

Exercise = black text; Key = blue text

Learning Objectives.

After completing these exercises, you will be able to...

- (1) Explain the relationship between a total ion chromatogram (TIC), an extracted (or selected) ion chromatogram (XIC), and a tandem (MS/MS) mass spectrum.
- (2) Interpret GC-MS and LC-MS/MS data and predict/explain simple fragmentation patterns

Exercise 1.

Within your group of three, assign and distribute a different color (RED, BLUE, YELLOW) “Duplo” molecule to each person.

In the table below, record the number of individual blocks that make up each color. These will represent the molecular masses of your group’s three molecules.

	Molecular Mass
RED	12 (compact)
BLUE	12 (branched)
YELLOW	16

- a) Predict the elution order of the three molecules following GC chromatographic separation. Assume that RED has the lowest boiling point and YELLOW has the highest.

Elution order: 1st RED 2nd BLUE 3rd YELLOW

- b) What logic could you use to predict how each Duplo molecule would break apart (“fragment”) if dropped on the ground?
 Figure out where the weak points are within the molecule and evaluate which parts appear to be more exposed
- c) How would this same logic apply to the fragmentation of actual molecules during GC-MS? (In other words, why do molecules break apart in certain places and not in others?)
 Should be related to bond strength and stability of molecules and fragments
- d) Fill in the table below with your predictions for the “masses” (# of blocks) of the likely fragments of each molecule. [Note: each molecule does not necessarily need to form five different mass fragments.]

		Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5
	Molecular Mass	Mass	Mass	Mass	Mass	Mass
RED	12 (compact)					
BLUE	12 (branched)					
YELLOW	16					

Once your group has agreed on an elution order and predicted fragment sizes, bring your intact molecules up to the front of the class and place them in the correct order. If another group has a different elution order than you, discuss your reasoning and come to a consensus.

Now let's look at how each molecule fragments in the mass spectrometer.

- e) Will every fragment be detected?

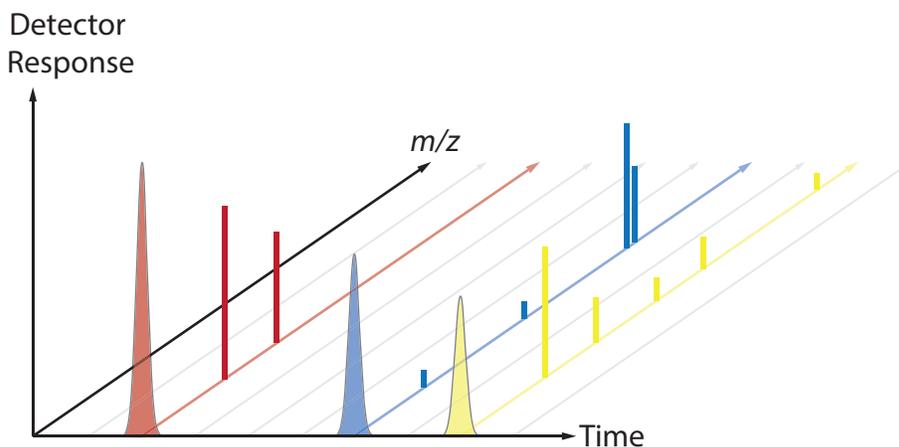
No, because only the ones with charge will get through the mass analyzer to reach the detector.

- f) What's wrong with the following description of the fragmentation pattern in a mass spectrum?
"Each fragment is formed by the breakdown of the next largest fragment."

Fragmentation is NOT sequential from largest to smallest, and individual molecules can fragment in different ways. In MS, many million molecules are being fragmented at once and the fragments that show up in the mass spectrum are simply the most probable.

- g) Describe how you would plot the total ion chromatogram (TIC) for this example and explain which ions contribute to the TIC signal.

To get the TIC you would sum up all of the charged fragments at every time point along the chromatogram. Ions contribute to the signal if they are within the range of m/z values selected by the mass analyzer.



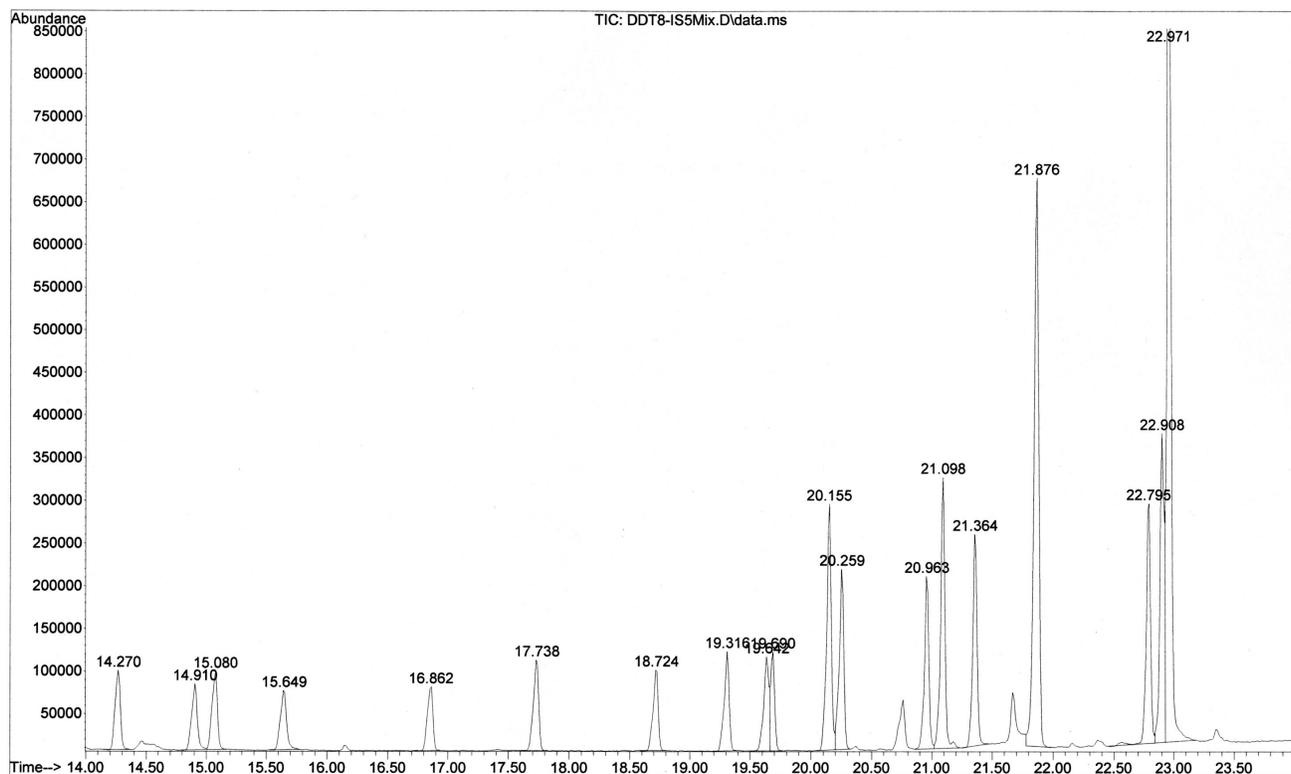
- h) Describe how you would plot an extracted ion chromatogram (XIC) for this example.

An "extracted ion chromatogram" (XIC) displays the intensity of only a particular ion (or ions) of interest over time.

Exercise 2. (Adapted from Bullen, Fitch, Kelly, and Larive, 2013, *Environmental Analysis – Lake Nakuru Flamingos: Pesticides*, Analytical Sciences Digital Library, Online.)

Mass spectrometry is widely used in analytical chemistry, forensics, bioanalysis, and environmental analysis. Some advantages of mass spectrometry include its sensitivity and low detection limits. Both qualitative and quantitative results can be obtained using a mass spectrometer. However, mass spectrometers are generally more costly than electrochemical and spectroscopic instruments.

A total ion chromatogram (TIC) was collected by GC-MS for a standard solution containing a mixture of 20 organochlorine pesticides:

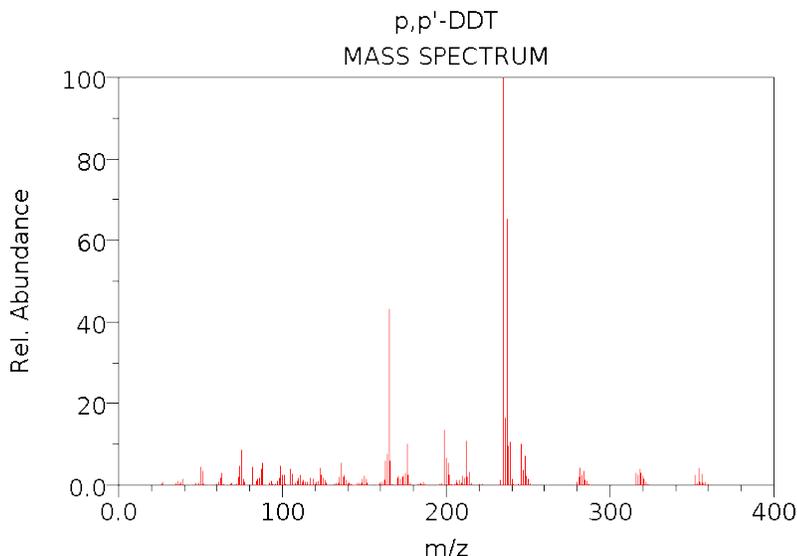


Consider the chromatographic peak at 21.876 min corresponding to the insecticide, DDT.

- List three factors responsible for the broadness of the peak.
Longitudinal diffusion, mass transport lags, multiple overlapping compounds
- What does the y-axis label “Abundance” refer to?
Number of ions that reach the detector (creating electrical signals that can be quantified or “counted”)
- What has to happen for a DDT molecule to create a detector response?
It has to be injected into the instrument, survive the GC column and inlet intact, create ions (molecular ions or fragment ions) in the source, make it through the mass analyzer (quadrupole, TOF, etc.), and reach the detector.
- Explain why neutral molecules are not detected in MS.
Neutral molecules are unaffected by the electromagnetic fields used in most mass analyzers.

To help confirm the identity of the peak at 21.876 min as DDT, it would be common to compare a mass spectrum (m/z vs. abundance) from the middle portion of the peak to a reference spectrum for DDT, taken under similar ionization conditions. A useful source for reference EI mass spectra is the NIST Chemistry WebBook.

The NIST mass spectrum (GC-MS; EI; 70eV) of DDT (MW = 354.5 g/mol) is given below.



- e) Predict how the mass spectrum of DDT-*d*8 would differ from the mass spectrum above. (DDT-*d*8 is an isotopically labeled DDT in which 8 hydrogen atoms have been replaced by 8 deuterium atoms.)
The masses of the molecular ion cluster would be 8 units higher. Depending on where the deuterium labels were placed, you would also expect increases (of 0 to 8 units) in fragment m/z values.

We often use internal standards to correct for instrument variability and losses during sample processing. Ideally, the internal standard is an isotopically labeled version of the analyte of interest. For example, if you were analyzing a water sample for DDT concentration, you might inject a known amount of DDT-*d*8 at the time of collection, then prepare your sample (*e.g.*, extract, concentrate, clean up) for injection on the GC-MS.

- f) Consider the TIC for a water sample extract that contained DDT as well as the internal standard DDT-*d*8 that you added. Is there a problem? If so, how would you solve it?
Yes, the problem is that DDT and DDT-*d*8 will probably co-elute (you might see only one peak in the TIC at 21.876 min), so you wouldn't be able to quantify either one. You could solve the problem by picking one or two fragments that are specific to DDT, and then have the computer sum them up at each time along the chromatogram, ignoring all the other fragment m/z values. This new (simplified subset) chromatogram is called an extracted (or selected) ion chromatogram (XIC). You could then do the same thing using fragment m/z values specific to DDT-*d*8. This would allow you to separate the DDT peak (XIC) from the DDT-*d*8 peak (XIC) and integrate (quantify) each.

Exercise 3.

In 2010, the Deep Water Horizon oil spill released 5 million barrels of crude oil into the bottom waters of the Gulf of Mexico. One strategy to minimize surface slicks was to release chemical dispersants (*i.e.*, surfactants) into the water by plane or into the deep water near the wellhead, 5000 feet below the surface. This was the first time that dispersants were released into deep water on a large scale (2.1 million gallons of dispersants), so there was considerable uncertainty about what would happen to the dispersant chemicals over time. The following figure was taken from a paper titled “*Fate of Dispersants Associated with the Deepwater Horizon Oil Spill*” by Elizabeth Kujawinski *et al.* (2011).

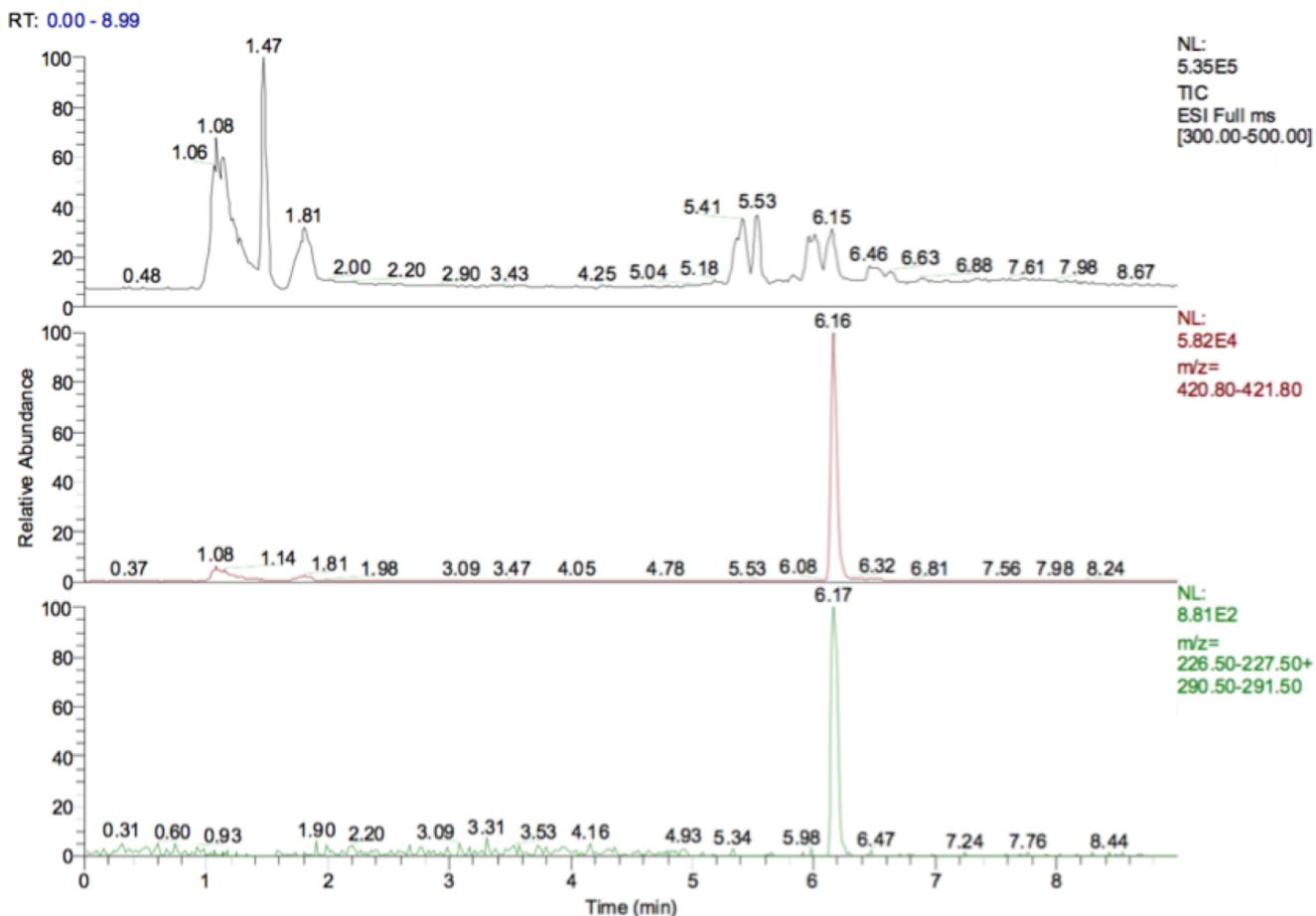


Figure S2. LC/MS spectrum of field sample (R/V *Cape Hatteras*, Cast 07, bottle 23, 10m).

- Have each person in your group pick a panel in the figure, take a couple minutes to study and annotate your panel (referring to other panels as needed), then take turns describing your panel to the other members of your group. In your explanations be sure to include a full description of the type of data plotted, the axes, and the meaning of the information on the right side of your panel. [Bonus: can you find the error in the figure caption?]

Top plot: TIC for m/z 300-500

Middle plot: XIC for m/z 421

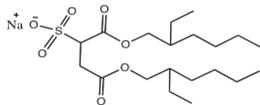
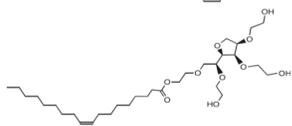
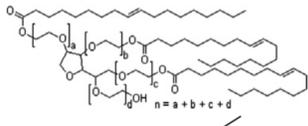
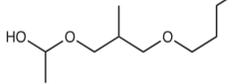
Bottom plot: Shows the XIC for two fragments (m/z 227 and m/z 291) of the m/z 421 molecular ion
x-axis: retention time

y-axis: relative abundance (signal intensity adjusted so that the highest value is 100)

NL: normalization level: the actual signal intensity (*e.g.*, counts per second) for the highest value

ESI: electrospray ionization (negative mode)

Bonus: The figure shows chromatograms, not spectra.

Compound	MW	Structure
DOSS	444	
Span 80	428	
Tween 80	604	
Tween 85	429	
DGBE	190	

- b) The table above (modified from Glover *et al.*, 2014, *Chemosphere* 111: 596-602) lists some of the components of Corexit, a dispersant mixture used during the Deep Water Horizon oil spill. Assuming the LC/MS analysis by Kujawinski *et al.* utilized electrospray ionization in negative mode, which component did they detect in their field sample? Explain your reasoning.

DOSS (dioctyl sodium sulfosuccinate): The observed m/z value for the molecular ion at 6 min is consistent with ionization via the removal of a hydrogen atom from DOSS, which we would write as [DOSS – H]⁻. The fragment ions' (m/z 227 and 291) retention time aligns with that of the molecular ion and both are plausible fragments based on the structure of DOSS. Kujawinski confirmed their assignment by injecting a pure standard solution of DOSS and observing the same fragments and retention times.

- c) How do you think Kujawinski *et al.* quantified the amount of DOSS in their field sample? They determined the peak area in the XIC for m/z 421 then used a DOSS calibration curve to calculate the concentration. Why not use the fragment ions? Because they give less signal, thus have lower signal to noise ratio, and likely a higher LOD.

In fact, the data from Figure S2 were collected using a liquid chromatograph coupled to an ion trap mass spectrometer operated in MS/MS mode. In this mode, the mass spectrometer uses the mass analyzer (an ion trap) to isolate ions of one particular m/z , fragment them, then sort/detect the resulting fragment m/z values. In this way Kujawinski *et al.* were able to create an MS/MS spectrum (*not shown*), which plots the m/z values for the fragments of the selected ion. The bottom panel in Figure S2 shows the XIC for two characteristic fragments (m/z 227 and m/z 291) of the molecular ion m/z 421.

- d) Describe a situation in which it would be advantageous to use an MS/MS (“tandem”) instrument? Explain your reasoning.
- When the matrix is complex (seawater, biological tissues, soil/sediment extracts, wastewater) and there are many more compounds in the matrix that could co-elute with and have the same molecular ion m/z as the analyte of interest. Notice that despite extensive clean up steps, the seawater sample TIC in figure S2 still shows interfering compounds at the RT of DOSS (~6 min).
 - To determine the structure of an unknown organic compound by fragmenting the molecular ion, collecting those fragments, fragmenting the fragment ions, collecting those fragments, and so on. This process can continue n times and is thus sometimes referred to as MSⁿ.