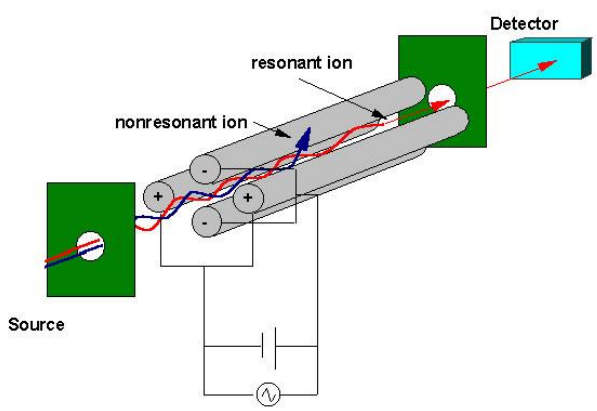
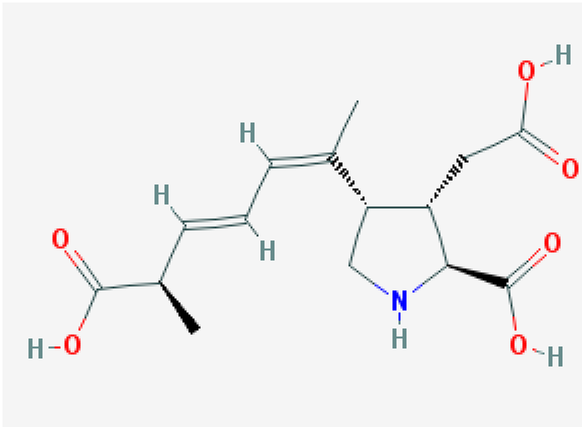
Obtaining Spectra **TMPL UAB**

- **Materials/equipment**
  - Infusion Pump(Harvard Apparatus)
    - Delivers a supply of sample into the MS
  - API 4000 Triple Quadrupole Mass Spectrometers(Sciex)
    - Ionization chamber
    - Electromagnetic Lenses/Quadrupole
    - Electronic detector – output in intensity
  - Gas-Tight syringe
  - Compound of interest
    - Preferably standard/pure quality
    - In solution 50% H2O/50% Organic or higher organic content with buffer.
    - Tend to want ~1 ug/ml or 100 ng/ml concentration for this process.
- **Vocabulary**
  - *Quadrupole*
    - Focuses & stabilizes ions through the machine while excluding those not selected.
    - 4 rods connected to Direct Current(DC) & Radio Frequency(RF) generators that are calibrated over a specified m/z range.



  - *Mass-to-charge ratio*(*m/z*)
    - The ratio of a molecule's mass(m) to the charge(z) on it.
    - Most small molecules have a signally charged state where as large biomolecules, such as proteins, can have multiple charged states.
    - Mass-to-charge can occur in positive and negative polarity depending on the compound of interest.
- **Analyte of interest**
  - *Domoic Acid*(DA, pictured below) is a toxin produced by algae blooms and is associated with Amnesic Shellfish Poisoning(ASP)[2]. DA is harmful to human health and exposure may occur through consumption of marine organisms that have bioaccumulated DA from their environment. CNS glutamate signaling is dysregulated due to DA's affinity and excitation of glutamate receptors(GluR) associated with neuronal synapses. DA's excitoxicity on the neuron leads to further disruption and damage to the CNS.[1] Due to a poisoning incident in Canada in 1987 the limit of DA in shellfish and crustacean tissue

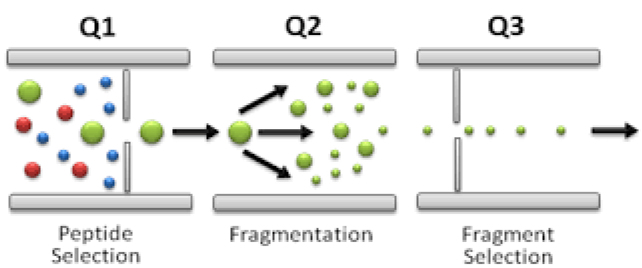


is set at 20 ug/g shellfish tissue[2]. Due to DA's relation to human health it is valuable to identify and quantify the amount food products.

Pictured: Domoic Acid [3]

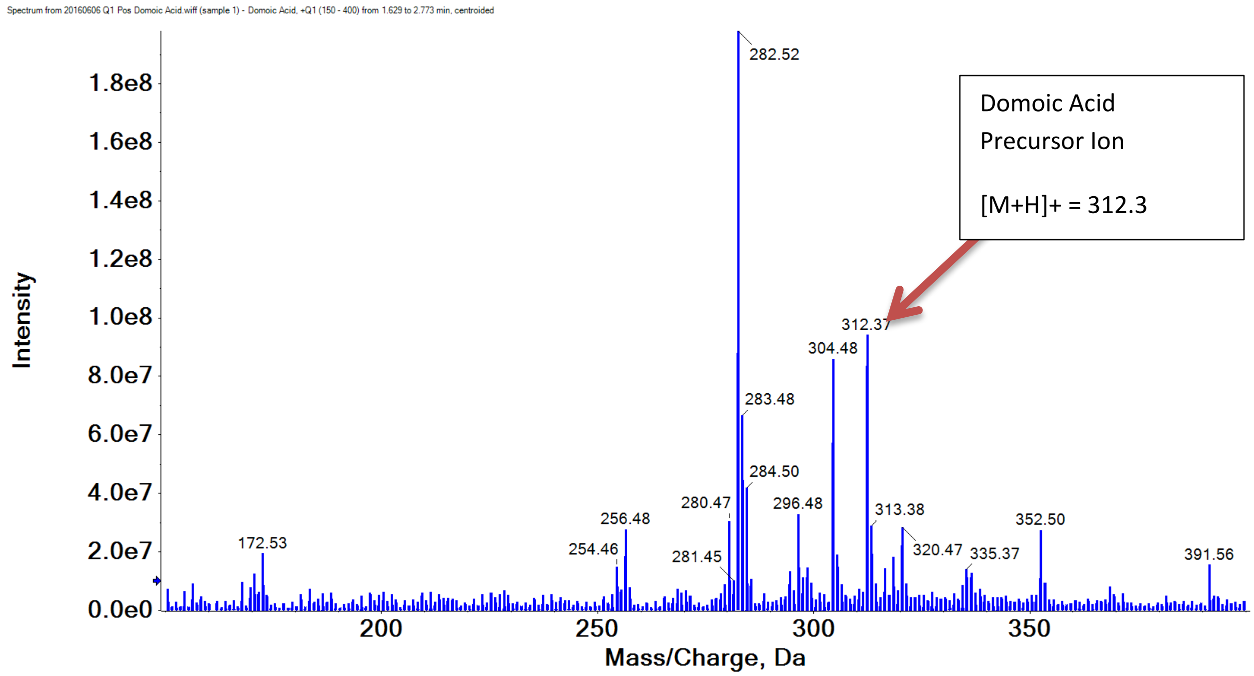
- DA MW = 311 g/mol
- Positive ion DA *m/z* = 312 charged

- **How to obtain spectra**
  - A triple quadrupole mass spectrometer, pictured above, consists of an ion source leading to an initial quadrupole – **Q1**- to a Nitrogen gas containing collision cell – **Q2**- and through a second set of quadrupoles – **Q3**- and finally to **detector**.



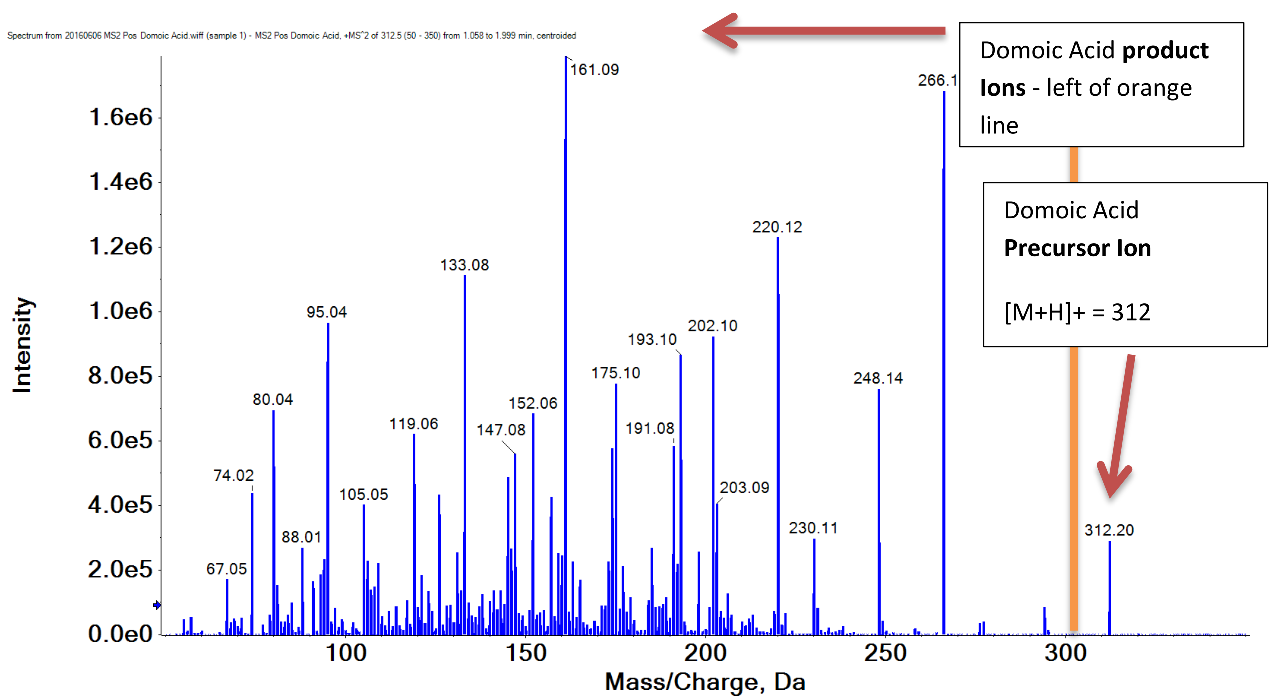
Pictured – Model of a triple-quadrupole instrument

  - For obtaining **spectra** for an a compound only Q1, Q2, and the detector will be utilized
  - **To observe the precursor ion – Survey scan**
    - Compound of interest is infused into the instrument at a rate of 10 ul/min in a 1 ug/ml solution.
    - **Q1** scan, or **survey scan**, of a compound will provide information about ions without fragmentation.



Pictured: **Q1**, or **survey scan**, of Domoic Acid – 312.37 m/z pictured. Other signal is generated from other ions in solution.

- **To obtain fragmentation of the precursor ion – MS2 scan**
  - **MS2** scan of a compound will provide structural information by fragmenting the **Precursor-ion** & detecting the **product ions** that form as a result.
  - Q1 focuses ion 312 *m/z* into collision cells, Q2, and Q3 transmits all fragments to the detector.



Pictured: **MS2**, or **fragmentation**, of Domoic Acid parent ion 312 m/z. Product ions, of lower *m/z* values, are generated and detected when fragmentation. The data generated by fragmentation is the **spectra** of the compound of interest.

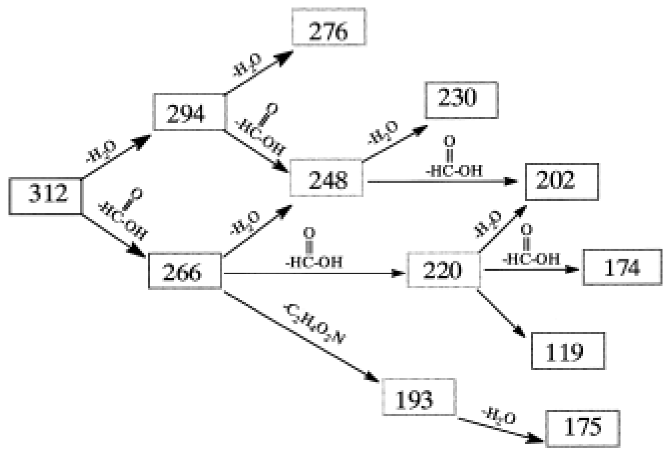
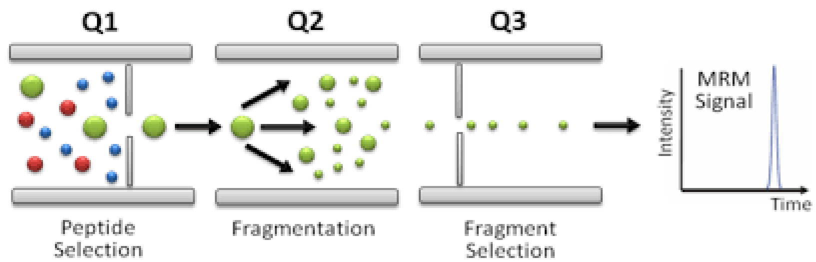


Fig. 2. MS fragmentation pathway for domoic acid (positive ion mode).

Pictured: Determination of domoic acid structure & fragmentation by interpretation of Spectra[4]

- **You have confirmed the compound of interest, now what?**
    - Need to verify the spectra obtained from compound of interest. Is it correct?
        - Literature sources
        - PubChem - https://pubchem.ncbi.nlm.nih.gov/
        - m/z Cloud - https://www.mzcloud.org/
        - METLIN - https://metlin.scripps.edu/metabo_search_alt2.php
            - Note – requires accurate mass
        - Etc.
    - The results of the survey scan and fragmentation scan are utilized to generate a '**mass transition**.'
    - Using the known precursor ion and major product ions the QQQ MS can be instructed to allow only the precursor ion through Q1 followed by the collision cell. Q3 will be instructed to allow only assigned product ions through to the detector.
        - Example of DA 312 *m/z* → 266 *m/z*



- Infusion & fragmentation alone ***will not*** allow determination quantity of domoic acid.
- **Mass transitions can be utilized for quantification purposes** using more advanced techniques. Ali will touch on the follow up techniques which are used for quantification.

Citations:
[1] Pulido OM. Domoic Acid Toxicologic Pathology: A Review. *Marine Drugs*. 2008;6(2):180-219. doi:10.3390/md20080010.

[2] Kathi A. Lefebvre, Alison Robertson, Domoic acid and human exposure risks: A review, Toxicon, Volume 56, Issue 2, 15 August 2010, Pages 218-230, ISSN 0041-0101, http://dx.doi.org/10.1016/j.toxicon.2009.05.034.

[3] https://pubchem.ncbi.nlm.nih.gov/compound/domoic_acid

[4] Determination of domoic acid in shellfish by liquid chromatography with electrospray ionization and multiple tandem mass spectrometry.
Furey A, Lehane M, Gillman M, Fernandez-Puente P, James KJ.
J Chromatogr A. 2001 Dec 14;938(1-2):167-74.