What's Mass Got To Do With It?
A Self-Guided Introduction to Mass Spectrometry

By Tracy Suggs
RET 2004
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Scenario:
You hurry into your chemistry lab only to find your teacher slumped unconscious over the lab table. The only sign of any chemical is one drop of a colorless liquid on top of the lab counter. What is the chemical? Is it responsible for the current state of your teacher? How can the small volume be analyzed? What good is one drop? Will your teacher live to give another assignment?

► 1. *Suppose the unknown liquid was diethyl ether, with a density of 0.70 g/cm^3 and assume that the approximation of 20 drops to 1 milliliter is reasonable. How many molecules would be in the one drop of the liquid found on the lab table?*

Objectives:

1. To experience how the structure of a substance can be determined by various analytical instruments commonly found in college labs.
2. To learn how a mass spectrometer works.
3. To learn how to analyze isotopic abundance from the mass spectrum of an element.
4. To learn how to identify a compound from a mass spectra.
5. To learn how fragmentation patterns of organic compounds contribute necessary information concerning the structure and hence the identity of the molecule.

Why Should You Care About The Mass Spectrometer?

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds. Even with masses as small as 10^{-12} g, chemicals can be identified using this instrument. Did you know that the Mass Spectrometer is used to……

- ✓ determine the chemical composition of substances retrieved from outer space?
- ✓ detect and identify the use of steroids by athletes?
- ✓ monitor the breath of patients under anesthesia?

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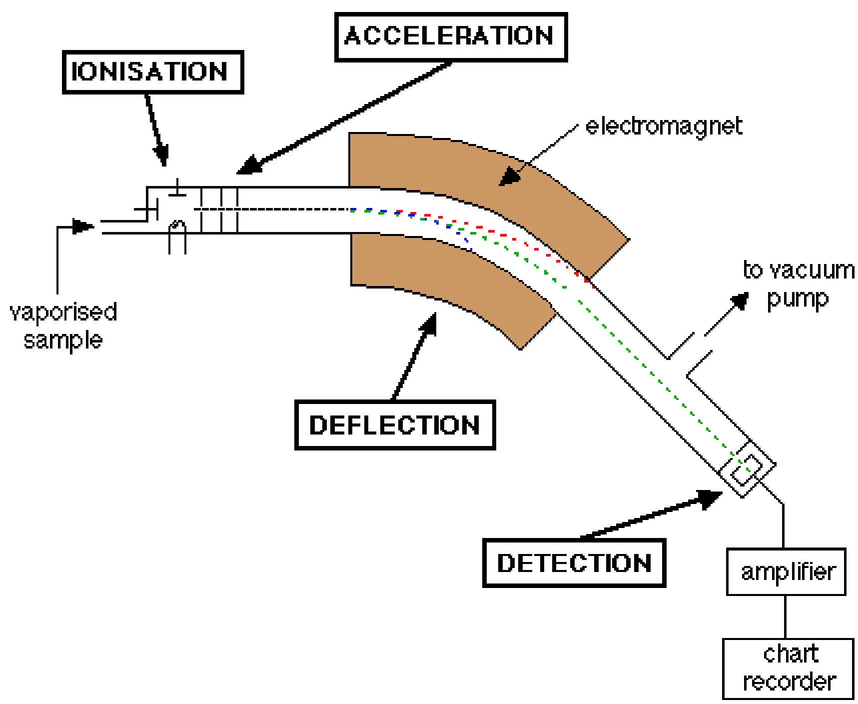
How Does a Mass Spectrometer Work?

Imagine trying to deflect the path of a ball being rolled in front of you by applying a sideways blast of water from a garden hose. The path of the ball will be deflected in a curved path depending on three variables.

► 2. *Name two of the three variables on which the degree of curvature depends.*

► 3. *Explain how material in a comet flying past planet Earth can also be an analogy for this kind of deflection. What is different about this analogy?*

Diagram of a Mass Spectrometer



Note; the sample to be analyzed in the mass spectrometer must be in the gaseous phase (vaporized) to be turned to an ion. Ionization is accomplished by using a stream of electrons to knock out one (or more) electron from the molecule. This species is now called the "molecular ion". With sufficient energies, the molecules can be broken into fragments that are also ionized. The molecular ions and fragments are accelerated to the same kinetic energy, and then deflected by an electromagnetic field. The ions passing through the spectrometer are detected electrically, and that signal is amplified.

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► 4. *If an electron were knocked out of a water molecule, what species would be formed? Include formula, mass, and charge.*

► 5. *If an electron were knocked out of a butane molecule, what species would be formed? Include formula, mass, and charge.*

► 6. *Which so you think is more likely, and why; to knock one electron out of a molecule, or to knock two electrons out of a molecule?*

► 7. *Charged particles travel from one side of the MS to the other. Can you imagine why the interior of the MS chamber must be kept at a high vacuum?*

Activity; A Mechanical Analog to the Mass Spectrometer

As the instructor demonstrates this instrument, draw and label the instrument in the space below.

► 8. *On your diagram above, draw the deflected pathways of 3 hypothetical balls, A, B, and C of increasing mass.*

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► 9. *Complete the chart of comparisons between the demonstration and a Mass Spectrometer.*

Factors That Influence Deflection of Particle	In Demonstration Analogy	In Mass Spectrometer
Mass		
Charge		
Force		

If we assume the electromagnetic force of the mass spectrometer remains constant during a particular run, then the 2 variables that influence deflection are mass and charge. These are combined into the mass/charge ratio and given the symbol m/z. Since it is so much more difficult to remove 2 electrons as opposed to one electron from the same molecule, we can safely assume during this introduction that the molecular ions will all have a +1 charge.

► 10. *In your labeled diagram with the trajectory of 3 balls indicated, label the trajectories with the highest and the lowest m/z ratio.*

What is Elemental Mass Spectroscopy?

In elemental mass spectroscopy, the elements in a compound are fractured, isolated and analyzed. This technique is usually done on inorganic compounds. Below is a sample of carbon dioxide in the MS. Note the molecular ion (M^+) is CO_2^+.

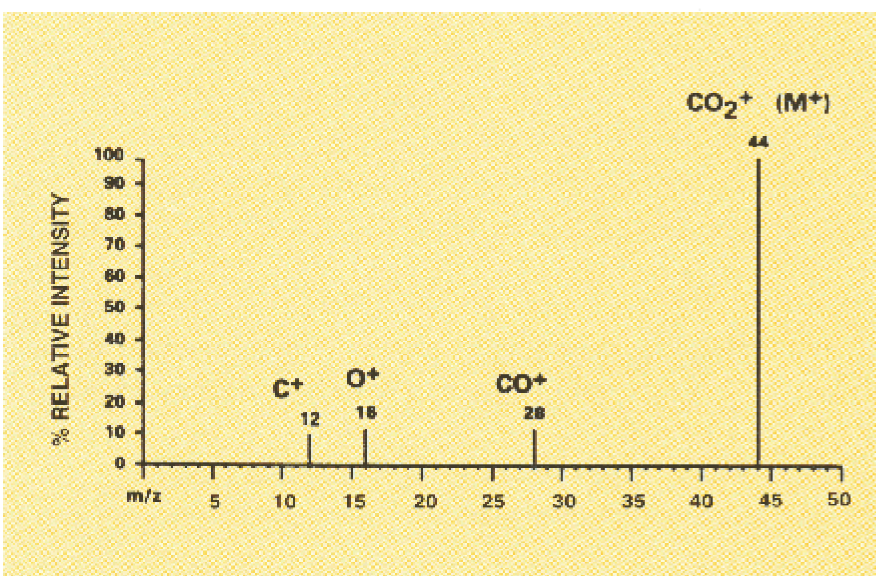

Figure 2 Mass spectrum of carbon dioxide, CO_2. Molecular ion is seen at m/z 44.

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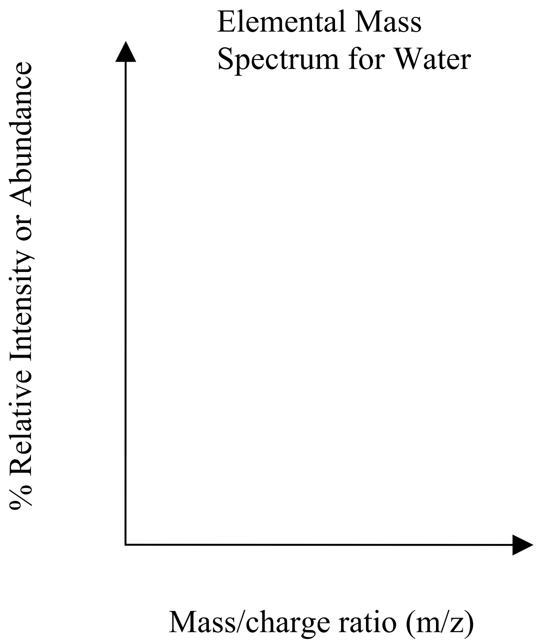
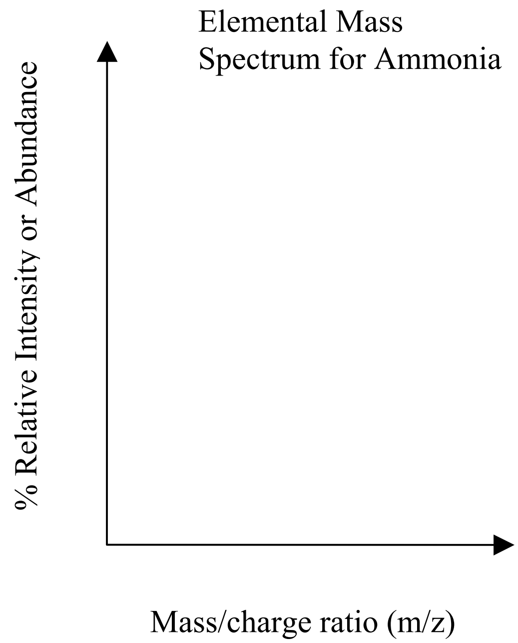
► 11. Now *you draw the elemental mass spectra of water and ammonia. Number the m/z axes. You will not know the relative intensities of the ions, but you can predict the m/z ratio of the possible charged particles for each of these compounds.*





A trained mass spectrometrist, and eventually you (!) can interpret the masses and relative abundances of the ions in a mass spectrum and determine the structure and elemental composition of the molecule. It has been said that "*a mass spectrometrist is someone who figures out what something is by smashing it with a hammer and looking at the pieces*".

► 12. *Notice that water and ammonia have very similar masses. Explain how a mass spectrometer can distinguish between these two chemical substances.*

What is Isotope Ratio Mass Spectroscopy?

► 13. *What is meant by an isotope and the isotopic abundance of an element? Why does boron have an atomic mass of 10.81, yet no atom of boron has that mass?*

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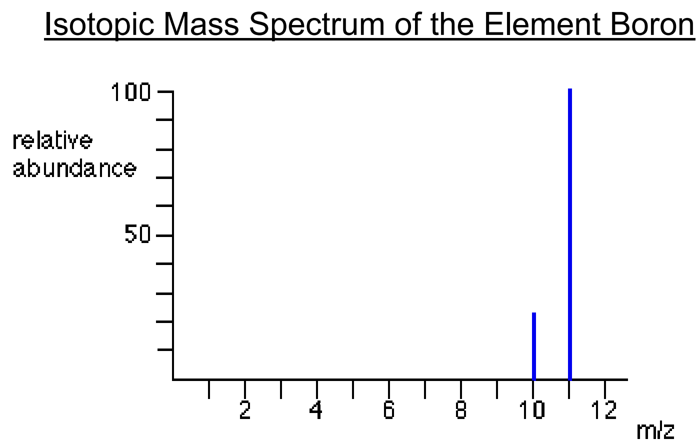

Note that on this graph the most abundant isotope is assigned the value of 100, and the other isotope is assigned a relative value. Do not confuse this with the real % abundance.

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Boron	B -10	10.012938	19.80%
Boron	B -11	11.009305	80.20 %

► 14. *Use the mass spectrum of the isotopes of zirconium found on the next page to first predict the atomic mass number of the element, and then actually calculate the atomic mass number from the masses of the isotopes.*

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Zirconium	Zr -90	89.9047026	51.54 %
	Zr -91	90.9056439	11.22 %
	Zr -92	91.9050386	17.15 %
	Zr -94	93.9063148	17.38 %
	Zr -96	95.908275	2.80 %

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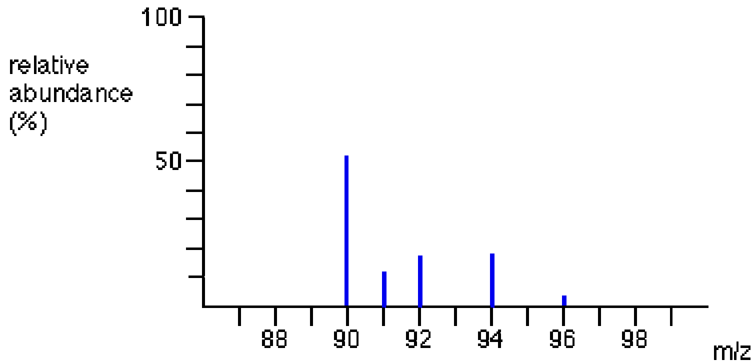
Isotopic Mass Spectrum of the Element Zirconium



What Are Some Common Elements Found in Organic Compounds?
and
What Are The Naturally Occurring Isotopes of These Elements?

(Note, numbers in parentheses are estimated and uncertain)

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Carbon	C -12	12.000 000 0	98.93(8)
	C -13	13.003 354 8378(10)	1.07(8)
Hydrogen	H -1	1.007 825 032 1(4)	99.9885(70)
	H -2	2.014 101 778 0(4)	0.0115(70)
Oxygen	O -16	15.994 914 6221(15)	99.757(16)
	O -17	16.999 131 50(22)	0.038(1)
	O -18	17.999 160 4(9)	0.205(14)
Nitrogen	N -14	14.003 074 005 2(9)	99.632(7)
	N -15	15.000 108 898 4(9)	0.368(7)
Chlorine	Cl - 35	34.968 852 71(4)	75.78(4)
	Cl - 37	36.965 902 60(5)	24.22(4)
Bromine	Br - 79	78.918 3376(20)	50.69(7)
	Br - 81	80.916 291(3)	49.31(7)

Does the Presence of Isotopes Affect the Mass Spectra of Molecules?

You bet! But the good news is we will just quickly look at the effect of isotopes on mass spectra and you will not be held responsible for this level of analysis. But it is still good for you to see what is complicating the mass spectra.

So, take the simplest organic compound methane. If you had 10 000 molecules, (remember, one drop of anything would have many more molecules than this!) a mass spectrometer would record the individual masses of each of these molecules.

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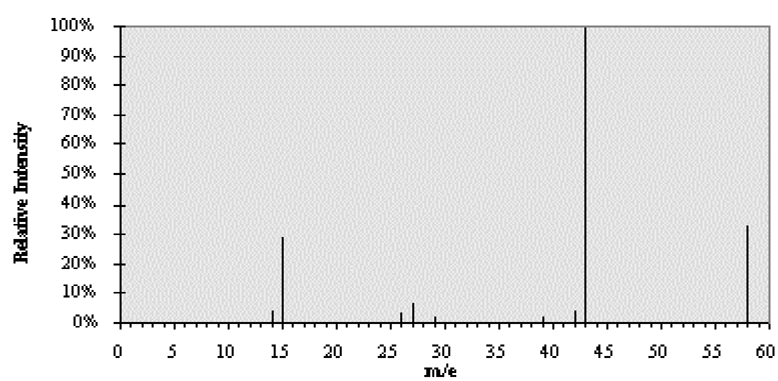
Now, would all the molecules of methane have the same mass? Unfortunately, no. Because of the existence of isotopes, the occasional methane molecule with C-13 (about 1% of all the molecules, or about 100 of the 10 000 molecules) would have larger mass, and the occasional methane molecule that had a deuterium atom (H-2) (about 0.01% or 1 molecule out of 10 000 methane molecules) would have a larger mass. Now you can imagine that with very large numbers of methane molecules, some molecules would have both a C-13 atom and an H-2 atom in the same molecule, some molecules could have two deuterium atoms, and a very small number could have a C-13 and two H-2 atoms making up the methane. All of this goes to say that when we look at the fragments of an organic compound in a mass spectrometer, we will see major peaks representing the commonly occurring isotopes, and minor peaks representing the other isotopic variations of masses.

► 15. *What masses of molecular ions could be identified in the mass spectrometer for the organic compound dichloromethane? Remember, the mass spectrometer records the actual mass of each molecule, not the average of the isotopic abundance. Using the provided table of isotopic abundances, list some possible masses for $C_2H_2Cl_2$ in the order of greatest to least frequency.*

How Do We Analyze Mass Spectra of Organic Compounds?

We have already looked at the mass spectra of simple molecules like carbon dioxide, water and ammonia. Now look at the mass spectrum of acetone, or 2-propanone or CH_3COCH_3.

Mass Spectrum of Acetone



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As you look at the above spectrum of acetone, note the following;

- ✓ The molecular ion at m/z = 58 represents the whole acetone molecule after being made into a +1 ion but without being fragmented.
- ✓ The mass spectrum shows many fragmented ions in addition to the molecular ion.
- ✓ The masses of the fragments have smaller masses around them due to isotopic abundances of the elements C, H, and O.

► 16. *Let's think about how an organic compound would most likely fracture. Not all the bonds in an organic molecule are of the same strength. Use reference materials to find the bond enthalpies of the following bonds commonly found in organic molecules. Then draw a structural formula for acetone (2-propanone) and identify where this molecule is likely to be fractured into smaller pieces.*

Bond Enthalpies of Bonds Commonly Found in Organic Molecules						
Bond type	C—C	C—H	C—O	O—H	C = O	C = C
Bond enthalpies (kJ/mol)						

What Does the Fragmentation Pattern Tell Us About an Organic Compound?

A lot! From the molecular ion we can determine the molar mass of the molecule. That is well and good, but hardly enough information to identity an organic compound. But if we include an analysis of the fragmentation pattern from the mass spectrometer, we can determine the structure of the molecule. And since structure identifies the organic compound, we can name that molecule!

► 17. *If you know an organic compound has a molecular formula of $C_5H_{12}O$ and a molar mass of 88.17 g/mol, what possible substances could this be? (I can think of two different isomeric functional groups and several different substances within each functional group family.)*

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► 18. *Draw the structures for functional group isomers ethanol and dimethyl ether. Identify one fragment each molecule would have common to each molecule, and one fragment that would characterize only one of the molecules.*

What Are Some Common Simple Fragments Found in Organic Molecules?

► 19. *The following list of fragments would be helpful to know. (Complete the mass expected to be found (considering only the most abundant isotopic species) for each of these common fragments.*

Common Fragments	—CH_3 Methyl group	—C_2H_5 Ethyl group	—CHO	—CH_2—	—COOH Carboxylic acid group	—OH Alcohol group
Mass of fragment						

► 20. *Imagine what a mass spectrum of ethanol looks like. What would be the m/z ratio for the molecular ion? The methyl group would break off the easiest. Then the alcohol group would break off. What would be some of the m/z ratios identified on the mass spectrum for this compound? (Do not take into consideration the isotopes of these elements)*

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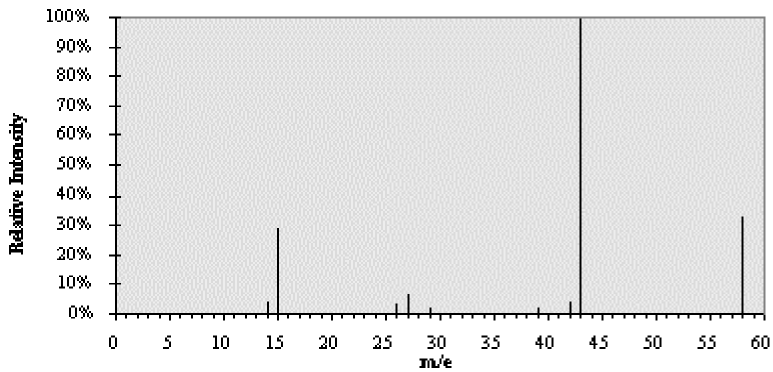
Here once again is the mass spectrum of acetone, otherwise known as 2-propanone.



► 21. *If the molecular ion represents acetone, what fragment was lost between the masses of 58 and 43? Identify what fragment broke off for the mass of 43 to be identified.*

► 22. *In the acetone MS, what fragment is represented by the mass lost between the fragment mass of 43 and the fragment mass of 27.*

How Do We Make Sense of the Fragmentation Pattern in Mass Spectra of Unknown Compounds ?

You can see how we can make sense of the fragmentation patterns in the mass spectra of organic compounds to confer structure and hence identify the substance. But with larger molecules the possible combinations of fragment losses becomes overwhelming. Consider a strand (molecule) of DNA with different fragments of amino acids being chopped off. We can actually sequence the amino acids in the protein by means of the mass spectrometer. This accomplishment, by the way, was the Nobel Prize in Chemistry awarded in 2002.

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Activity
An Analogy for Protein Amino Acid Sequencing Using the Mass Spectrometer

The Problem

A protein is one big molecule. It consists of huge numbers of amino acids bonded end-to-end in a long chain. Using the mass spectrometer, we can determine the molar mass of the protein, but the possible combinations of fragments is overwhelming. How is the sequencing of amino acids accomplished to determine the structure of a protein? Consider a small fragment of protein with a sequence of 9 amino acids. Because each one of the amino acids in the sequence could be one of 20 amino acids, the possible number of sequences is 20^9. That is 512 000 000 000 possible combinations, and you do not want to spend your evening figuring them all out. But with the aid of computers and statistical analysis, this problem can be solved. Let's look at an analogy.

Your Turn

You will need a stack of puzzle cards from the teacher for puzzles 1 and 2.

Puzzle 1

Your puzzle is a word made up of letters that have been fragmented as if they were passed through a mass spectrometer. Each word has been broken into one, two, or three-letter fragments. These have been written on cards in your stack. The idea is that the letters next to each other in the word are like atoms bonded to each other in a molecule. You should shuffle the cards in the stack, and turn one card over at a time until you can break through the code and solve the puzzle. For example, if the word is helium, you would see represented the following possible one, two, and three letter fragments; h, he, hel, e, el, eli, l, li, liu, i, iu, and ium. (This would be harder if these cards were shuffled.) Now you try puzzle #1.

Puzzle 2

This is the same idea as puzzle #1, but let's kick it up a notch.

Puzzles 1 and 2 are easier than the clues given by a mass spectrometer, because you were looking at the letters in the words, and not some property of the letters that make up the word. So now let's bring the analogy closer to a real mass spectrometer and identify a numerical property of the letter instead of the letter itself. This would be like the mass-to-charge ratio provided by the MS rather than the specific atom fragments. So we will assign each letter of the alphabet a corresponding "mass" using the first 26 prime numbers.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	2	3	5	7	11	13	17	19	23	29	31	37	41	43	47	53	59	61	67	71	73	79	83	89	97

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A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	2	3	5	7	11	13	17	19	23	29	31	37	41	43	47	53	59	61	67	71	73	79	83	89	97

Now the next puzzles can be challenging. As an example consider the word CAT. For CAT the letter fragments are C, CA, CAT, A, AT, and T. These fragments correspond to the "masses" of 3, 4 (3 + 1), 71 (3 + 1 + 67), 1, 68 (1 + 67), and 67. Notice that the mass of 71 is complicated by the fact that "U" also has a mass of 71. When the puzzles are presented, the order of the "masses" will be in increasing mass, as in 1, 3, 4, 67, 68, and 71. In puzzles 3, and 4, you are given all the possible 1, 2, and 3 letter fragments for a word, including the entire word ("molar mass"). Puzzle 5 gives you ALL possible combinations of letter fragments. Use the table to solve each of the following puzzles.

<u>Puzzle 3</u> 11, 41, 71, 82, 112, 123

<u>Puzzle 4</u> 1, 7, 8, 37, 38, 45, 67, 74, 75, 112

<u>Puzzle 5</u> 3, 7, 10, 31, 37, 38, 41, 43, 71, 74, 80, 81, 84, 102, 105, 109, 111, 112, 118, 119, 121, 143, 150, 155, 186, 192, 193, 223, 230

► 23. *What are the answers to Puzzles 1, 2, 3, 4, and 5? Record answers by puzzles.*

► 24. *The letters that make up the answer to puzzle #4 came from 4 different words. What are the words? Are the fragment masses the same for each of these words? Explain.*

► 25. *The words TON and NOT have very different meanings in the English language. Do these two words have different fragment masses? Would this make a difference in a mass spec of a molecule contained three elements that were all different from one another?*

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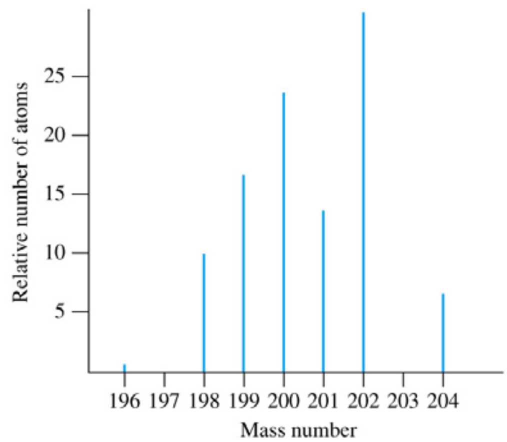
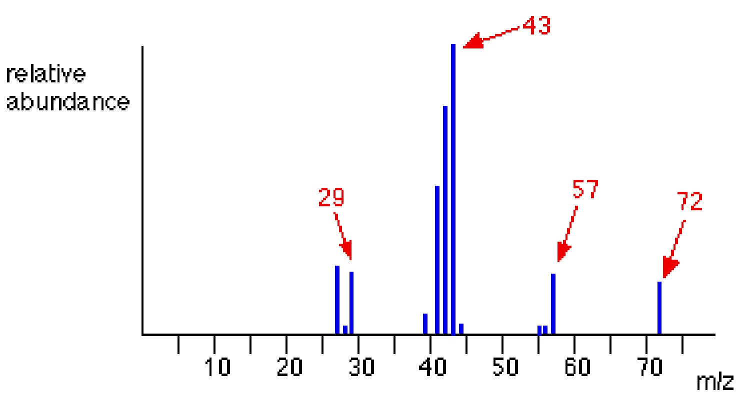
► 26. *When mercury is placed in a mass spectrometer, multiple masses are observed in the mass spectrum. How do you explain this?*

Mass Spectrum for Mercury



What Do Mass Spectra of Simple Organic Compounds Look Like?

simplified mass spectrum of pentane - $CH_3CH_2CH_2CH_2CH_3$



► 27. *What is the mass of the molecular ion in the spectrum of pentane? (We will always assume a charge of +1, so the mass/charge ratio becomes the same number as the mass.)*

It is important to realize that the pattern of lines in the mass spectrum of an organic compound tells you something quite different from the pattern of lines in the mass spectrum of an element. With an element, each line represents a different *isotope* of that element. With a compound, each line represents a different *fragment* produced when the molecular ion breaks up.

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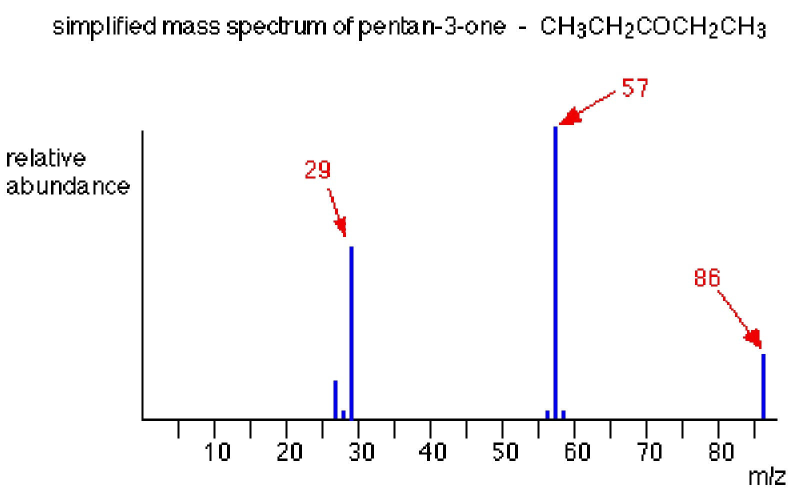
► 28. *What fragments are represented by the breaking of the molecular ion as it forms the fragments of mass 57, mass 43, and mass 29? Look at the Δ mass to determine fragments.*

► 29. *Pentane is isomeric with 2,2-dimethylpropane. Would these two compounds have the same mass of the molecular ion? Predict the fragmentation pattern you would see with 2,2-dimethylpropane.*

Here is a MS for 3-pentanone; otherwise known as pentan-3-one.



► 30. *In the above spectra of 3-pentanone the molecular ion has the mass of 86. Identify the loss fragments that resulted in the recorded masses of 57 and 29. Also identify and draw the fragments that are actually detected and producing the masses of 57 and 29.*

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In Summary;

Mass Spectrometry at its simplest is a technique for measuring the mass and therefore the molecular weight of a molecule. In addition, it is often possible to gain structural information about a molecule by measuring the masses of the fragments produced when molecules are fragmented in the mass spectrometer. This instrument has minimized the need for time consuming chemical and physical tests to identify functional groups and determine molar mass. When mass spectrometry in used in conjunction with other instruments such as Infrared Spectrometer and Hydrogen Nuclear Magnetic Resonance (H-NMR), chemical characterization becomes even faster and more confident. Complex molecules, such as proteins, can be readily sequenced by using the Mass Spectrometer to fragment and analyze the fragment masses. Nobel Prizes honor such recent accomplishments.

Final Scenario;
The rush of students to the care of the teacher caused the teacher to arouse from her seemingly unconscious state. It was only the grading of mountains of lab reports the night before that had diminished her quantity of required sleep. She nodded off while trying to finish her grading. On the third bob of her head before dropping off to a deep sleep, one drop of drool had dribbled from her open mouth, and sat unidentified by the side of her head.

Which goes to prove the old saying….

Old teachers never die, they just pass everyone.

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Crute, Thomas D.; Myers, Stephanie A. **1995**, *72*, 232

Journal of Chemical Education
Mass Spectra
JCE Editorial Staff **2003**, *80*, 176A

Websites

American Society for Mass Spectrometry

What is Mass Spectroscopy tutorial? Why should you be interested? What can MS do for you? How did it originate? How can MS data be used for structural analysis?
http://www.asms.org/

Integrated Spectral Data Base Systems for Organic Compounds

From this web site you can find and print MS, IR, and HNMR spectra from a data base of organic compounds.
http://www.aist.go.jp/RIODB/SDBS/menu-e.html

National Institute of Standards and Technology
NIST Standard Reference Database

Here you can view MS or IR Spectrum a digitized image, then copy all, and paste into document.
http://webbook.nist.gov/chemistry/

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Commercial site for selling MS

Here is a "short explanation for the absolute novice" and good MS spectra of water vs ammonia.

http://www.jeol.com/ms/docs/whatisms.html

Nobel Prize site for 2002 awarded for Mass Spec and NMR

http://www.nobel.se/chemistry/laureates/2002/press.html

Problems in HNMR and IR Spectroscopy

Problems for solving HNMR and IR Spectra

http://www.chem.ucla.edu/~webspectra/

Organic Chemistry Online by Paul Young

Problem sets using a variety of spectra for analysis. Interactive.

http://www.organicchemistryonline.com/OCOL.HTM#

Jim Clark's UK website to chemistry. Select Instrumental Analysis for info on Mass Spec, IR Spec, and HNMR

http://www.chemguide.co.uk/index.html#top

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CCMR

Teacher Notes for

"What's Mass Got To Do With It?"

A Self-Guided Introduction to the Mass Spectrometer

By Tracy Suggs
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RET 2004

Summary Page

Up until the early 1900's scientists had to perform time-consuming chemical and physical tests to determine the identity of an unknown substance. Today, instruments such as the Mass Spectrometer, Infrared Spectrometer, and Nuclear Magnetic Resonance give detailed information to allow structural and compositional analysis. Each instrument provides a piece of the puzzle in determining the identity of a chemical, and if used in concert, the specific identity of the substance can be determined. The Mass Spectrometer can be used to determine relative abundance of elemental isotopes, or the molecular mass of a compound. In addition, the Mass Spectrometer is used extensively in organic chemistry to analyze the fragments when the molecule is smashed. This fragmentation pattern gives insight into the structure of the molecule.

High School Students can understand the Mass Spectrometer with the help of several hands-on analogies. These learning tools will be examined in this lesson.

New York State Standards Core Curriculum — **Physical Setting/ Chemistry Major Understandings and Major Skills**

I.13 The average atomic mass of an element is the weighted average of the masses of its naturally occurring isotopes.

The student should be able to:

- ✓ given an atomic mass, determine the most abundant isotope.
- ✓ calculate the atomic mass of an element, given the masses an ratios of naturally occurring isotopes.

VII.1 Organic compounds contain carbon atoms which bond to one another in chains, rings, and networks to form a variety of structures.

The student should be able to:

- ✓ classify an organic compound based on its structural or condensed structural formula.

VII.2 Hydrocarbons are compounds that contain only carbon and hydrogen. Saturated hydrocarbons contain only single carbon-carbon bonds. Unsaturated hydrocarbons contain at least one multiple carbon-carbon bond.

The student should be able to:

- ✓ draw structural formulas for alkanes, alkenes, and alkynes containing a maximum of ten carbon atoms.

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VII.3 Organic acids, alcohols, esters, aldehydes, ketones, ethers, halides, amines, amides, and amino acids are types of organic compounds that differ in their structures. Functional groups impart distinctive physical and chemical properties to organic compounds.

The student should be able to:

- ✓ classify an organic compound based on its structural or condensed structural formula.
- ✓ draw a structural formula with a functional group on a straight chain hydrocarbon backbone, when given the correct IUPAC name for the compound.

Syllabus statements for International Baccalaureate Standard Level Chemistry
Organic Chemistry

Option A.1.1 State that the structure of a compound can be determined using information from a variety of spectroscopic and chemical techniques.

Option A.1.3 Describe and explain how information from a mass spectrum can be used to determine the structure of a compound.

Restrict this to using mass spectra to determine the relative molecular mass of a compound and to identify simple fragments, for example: $(M_r - 15)^+$ loss of CH_3; $(M_r - 29)^+$ loss of C_2H_5 or CHO; $(M_r - 31)^+$ loss of CH_3O; $(M_r - 45)^+$ loss of COOH.

Grade: 10-12 graders
Subject; Introductory Honors Chemistry (IB/SL Chemistry)

List of equipment;

3 by 5 note cards
Plexiglas sheet, about 24 inches by 24 inches
Variety of nickel ball bearings, varying in mass and size
Strong niobium magnet
Inclined plane or ramp made from a piece of wood.

Note; this demonstration is available from Sergeant-Welch
Deluxe Mass Spectrometer Kit
WL0624 $179.00

Student and instructor handouts

Answers to Amino Acid Sequencing Activity.

Puzzle #1 answer; GEOMETRY (8 letters)
Puzzle #2 answer; PRECIPITATE (11 letters)
Puzzle #3 answer; FUN
Puzzle #4 answer; TEAM
Puzzle #5 answer; MOLECULE

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