991

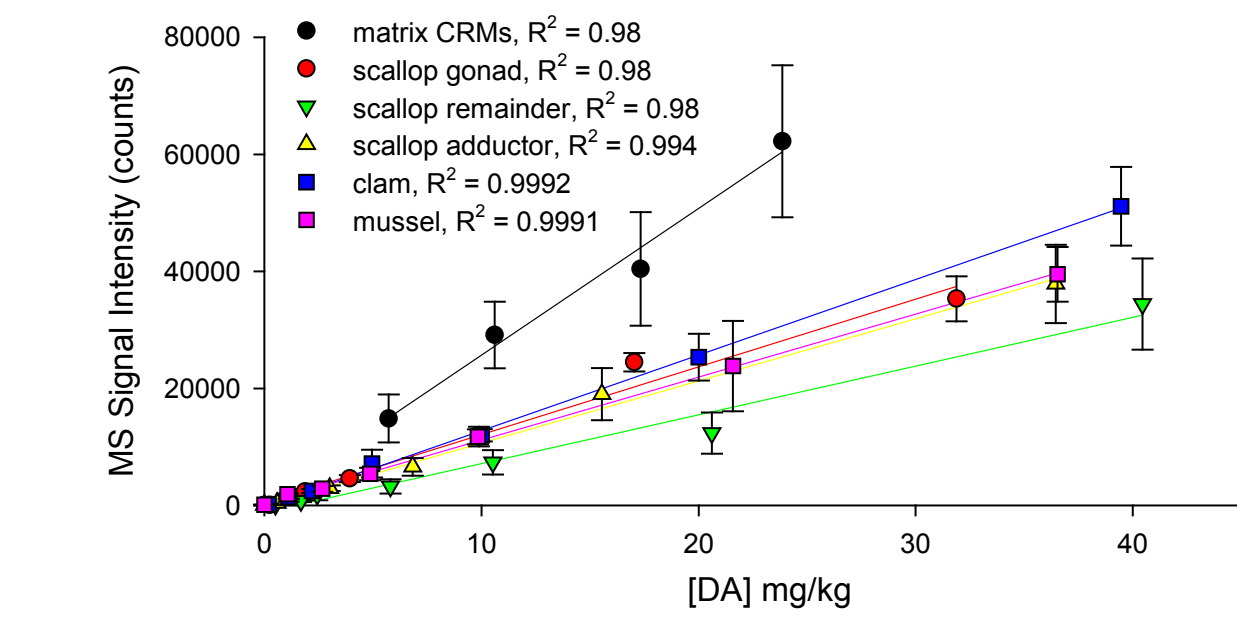


Figure 3: Rapid analysis of domoic acid in shellfish homogenates. A) Overall shellfish sample analysis workflow for LAESI-MS and traditional LC-UV/LC-MS. B) LAESI-MS matrix matched calibration curves per homogenate sample matrix type over 1-40 mg/kg range. The LOD of domoic acid per matrix is reported here includes the 1:1 dilution factor used in sample preparation. Matrix matched 5 mg/kg domoic acid was analyzed throughout the analyses as a quality control and the precision is reported here as %RSD. C) Extracted ion chromatogram (XIC) of LAESI-MS analysis of domoic acid m/z 312.1440. The green trace depicts the LAESI laser analog at each well 50 pulses at 10-20 Hz. D) LAESI-MS comparison to LC-UV and LC-MS. The overall correlation coefficient was calculated to have R² 0.89 for the linear regression between LAESI-MS and LC-UV/LC-MS techniques. All samples (n=17) above the 20 mg/kg regulatory action level (red line) were correctly identified by LAESI-MS. Eight false positives were incorrectly identified by LAESI-MS to be above 5 mg/kg (4% false positive rate).

Conclusions:

- LAESI-MS performed well as a robust high-throughput rapid screening tool between 1 and 200 mg/kg domoic acid in a variety of shellfish homogenate matrices.
- LAESI-MS required very minimal sample preparation for direct analysis.
- Over 2,500 shellfish samples were analyzed in triplicate over 2 days. Analysis time per sample was 30 seconds.
- LAESI Bridge software (Gubbs v8.4.32) could be used to rapidly extract average signal intensity for rapid quantitation of up to 11 m/z per analysis.
- This technique could result in significant cost and time savings for 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.

Acknowledgements:

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LAESI Bridge Software

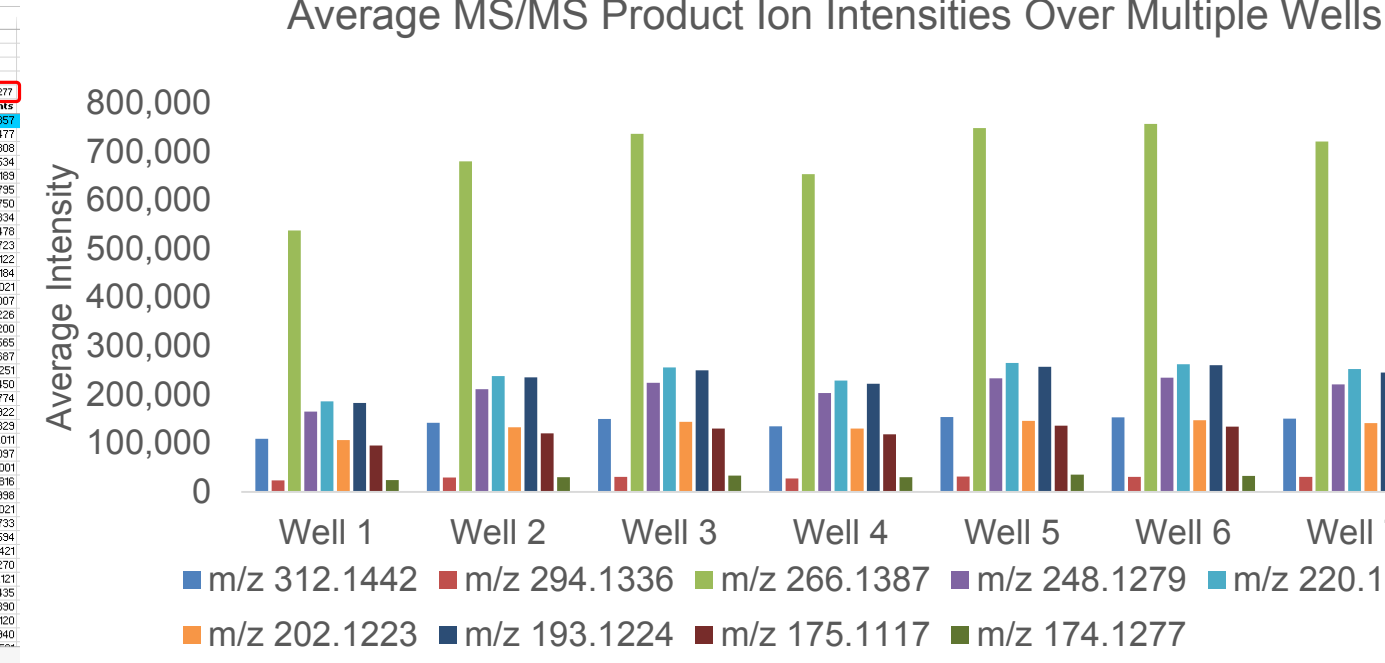
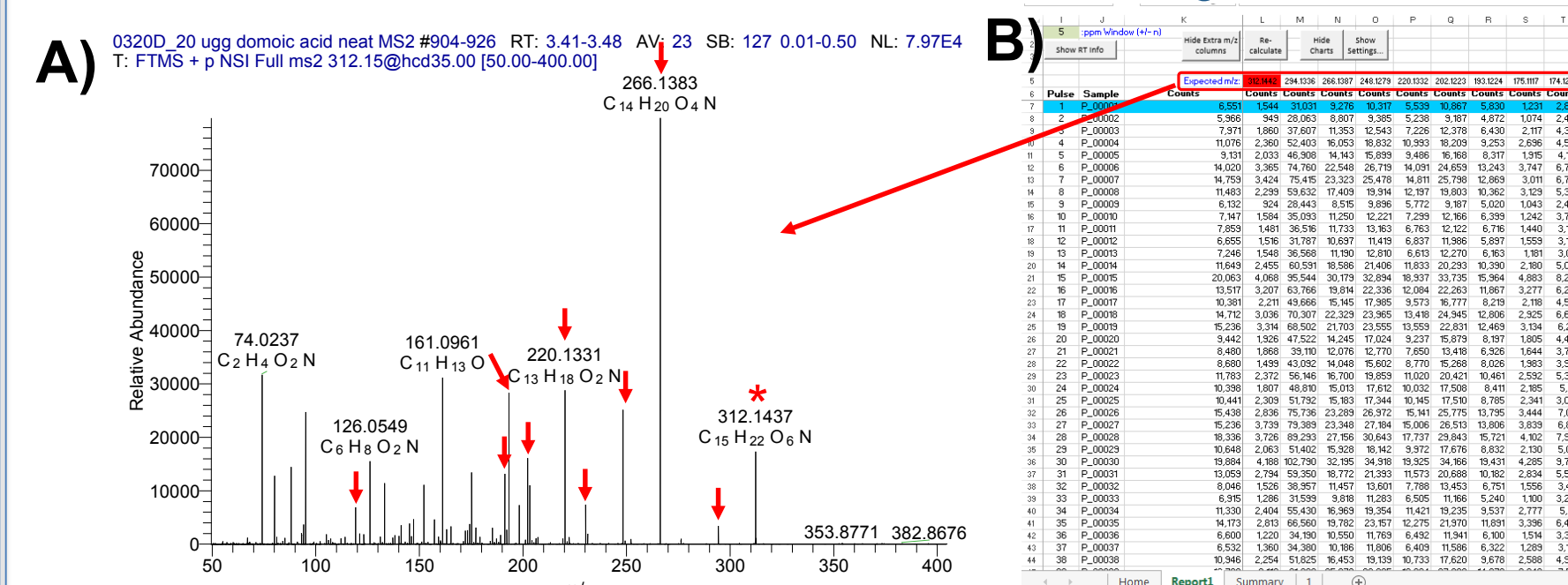


Figure 6: LAESI-MS/MS analysis of domoic acid (targeted m/z 312.15, HCD35) 20 μg/g neat standard in 96 wells. A) LAESI-MS/MS average spectrum from representative well analysis, with product ions denoted with red arrows. B) LAESI Bridge (Gubbs software v8.4.32) is Excel based that can rapidly extract up to 11 m/z intensities per well per analysis.