MD/VA Joint Harmful Algae Task Force Meeting
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Novel DSP-Like Toxins Associated with Blooms of *Dinophysis norvegica* in Maine, USA with Implications for Shellfish Management

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Collaborators

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  - US FDA Center for Food Safety and Applied Nutrition
- **Pearse McCarron**
  - National Research Council of Canada
- **Juliette Smith**
  - Virginia Institute of Marine Science
- **Jerry Borchert**
  - Washington State Department of Health
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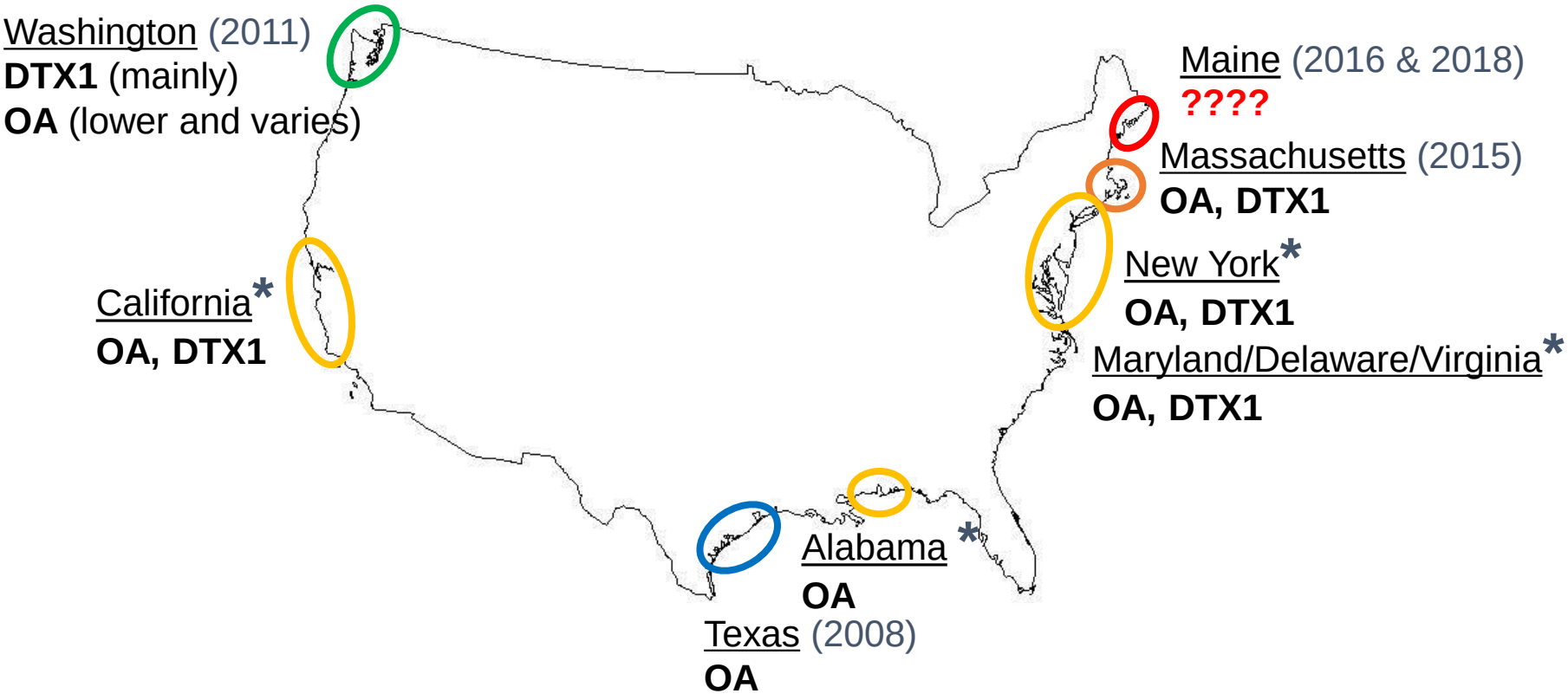
  - Washington State Department of Health Public Health Laboratories
- **Bich-Thuy Eberhart**
  - NOAA Northwest Fisheries Science Center

DSP Management in the US

- In the US marine biotoxin hazards in shellfish are managed through the National Shellfish Sanitation Program (NSSP).
- In 2017 a Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method using acidic chromatography was adopted as the reference method for DSP toxin monitoring under the NSSP.
- States are allowed to use alternate methods for the purposes of screening and precautionary closures.
- A commonly used alternate method is the Protein Phosphatase Inhibition Assay (PPIA) [Developed by ZEU Imnumotec, Spain and distributed in the US by Abraxis]

History of DSP Shellfish Harvesting Closures in the US



* Non-commercial product and/or below guidance level

2016 Maine *D. norvegica* Bloom

- From July 5 – August 29, 2016 a large monospecific bloom of *D. norvegica* occurred on the central coast of Maine.
- Maine Department of Marine Resources (ME DMR) found levels of DSTs in excess of the 16 µg/100g OA eq.US guidance level by PPIA and closed shellfish harvesting on July 20.
- Samples sent to Bigelow Analytical Services for confirmatory LC-MS/MS testing only detected trace amounts of DTX1.
- Subsequent re-testing by both PPIA and LC-MS/MS by the FDA and the Washington Department of Health confirmed these findings.
- High resolution LC-MS testing by NRC Canada found no other lipophilic shellfish toxins to be present.
- ME DMR re-opened shellfish harvesting on August 5 and PPIA results were considered to be false positives.

Analysis of Shellfish Samples from 2016 Maine *D. norvegica* Bloom

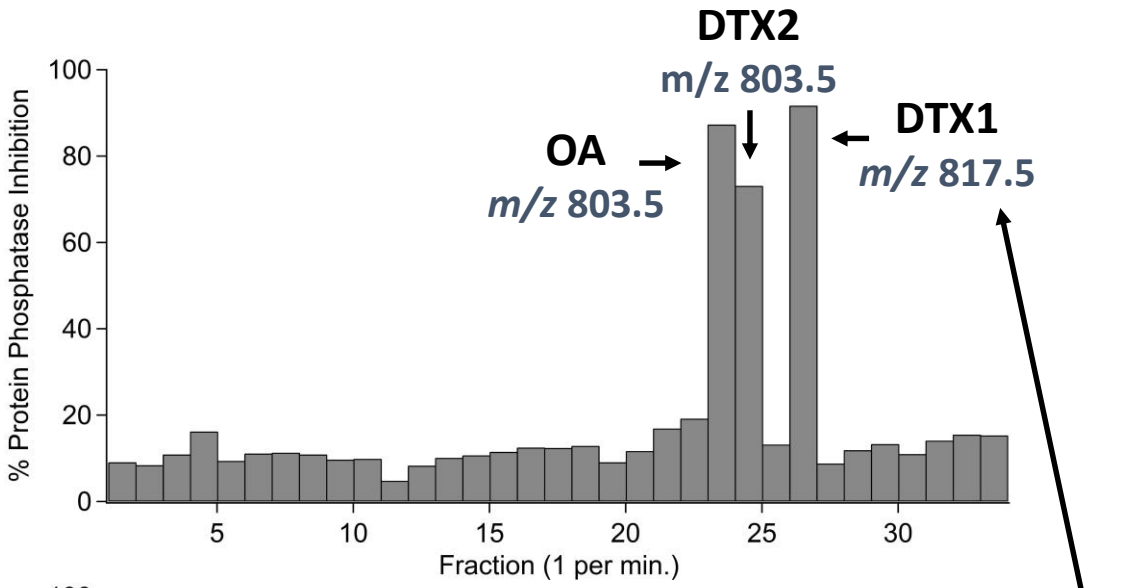
Sample	PPIA		LC-MS/MS *		ELISA	
	FDA	ME DMR	BIGELOW	WA DOH	NEOGEN	BIOO
1	28	17	ND	ND	Positive	27
2	< 6	NT	<1	<2	Negative	2
3	14	12	ND	ND	Negative	12
4	< 6	< 6	4	5	Negative	5
5	20	18	ND	<2	Positive	19
6	17	31	ND	ND	Positive	13
7	> 35	> 35	ND	ND	Positive	53
8	10	7	ND	ND	Negative	6
9	19	20	ND	ND	Negative	11
10	8	7	ND	ND	Negative	6

Red: Exceeds Guidance Level *Tests for OA, DTX1, DTX2 only

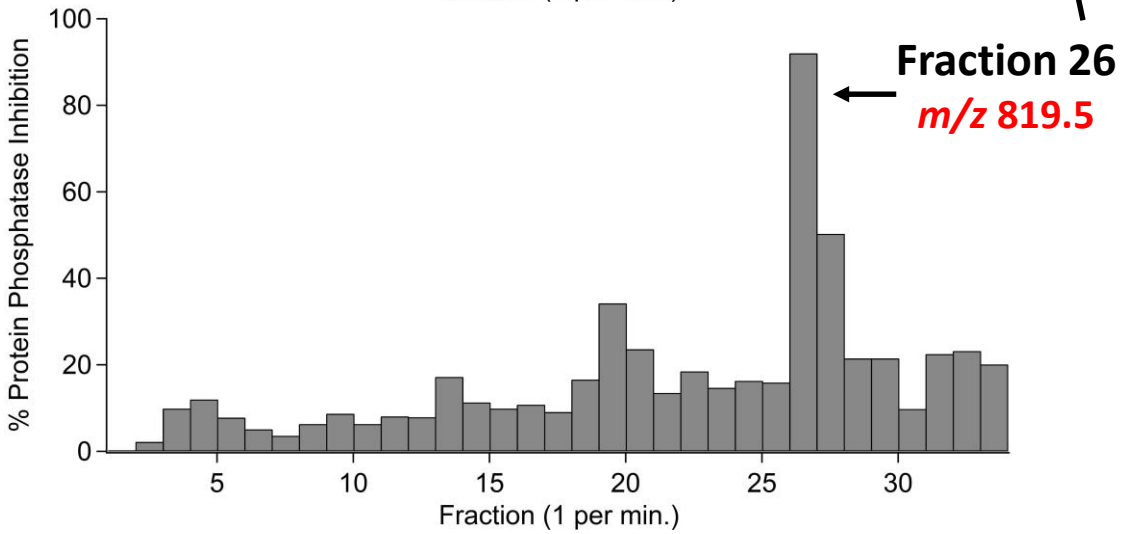
PPIA-Based Bioassay Guided Fractionation

(following *Holmes, 1991, Toxicon 29: 469-477*)

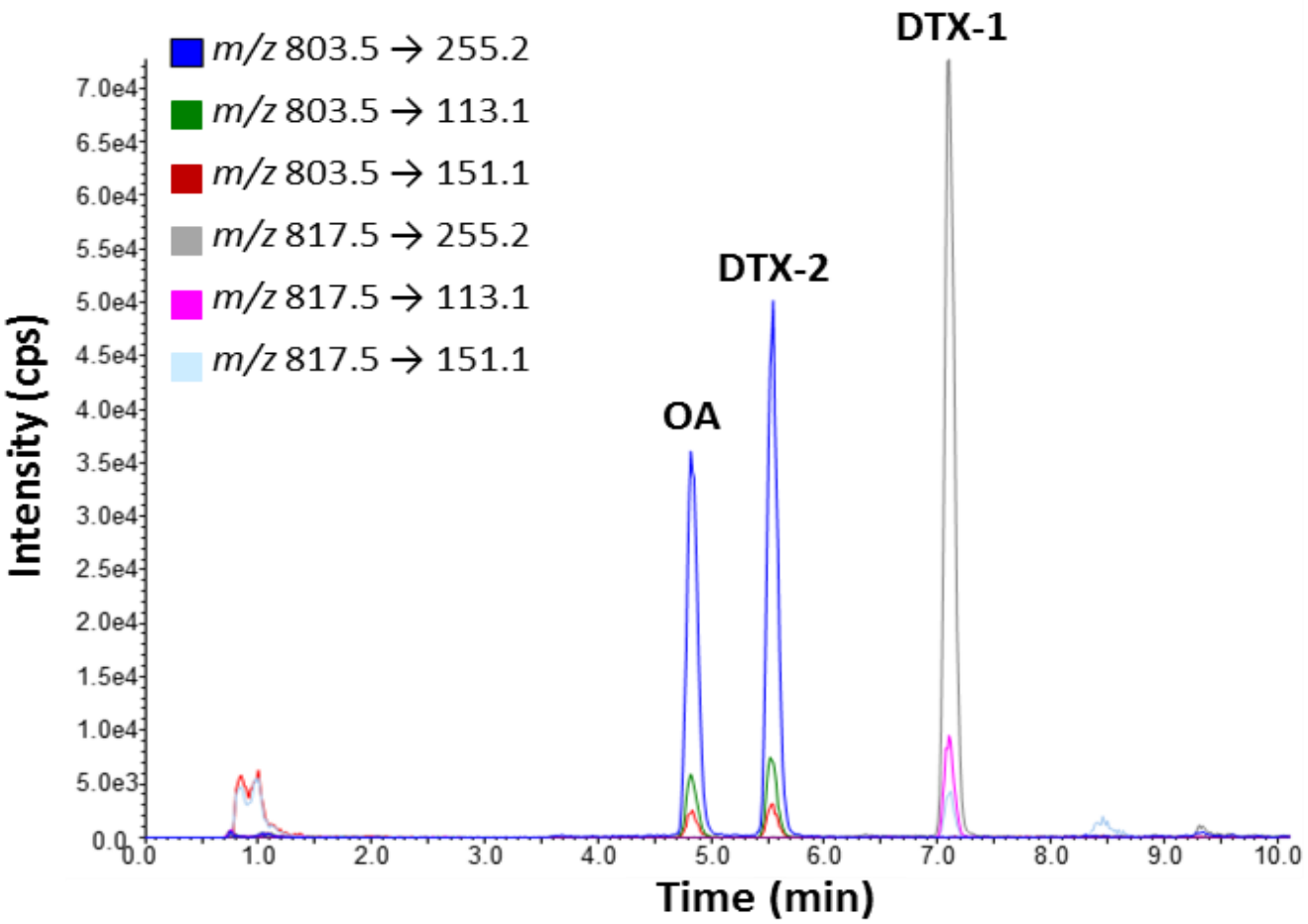
Shellfish Spiked with 16 µg/100g OA, DTX1, DTX2



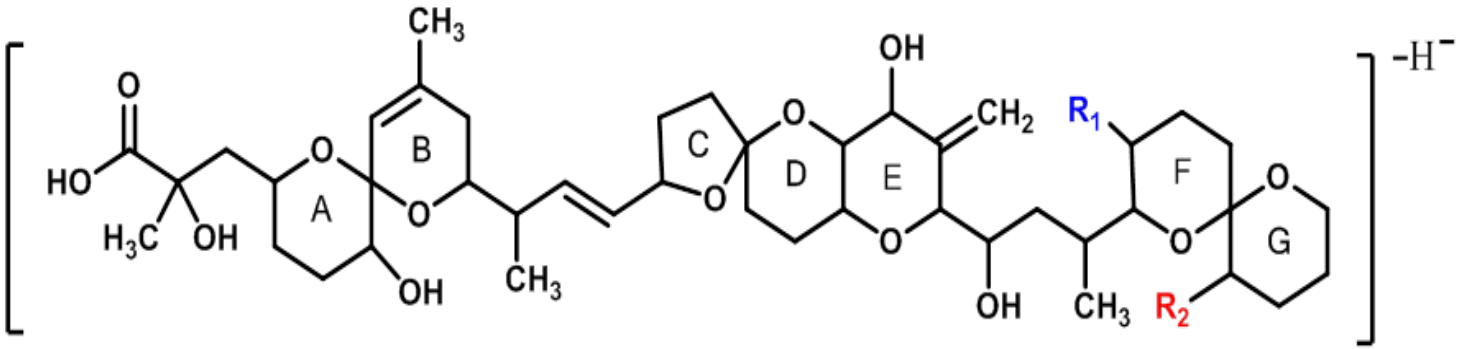
2016 Shellfish Sample from Maine *D. norvegica* Bloom



LC-MS/MS Multiple Reactions Monitoring Method for DSP

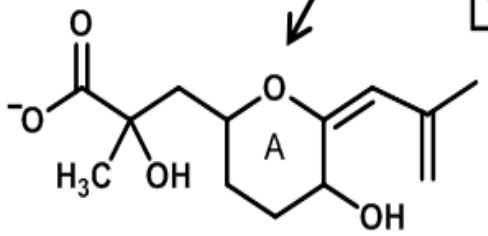


LC-MS/MS Multiple Reactions Monitoring Method for DSP



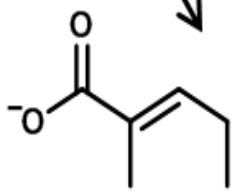
	R_1	R_2	*m/z*
OA	CH_3	H	803.5
DTX1	CH_3	CH_3	817.5
DTX2	H	CH_3	803.5

Quantitation Ion

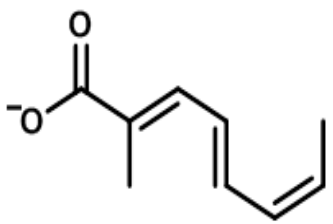


m/z = 255.2

Confirmatory Ions

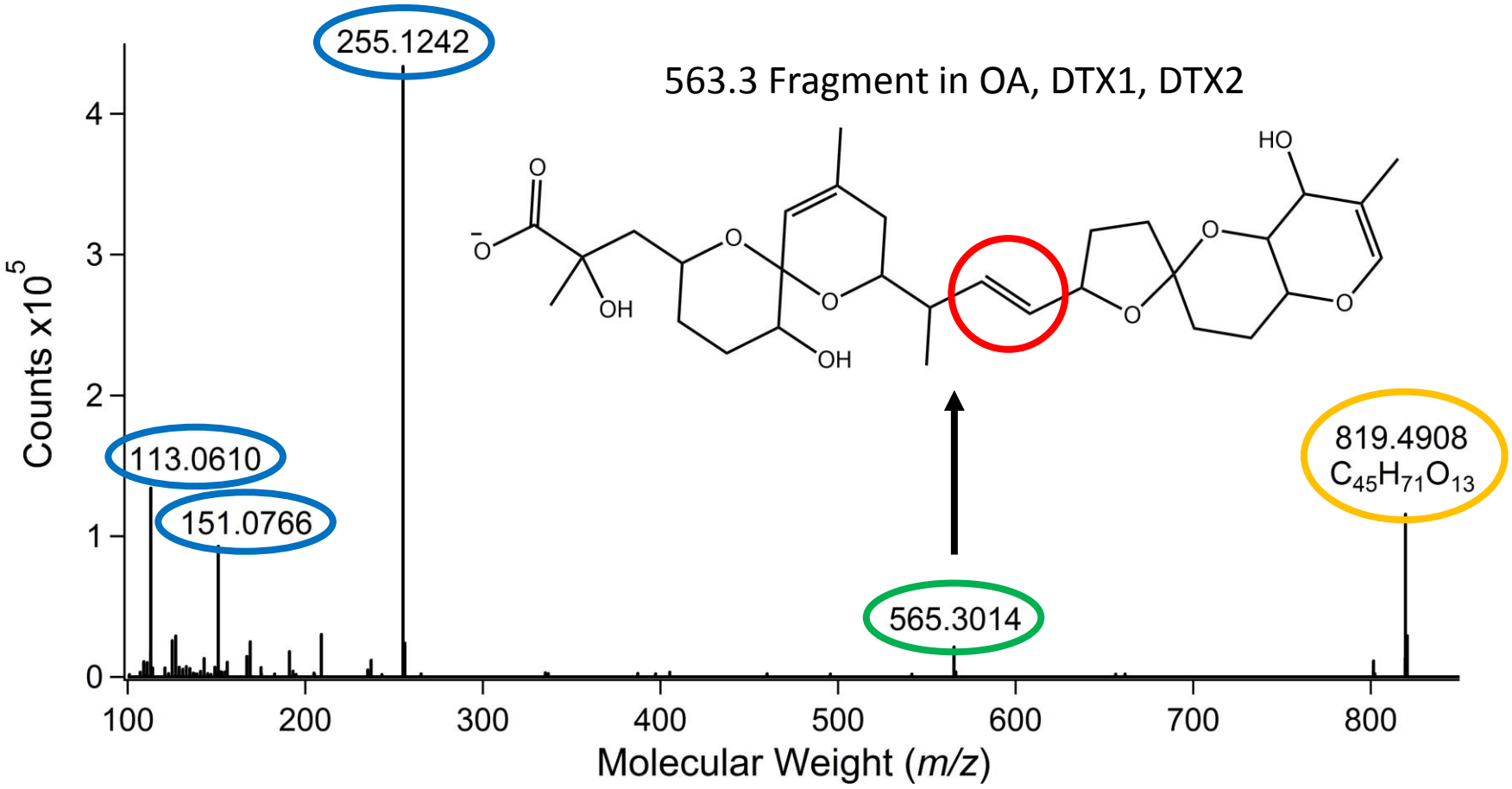


m/z = 113.1



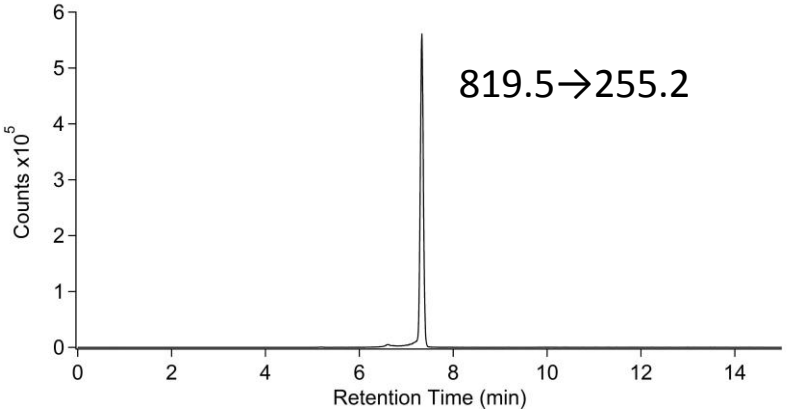
m/z = 151.1

Dihydro-DTX1

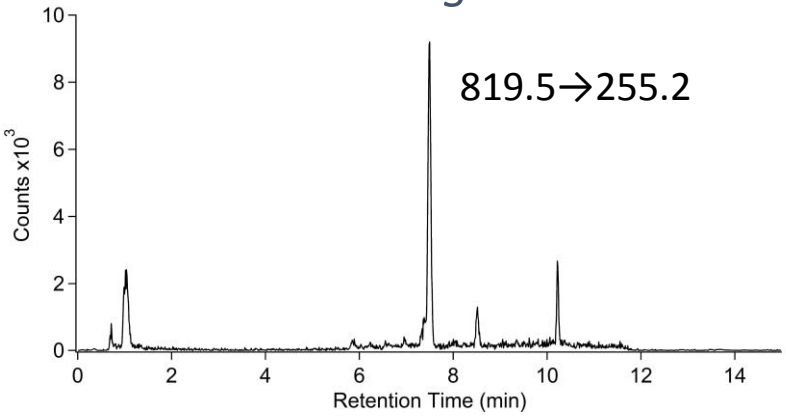


LC-MS/MS Multiple Reactions Monitoring for OA, DTX1, DTX2, dihydro-DTX1

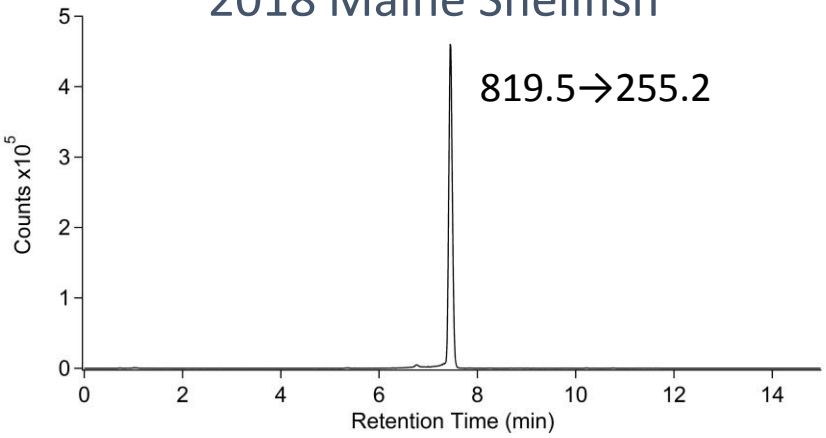
Fraction 26



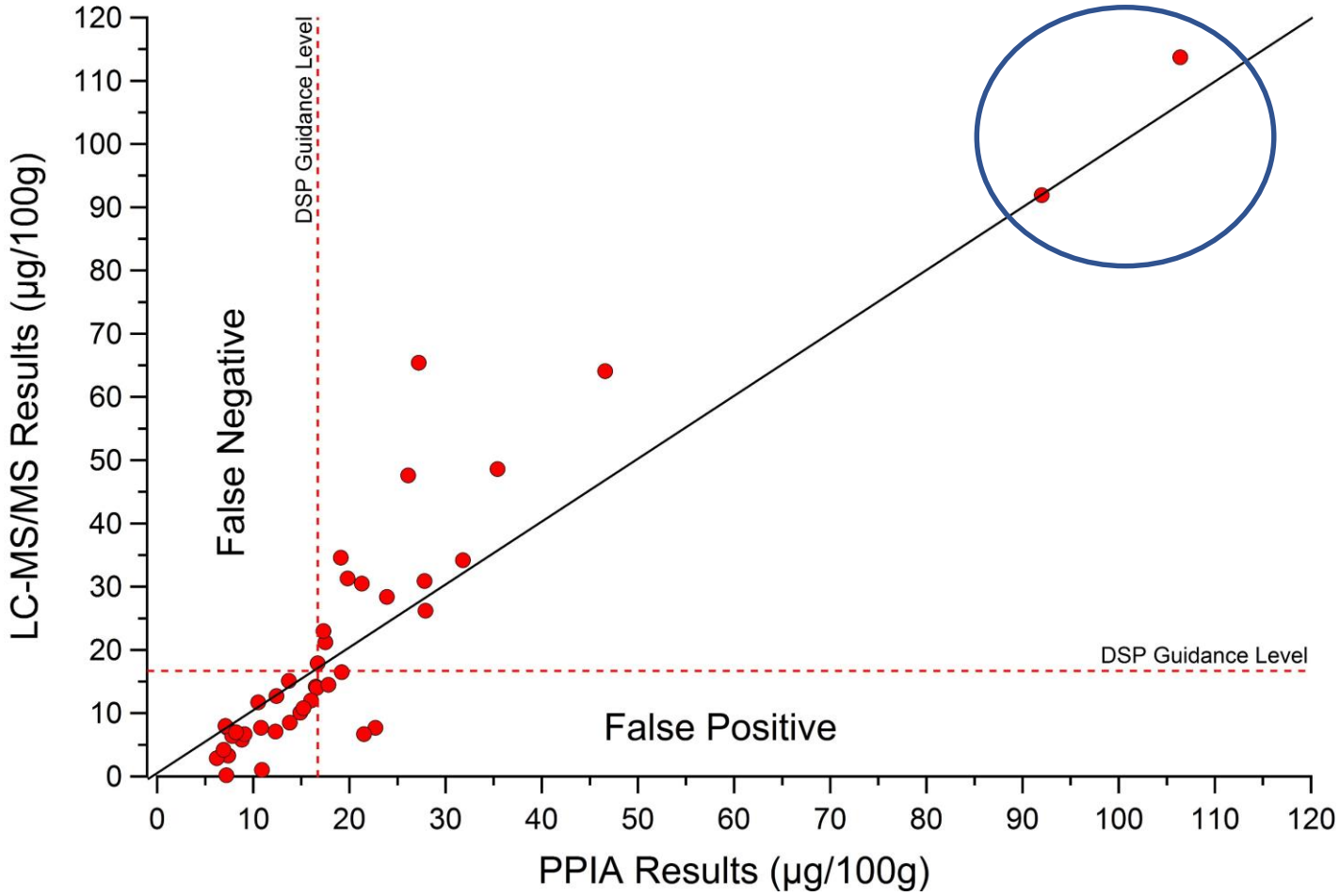
2018 *D. norvegica* Bloom



2018 Maine Shellfish

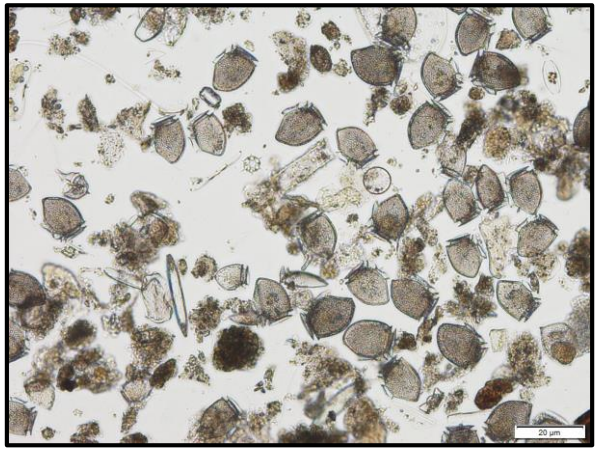


LC-MS/MS vs. PPIA for Measurement of dihydro-DTX1



Next Steps…

- Confirm production of dihydro-DTX1 in *D. norvegica* in culture
- Determine TEF
- Need to produce a standard
- Determine if *D. norvegica* produces dihydro-DTX1 in other regions of the world



Photos: J. Wolny