**Isotopomers and Isotopologues**

**Learning Objectives:**

* Isotopomers and isotopologues are analogous molecules that differ by isotope composition and/or position within a molecule. Isotopomers have known isotopic position, while isotopologues have unknown isotopic position with a molecule.
* Determine which analytical tools can be used to identify isotopologues and isotopomers.
* The International Chemical Identifier (InChI) can be used to store isotopomer information for retrieval in a chemical database.
* A nonstandard InChI layer can be used when the isotopic positions are unknown.

**Success Criteria:**

* Given a molecule, draw an isotopomer and an isotopolog
* Explain how mass spectral data can identify isotopologues, but NMR is typically necessary to identify isotopomers.
* Use the InChI isotopic /i layer to identify specific isotopomers and the nonstandard /a layer for the identification of isotopologues.

**Prerequisite Knowledge:**

* *From General Chemistry:* An **isotope** is a variant of a chemical element that has the same number of protons but a different number of neutrons in its nucleus, resulting in different atomic masses.
* *From organic chemistry:* **Constitutional isomers**, also known as **structural isomers**, are compounds with the same molecular formula but different connectivity, or atomic organization.
* *From organic chemistry*: **Mass spectrometry** is an analytical technique used to measure the mass-to-charge ratio of ions. It is widely employed to identify the composition of a sample by generating a mass spectrum, which displays the masses of the various components within the sample. The process involves ionizing chemical compounds to generate charged molecules or molecule fragments, which are then separated based on their mass-to-charge ratios.
* *From organic chemistry:* **Nuclear Magnetic Resonance (NMR) spectroscopy** is an analytical technique used to determine the structure, dynamics, reaction state, and chemical environment of molecules. It relies on the magnetic resonance properties of certain atomic nuclei. When placed in a strong magnetic field, nuclei of certain isotopes (such as 1H, 13C, and 31P) resonate at characteristic frequencies when exposed to radiofrequency radiation. This resonance is influenced by the local chemical environment, making NMR spectroscopy a powerful tool for elucidating molecular structure.

**Model 1:** [**Isotopologues**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3679491/#:~:text=%E2%80%93%20Isotopologues%20are%20molecules%20differing%20only%20in%20their%20isotopic%20composition.%20Two%20isotopologues%20are%20the%20same%20chemical%20species%2C%20but%20with%20at%20least%20one%20atom%20containing%20a%20different%20number%20of%20neutrons.%20The%20term%20isotopologue%20is%20derived%20from%20%E2%80%98isotope%E2%80%99%20and%20%E2%80%98homologue%E2%80%99.)

Monoisotopic mass is a type of molecular mass used in mass spectrometry to calculate the exact mass of an ion or molecule. It's calculated by adding the accurate masses of the most abundant naturally occurring stable isotope of each atom in the molecule. For most organic molecules, this means adding the masses of the lightest naturally occurring isotopes.

*Isotopologues* are molecules differing only in their isotopic composition. Two isotopologues are the same chemical species, but with at least one atom containing a different number of neutrons. Isotopologues have differing molecular masses. The term isotopologue is derived from ‘isotope’ and ‘homologue’.

Table : Isotopologues of Methanol

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Structure |  |  |  |  |
| Monoisotopic Mass (rounded amu) | 32 | 33 | 34 | 35 |

*Critical Thinking Questions:*

1. Looking at each of the structures in Table 1 how can you tell they are isotopologues of the same molecule?
2. Using the data in Table 1, identify the most abundant mass (in rounded amu) for each of the following elements:

|  |  |  |  |
| --- | --- | --- | --- |
| Element | Hydrogen | Carbon | Oxygen |
| Most abundant mass |  |  |  |

1. Carbon has three naturally occurring isotopes: carbon-12 (12C), carbon-13 (13C), and carbon-14 (14C). 12C and 13C are stable isotopes of carbon, while 14C undergoes radioactive decay. 14C is often used in radiolabeling drugs, because of the ability to produce molecules with almost identical chemical properties that can generate measurable radioactivity. Draw two different radiolabeled isotopologues of ethanol.
2. Draw two different isotopologues of ethanol that have atomic masses of ~49 amu.

**Model 2:** [**Isotopomers**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3679491/#:~:text=%E2%80%93%20Isotopomers%20are%20defined%20as%20molecules%20having%20the%20same%20number%20of%20each%20isotopic%20atom%2C%20but%20differing%20in%20their%20positions.%20The%20term%20isotopomer%20is%20a%20portmanteau%20of%20%E2%80%98isotope%E2%80%99%20and%20%E2%80%98isomer%E2%80%99.)

*Isotopomers* are defined as molecules having the same number of each isotopic atom, but differing in their positions. Isotopomers have the same molecular mass. The term isotopomer is a portmanteau of ‘isotope’ and ‘isomer’.

Table : Isotopomers of 1-butanol

|  |  |
| --- | --- |
| Isotopomer Set A | Isotopomer Set B |
|  |  |
|  |  |
|  |  |
|  |  |

*Critical Thinking Questions:*

1. Looking at each of the structures in Set A in Table 2 how can you tell they are isotopomers of the same molecule?
2. Looking at each of the structures in Set B in Table 2 how can you tell they are isotopomers of the same molecule?
3. What is the relationship between molecules in Set A and Set B?
4. The average atomic mass of bromine is 80, with relative abundance of 79Br and 81Br of about 50% each.
   1. Draw two structural isomers of molecules that have the molecular formula C3H6Br2. Add atomic masses as appropriate.
   2. Draw 2 isotopologue structures of molecules (with respect to bromine) that have molecular formula C3H6Br2. Add atomic masses as appropriate.
   3. Draw 2 isotopomeric structures of molecules that have molecular formula C3H6Br2. One isotopic atom should be a 2H, one should be 13C, and one should be 79Br. Have the bromine on a terminal carbon to limit the possibilities.

**Model 3:** 1H-NMR spectra of Ethanol with 2H Isotopes

The following simulated spectra explore isotopologues of ethanol. Integration is indicated next to each signal in the spectra.

A graph of a chemical reaction

Description automatically generated

2H

1H

3H

Figure : 1H NMR Spectrum of CH3CH2OH

A diagram of a chemical formula

Description automatically generated A graph of chemical formula

Description automatically generated

1H

3H

1H

1H

3H

Figure : Simulated 1H NMR Spectrum ofCH3CH2H2OH Figure :Simulated 1H NMR Spectrum ofCH3C2H2OH

*Critical Thinking Questions:*

1. Explain the splitting patterns shown in Figure 1 of CH3CH2OH. Be sure to identify which protons (1H) are associated with each chemical shift δ and why they are split that way.
2. Why is there a signal at δ 3.4 ppm in Figure 2, but none found in Figure 3?
3. What accounts for the difference in splitting of the peaks found at δ 1.2 ppm in Figures 1-3?
4. How might isotopologues be identified via NMR?

**Model 4:** Mass Spectrometry of Ethanol with 13C Isotopes

A graph of a chemical reaction

Description automatically generated

Figure :Simulated Mass Spectrum of CH3CH2OH with potential fragmentation patterns.

**A graph of a chemical reaction

Description automatically generated**

Figure : Simulated Mass Spectrum of CH313CH2OH with potential fragmentation patterns.

*Critical Thinking Questions:*

1. Explain how the shown *m/z* peaks in Figure 4 correspond to the structures adjacent to the peaks.
2. Why do some of the peaks in Figure 5 change compared to Figure 4, but not all?
3. How do the shown m/z peaks in Figure 5 indicate the 13C atom is on carbon 1 and not on carbon 2?
4. How would the spectrum change if the molecule was 13CH3CH2OH?
5. How can mass spectrometry be used to differentiate between isotopologues?

**Model 5:** InChI Line Notation

The International Chemical Identifier (InChI) is a standardized textual representation of chemical substances, developed by the International Union of Pure and Applied Chemistry (IUPAC). It provides a unique, machine-readable code that encapsulates the structural information of a molecule. Traditional names and graphical representations of molecules can be ambiguous and inconsistent, leading to difficulties in data sharing and retrieval. InChI addresses these challenges by offering a uniform and precise way to represent chemical structures, facilitating efficient data integration, searchability, and interoperability among different databases and software tools. This standardization is crucial for advancing research, enabling accurate communication, and ensuring reliable access to chemical information across disciplines.

![A diagram of a chemical structure

Description automatically generated]()

Figure : The main layers for a standard InChI of [(R)-carboxy(chloro)methyl]azanium, the protonated form of 2-(35Cl)chloro-R-glycine . Note each layer or sublayer is separated by a forward slash [/].

*Critical Thinking Questions:*

1. What information is provided in the main layer of an InChI string?
2. What is the overall charge in the molecule shown in Figure 6 and how is this indicated in the InChI?
3. Is the molecule shown in Figure 6 chiral? If so, how is this indicated in the InChI?
4. Are there any isotopes shown in the molecule? If so, how is this indicated in the InChI?

Information: The InChI Trust maintains a web demonstration of the InChI code. It is located at <https://iupac-inchi.github.io/InChI-Web-Demo/> Open a web browser and go to the link.

1. In the InChI Web Demo, draw ethanol and obtain the InChI.
   1. What is the InChI for ethanol?
   2. Which layers are included in the InChI?
   3. Which layers are excluded from this InChI? Why?
2. Explore the following InChI strings for isotopomers of ethanol:

|  |  |
| --- | --- |
| Structure | InChI |
|  | InChI=1S/C2H6O/c1-2-3/h3H,2H2,1H3/i2D |
|  | InChI=1S/C2H6O/c1-2-3/h3H,2H2,1H3/i1D |
|  | InChI=1S/C2H6O/c1-2-3/h3H,2H2,1H3/i3D |

* 1. What information is consistent in the InChI?
  2. What part has changed in the InChI? How is the location of the deuterium atom indicated?
  3. Does the numbering of the location of the deuterium atom follow standard IUPAC nomenclature numbering rules?

1. Using the InChI web demo, draw the following isotopic form of ethanol:



* 1. What is the InChI of this molecule?
  2. How is the 13C isotope identified in the InChI?
  3. Predict the InChI for the following isotopologue of ethanol based on answers to parts a. and b.



* 1. Check to see if your predicted InChI is correct by drawing the molecule in the web demo.
  2. How would your InChI isotope layer change if you had 14C instead of 13C in the previous example?

**Information: To represent isotopologues, a new ambiguous isotope localization “/a” layer specification has been added to the InChI. The isotopologue layer starts with “/a” followed by an encoded description within parentheses.**

1. How are isotopologues and isotopomers distinquished in a standard InChI?

**Homework**

Consider the 13C NMR spectra of ethanol:

A graph of a chemical formula

Description automatically generated

1. Explain why there are two different peaks found in the spectrum and why one is more downfield than the other.
2. Is the spectrum the result of isotopomers or isotopologues? Explain.
3. What is the relationship between 13CH3CH2OH and 13CH313CH2OH?
4. Using the InChI Web Demo, generate InChI for the three 13C isotopologues of ethanol.