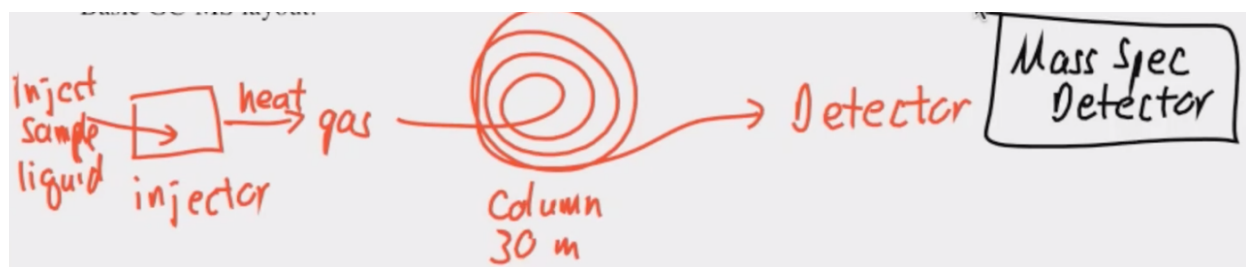


## Chem 355 Jasperse Gas Chromatography-Mass Spectroscopy

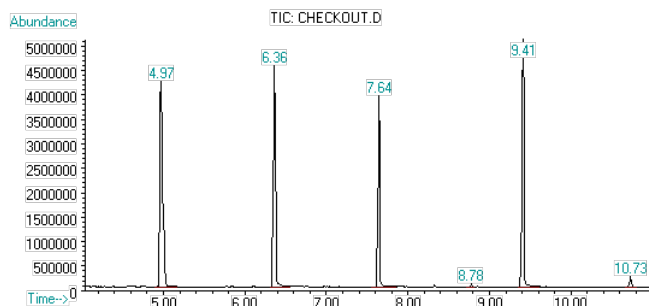
**BACKGROUND** Every type of chromatography depends on the distribution of a substance between two phases, a **mobile phase** and a **stationary phase**. Recall that in the liquid/solid chromatography the mobile phase was liquid flowing over a stationary solid phase.

In **gas chromatography**, the mobile phase is a stream of gas that flows through a narrow tube, which is coated on the inside with a non-mobile liquid film. A solution is syringed into a high-temperature injector where it is vaporized then carried through the column in a high-pressure gas (the mobile phase). For this lab, the injector will be 300°C; **the column will be 30m long with an internal diameter of 0.25mm**; the **film** will be **moderately polar** (with hydrogen bonding) with a 0.25µm thickness; and we will use 40 psi of helium gas as the mobile phase.

Basic GC-MS layout: (to be described and sketched in during the pre-lab video...)



Typical Look for a Gas Chromatogram:



The solutions injected will be mixtures of three substrates diluted in ethyl acetate solvent. Each of the three solutes will have distinct “retention times”. **GC retention times depends on several factors:**

1. **Sample polarity.** **More polar** samples stick to the polar film better, and **move slower**.
2. **Sample size:** **Larger molecules** are more likely to condense into the liquid phase and **move slower**. Smaller molecules vaporize more easily and remain in the mobile phase.
3. **Boiling point:** **Lower-boiling samples** boil more easily, and in the gas phase get carried through the column **faster**. Samples with higher boiling points spend are harder to get and to keep in the mobile vapor phase, so they move slower. Both the sample polarity and the sample size really relate to the boiling point.

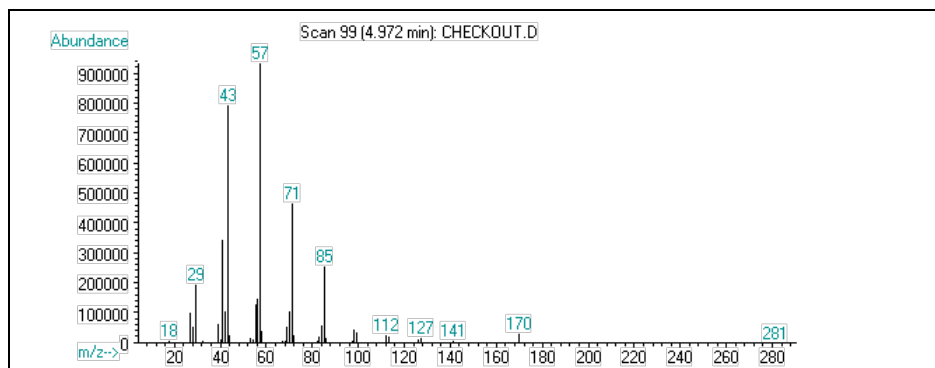
GC has **extraordinary separating power**. With a 30-meter column, there is a lot of opportunity to achieve separation! The retention times are very precise and characteristic, and the ability to separate is much greater than with most other forms of chromatography.

GC is **very sensitive**. Very dilute samples can be injected and trace amounts can be detected (as little as  $10^{-9}$  g).

**DETECTION** can involve either **mass spectrometry** (“MS” = “mass spec”) or **flame ionization detection** (“FID”). Our instrument will use both methods, but the primary focus will be on the mass spec detection.

**Flame ionization detection** involves passing the sample through a flame (both hydrogen gas and compressed air must be supplied.) The flame ionizes the molecules and the ions are detected. The process is highly sensitive for carbon-containing molecules. FID is nice in that it is relatively inexpensive, and any organic chemical will be detected.

**Mass spectrometry** is more helpful in that it provides the molecular weight and valuable **information about the molecular structure**. MS knocks an electron out of a molecule. The result is both a cation (it lost an electron, so it becomes charged) and a radical (it lost one electron, so now there is an unpaired electron left somewhere.) The energy of the process and the instability of the radical cation usually result in fragmentation into multiple pieces. A typical mass spectrum is shown below.



Mass spectrometry is invaluable for sample identification. **No two molecules give exactly the same mass spectrum**. Thus the MS provides an unambiguous identification; the mass spectrum is like a molecular fingerprint. The sensitivity and identifying power of GC-MS make it a primary tool in forensic science and in Olympic drug testing. Our mass spectrometer is what's called a "**low resolution**" mass spec; it can determine molecular weights to the nearest tenth of a gram. More expensive "**high resolution**" mass spectrometers can give molecular masses to the nearest 0.0001 gram, and are even more powerful for identification purposes.

**Mass spectrum interpretation is both simple and hard, depending**. The hard part is that molecules are often ionized with so much energy that they fragment into many pieces and in sometimes unpredictable ways. (The process has been compared to shooting a bullet through a vase and looking at the pieces afterwards.) Analysis of some fragmentation patterns is discussed later.

### NIST Library

**Matching.** Many molecules have been analyzed by mass spectrometry before, and their spectra have been filed with the National Institute of Standards and Technology (NIST). The 2008 NIST library includes over 220,000 mass spectra. When the NIST library is available, the computer can check for spectra that might match.

**Molecular Ion, Molecular Weight and Molecular Formula:** While ionization often leads to fragmentation, usually some of the parent molecular ion will appear. (Not always.) The molecular ion ( $M^+$ ) provides the molecular weight for the molecule. With high resolution mass spec, the molecular ion gives not only the molecular weight but also the exact molecular formula.

**Molecular Ion recognition and Isotope lines.** The molecular ion should be the highest line in the spectrum, because the fragments are smaller. However, while most carbons are  $C^{12}$ , 1.1% of carbons are  $C^{13}$  isotopes. Thus a molecule with 10 carbons will have not only an  $M^+$  line but also an  $M+1$  line that is about 11% as tall as the main  $M^+$  line. While this is often too small to register, it is often visible and should not be mis-identified as giving the molecular weight. Several finesse notes:

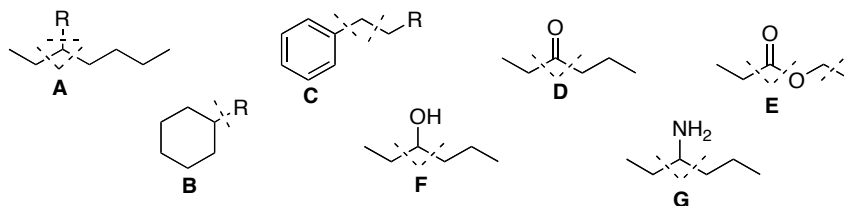
- For molecules that have **C, H, and O only**, the molecular ion  $M^+$  will always have an **even number**. An odd number either implies that either some other element is included, or that you are looking at  $M+1$  ion rather than  $M^+$ , or that it isn't really the molecular ion at all but is actually a fragment.
- Normally the  $M^+$  and  $M+1$  ions will be the lines furthest to the right in a mass spectrum. But sometimes there is some "bleed" from the column film. In our case, **if you see lines at 207, 191, or 177, ignore these**. They are background bleed signals and not your molecular ion.

**Even-versus-Odd Numbered Fragments: Even-numbered fragments are the result of multiple processes. For this week's lab, don't analyze or try to figure out any even-numbered fragments (other than the molecular ion line).**

- For molecules that have **C, H, and O only**, the unbroken molecule will always have an even molecular weight.
- But whenever a break happens, both resulting fragments ("primary fragments") will have odd-numbers.
- Primary fragments sometimes undergo secondary reactions, resulting in new pieces ("secondary fragments") that may be even-numbered.
- **For today's lab, don't think about or try to analyze any even-numbered secondary fragments**
- **Focus only on odd-numbered fragments that might be understandable primary fragments.**

**General mass spectrometry fragmentation guidelines:**

1. For today, look for odd-numbered “primary fragments” only; other than the molecular ion don’t spend any time on even-numbered secondary fragments.
2. **It is common to fragment either at a functionalized carbon or at the adjacent C-C bond.**
3. **Cyclic molecules with side chains tend to fragment at the connection point.**
4. More substituted bonds fragment more easily.
  - cations and radicals are stabilized by substitution. Thus fragmentation at substituted bonds leads to stabilized cation and radicals fragments.
5. **Size** increases fragmentation, all else being equal.
6. Double bonds and especially **aromatic rings** reduce fragmentation.
  - This is due to charge delocalization
  - Aromatic molecules tend to have relatively strong molecular ion signals.
7. Cyclic molecules with no side chains tend to have strong molecular ion signals. But when they do fragment, it can be difficult to recognize the pieces.
8. Oxygenated primary fragments will often subsequently add one or even two extra hydrogens, leaving secondary-fragment lines one or two mass unit heavier than the primary fragment.

**Some General Fragmentation Notes for Famous Families:**

1. **Acyclic alkanes:** break wherever but especially at branches. (See A). Usually show M<sup>+</sup>.  
**Cyclic alkanes with branches:** tend to break at the branch point. (B).
2. **Acyclic alkenes:** typically break at the allylic site. Usually show M<sup>+</sup>.
3. **Substituted Aromatics:** tend to break at the ring or at the benzylic site. (C). Usually show strong M<sup>+</sup>.
4. **Ketones:** typically break at the carbonyl bond (see D), and sometimes also one bond further. Usually show strong M<sup>+</sup>.
5. **Acyclic esters:** sometimes break at the carbonyl bond (see E), but have other break points. Sometimes don’t show any molecular ion or only a weak molecular ion.
6. **Alcohol and amines:** often break rapidly at the functional carbon (see F and G). Often show no molecular ion.
7. **Cyclic components can sometimes make things crazy! Subdivision within the ring requires two breaks, so often show strong M<sup>+</sup>.**
8. **Oxygenated fragments often add one or even two H’s after the initial break.**

**Basic GC-MS Operation    Compressed Draft 4            For Chem 355 Organic Unknowns Lab**

Note: The following assumes the gc/ms program has been opened and warmed up; that an appropriate “method” and “sequence” have been selected; and that Jasperse will turn things off.

**Sequenced Data Acquisition: Using the Autosampler to Sequence Runs Automatically**

Note: this assumes that Jasperse has already prepared a “sequence”, but you are trying to add your sample to the lineup.

- **If you’re first in line, get Jasperse to come and help. Or hit “OK” and “Run Sequence”.**

**1. Add your sample to the back of the line in the autosampler.**

- Do NOT leave any open holes (unless the sample belonging in that hole is being sampled and is in the injector tray.)
- Filling a “sample-is-in-the-injector-tray” hole will cause a system freeze. When the machine tries to put the injection sample back, it will have no place to go.

**2. Open “edit sequence” by clicking the “edit” icon on the yellow panel high on the computer screen.**

- This will open a spreadsheet that you can edit.
- Add your names in the “sample” box that goes with your vial number.
- **Click OK.** Note: if you don’t click “OK”, the machine will freeze at the end of the current run. NEVER leave the spreadsheet page open unless somebody behind you is going to close it.

**Data Processing/Analysis: Getting and Printing the GC Graph, % Report, and/or Mass Spec.**

- Note: data analysis can be done while acquisition is ongoing.
- Note: Data files are in a Data Folder, usually open on the left. Or, you can click “Data Analysis from the yellow panel on top of the GC software field.

**3. Open a data file: double click with the left mouse button to.**

- Data file will have the names “Vial-1” or “Vial-2”, so **remember which vial was yours.**
- Your data files should be within an Organic Lab folder.

**4. Printing GC Graph, % report, and retention times: Click Method>Run Method**

- Repeat as many times as needed to provide prints for each student in your group.

**5. Printing Mass Specs: Click the 5th Hammer button.**

- Click the 5th hammer button as many times as needed to provide prints for each student in group.
- Note: You don’t need to wait for a print to finish before clicking the hammer again. If you’ve got 5 partners, just click the hammer five times and the prints will come out one by one....

## **The Experiment**

Time overview:

1. Instructor will give each student printout data for Sample A
2. Each student or pair-of-students will prepare one sample.
3. We will all go over to the GC-MS and instructor will show the instrument, how it works, how to run it, how to use the software, how to print extra copies of the GC data and the mass spec data, etc..
4. Students will bring along their sample printout for Sample A, and their Basic GC-Operation instruction sheet. (Previous page.)
5. We will all return to lab, and discuss how to analyze and report on Sample A.
6. As the unknown samples complete, you will then be able to get data and printouts for your unknown samples, and come back to lab to analyze and process them.

**Sample A Data Analysis:** The instructor will give each student a copy of the GC-MS data for Sample A.

- Everybody will be responsible for analyzing it.
- Bring this with you to the GC-demo with the instructor, so that you have a reference sample data and printout to look at when the instructor is discussing aspects of a printout.

## **Unknown Sample**

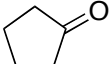
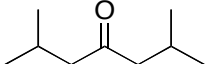
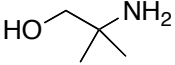
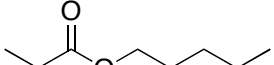
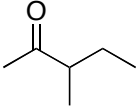
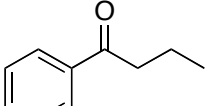
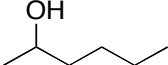
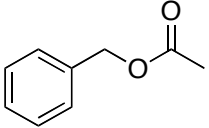
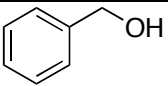
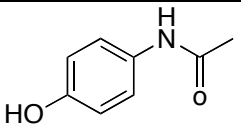
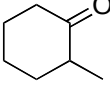
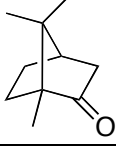
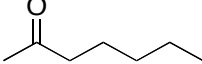
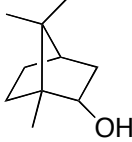
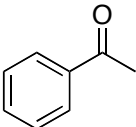
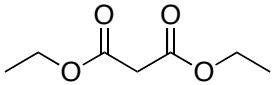
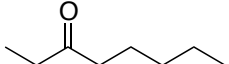
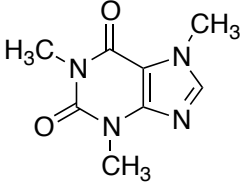
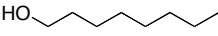
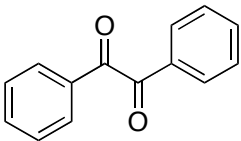
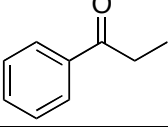
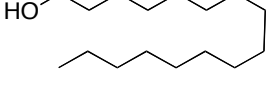
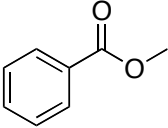
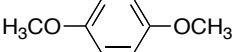
1. You may work with a partner (prepare one sample between the two of you); or you may work individually (prepare and analyze your own sample.)
2. Sample preparation: Using the pipets attached to each unknown sample, fill a GC-MS sample vial to  $\geq 0.5$  mL. Filling it deeper won't do any harm.
  - Note: Samples A-G are already highly dilute.
  - Ethyl acetate is the solvent.
3. Sample submission: Using the Basic GC-Operation instruction sheet as a guide, place your vial into the autosampler, and enter needed submission information on the computer.
4. **Record your autosampler number spot; finding your data later will depend on knowing your number!**
  - The data is filed by Vial number, not by student names....
  - So when you go back to get your data, you need to know whether you're Vial-14 or Vial-12 or whatever!!!!)
5. After the GC-MS queue has run your sample, you will need to find and open the data file, and print needed data. (Each partner will need to have a copy of the GC data AND the MS data.)

**Notes: the unknown components are all taken from the table shown on the following page.**

## **Tips:**

1. Interpretation is best done by:
  - Finding the molecular ion. If you can figure the molecular weight, in most cases that's as good as identifying your candidate.
2. Ignore any peaks at 207, 191, and 177. These are released from the column and are not produced from your molecule.
3. Once you know what your molecules are, look at the mass spectra for each one and try to find one or two fracture points leading to major recognizable peaks.
4. Look for odd-numbered fragments that correlate to breaks next to a functional group, and that would have mass of  $\geq 45$ .

**GC-MS Candidates, with Molecular Weights and Structures**

Compound	MW	Structure		Compound	MW	Structure
Cyclopentanone	84			2,6-Dimethyl heptanone	142	
2-amino-2-methyl-1-propanol	89			n-Pentyl propionate	144	
3-Methyl-2-pentanone	100			Butyrophenone	148	
2-Hexanol	102			Benzyl acetate	150	
Benzyl Alcohol	108			Acetaminophen	151	
2-Methyl Cyclohexanone	112			Camphor	152	
2-Heptanone	114			Isoborneol	154	
Acetophenone	120			Diethyl malonate	160	
3-Octanone	128			Caffeine	194	
Octyl Alcohol	130			Benzil	210	
Propiophenone	134			Hexadecanol	242	
Methyl Benzoate	136					
Dimethoxybenzene	138					

**See hand-in sheets on following pages.**



GC-MS Lab Report

Name:

**Part 1: Group SAMPLE A**

1. Record the retention times, the molecular weights, and the structure for the three components. (See the list on the earlier page).

	Retention Time	Molecular Weight	Unknown Structure
--	----------------	------------------	-------------------

First Peak

Second Peak

Third Peak

2. Redraw the three unknown structures, and write in the molecular weight under each one.
- Look at your mass spec for each unknown and the lines that actually appear in the mass spectra, and for each unknown **draw out two** "fragments" that match up with actual fragmentation peaks that are actually visible in the mass spec. (Given the original structure of the unknown, the mass of the fragment, and typical fragmentation patterns.)
  - Draw the fragment number beneath each figure.
  - Note: each fragment must be a cation, and must have a molecular weight that matches a line that you actually see in the mass spectrum.
  - Note: Look for odd-numbered fragments, since even-numbered fragments are not primary fragments.

Unknown Structure	One Fragmentation Peak and What it Might Be:	Second Fragmentation Peak and What it Might Be:
Unknown 1	1.	2.
Unknown 2	1.	2.
Unknown 3	1.	2.

**Part 2: Individual/Partner GC-MS**

3. Which sample did you and your partner run? \_\_\_\_\_
4. Attach a printout of BOTH the chromatogram and a printout of each of the three mass spectra.
5. Record the retention times, the molecular weight, and draw the structure for the three unknowns. (See the list on the earlier page).

	Retention Time	Molecular Weight	Unknown Structure
--	----------------	------------------	-------------------

First Peak

Second Peak

Third Peak

6. Redraw the three unknown structures, and write in the molecular weight under each one.
- For each unknown **draw out two** “fragments” that match up with actual fragmentation peaks that are actually visible in the mass spec.
  - Draw the fragment number beneath each figure.
  - Reminder: each fragment must be a cation; must have a molecular weight that matches a line that you actually see in the mass spectrum; and you should look for odd-numbered fragment.

Unknown Structure	One Fragmentation Peak and What it Might Be:	Second Fragmentation Peak and What it Might Be:
Unknown 1	1.	2.

Unknown 2	1.	2.
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Unknown 3	1.	2.
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