Gas Chromatography - Mass Spectrometry

GC-MS ANALYSIS OF ETHANOL AND BENZENE IN GASOLINE

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INTRODUCTION

The United States and most of the world are exceedingly dependent on fossil fuels for their energy needs. For Americans, gasoline is the most common energy source for transportation. Due to the large quantities of pollutant species emitted and formed from regular and diesel fuel combustion (CO, NO, unburned hydrocarbons, particulate matter, and polycyclic aromatic hydrocarbons, to name a few), there is an increasing number of air pollution regulations in the U.S. and worldwide.

Oxygenated compounds are now added to gasoline in many parts of the U.S. They are added to increase the octane number, compensate for the reduction of aromatic and olefinic contents, and to decrease emissions of CO (Calvert et al., 1993; National Academy of Sciences, 1991). Common oxygenates added to gasoline include methanol, ethanol and methyl-*t*-butyl ether (MTBE). Due to some gasoline leakage from underground storage tanks into drinking water supplies, MTBE has been, or is in the process of being phased out in many areas.

The octane number is a measure of the burning characteristics of the fuel, such as its ability to resist early ignition. The octane ratings are based on isooctane (2, 2, 4 – trimethylpentane), which is assigned an octane number of 100, and heptane, which is assigned a value of 0. So gasoline with an octane rating of 87 would have similar performance characteristics of a standard fuel mixture consisting of 87% isooctane and 13% heptane. The higher the octane rating, the better the fuel performance and the greater the price per gallon (i.e.: 89 and 91 premium fuels).

In addition to a variety of non-aromatic hydrocarbons in gasoline, there are many aromatic hydrocarbons, some of which are classified as toxic chemicals (this is why you see warnings at gasoline stations). One of the major ones is benzene (Kelly et al., 1994).

The goal of this experiment is to separate the components in a sample of gasoline using Gas Chromatography. Mass Spectrometry will then be used to identify as many components in the gasoline as possible and to determine the concentration of ethanol and benzene in the sample.

BACKGROUND

GC-MS is a "hyphenated" experimental technique that incorporates two widely used methods in tandem. The GC portion is the *Gas Chromatography* used for separating components in a mixture, and the MS portion is the *Mass Spectrometry* used in the qualitative and quantitative analysis of each component that was separated by the GC. The combination of these two highly applicable techniques creates possibly the most commonly used instrument for analytical scientists. Each technique will be briefly discussed below. A schematic layout of a GC/MS instrument is shown in Fig. 1.

I: Gas Chromatography

Gas chromatography is the most powerful and applicable separation technique for complex mixtures of volatile chemicals. Gas chromatography uses a gaseous mobile phase, or *eluent*, to carry the analyte being analyzed through a column packed or coated with a stationary

phase. Some GC columns are up to 100 meters long! The column you will be using in this lab is about 30 meters long.

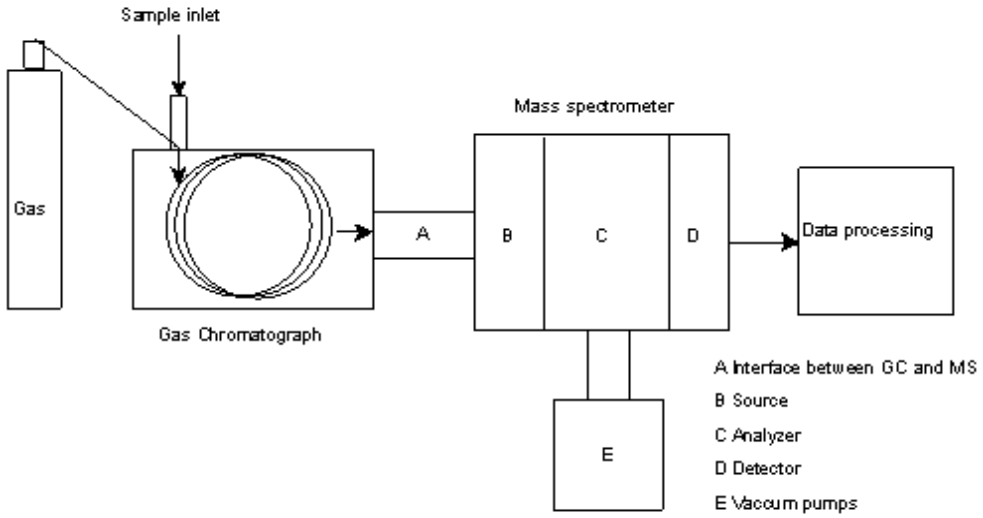
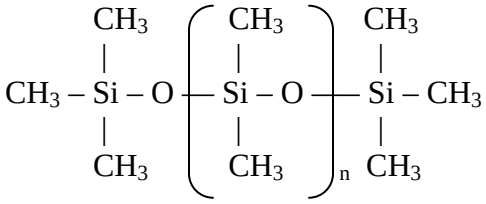


Figure 1: Schematic layout of a GC/MS instrument

The stationary phase in Gas Chromatography is commonly a packing of inert, small diameter particles (such as diatomaceous earth) with a nonpolar liquid coating them, or just a liquid coating on the inner surface of the column. This liquid is a very thin layer (0.1 to 5 μm), usually a polydimethyl siloxane (shown below) where some of the $-CH_3$ groups can be altered so as to match the polarity of the analytes. A parameter common in chromatography used for this is called the *Partition Coefficient (or Ratio)*, K, which is the ratio of the concentration of the analyte in the stationary phase to that in the mobile phase.



The mobile phase is an inert gas such as Argon, Helium or Nitrogen that only carries the analyte molecules through the column. The carrier gas does not interact with the analyte and column packing material. In this lab, ultrahigh purity Helium is used as carrier gas.

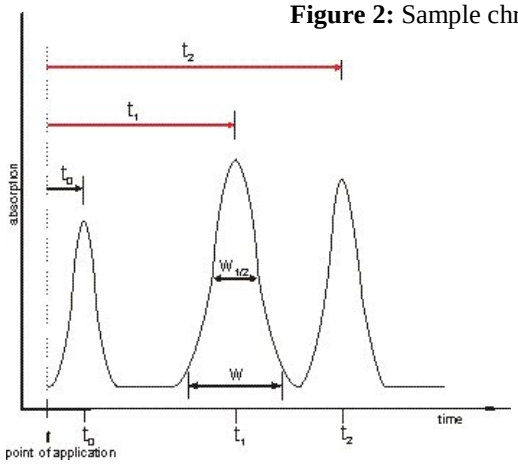
The *retention time* (time it takes to pass through the column) for an analyte is based on the time spent in the stationary phase vs. the mobile phase, with longer retention times for analytes with polarities closer to that of the stationary phase. In the sample chromatogram shown in Fig. 2, two different molecules have distinct retention times, t_1 and t_2. Dead time, t_0, is the time it takes for the carrier gas to go through the column.

The analyte peaks tend to broaden as they pass along the column, resembling Gaussian peaks. This is due to the random motions of molecules as they migrate down a column, passing in and out of the stationary phase. This peak broadening affects the efficiency of the column as well as its ability to distinctly separate the peaks of two different analytes (the *resolution*). Another common parameter used in chromatography is the *Selectivity Factor*, which is the ratio

of the migration rates between two different analytes, A and B, and provides a measure of how well the column separates A from B. In Figure 2, molecules 1 and 2 are well separated in spite of the substantial peak broadening.

In order to optimize the column resolution and efficiency, one can change the column dimensions and/or the stationary phase. However, altering the temperature has the greatest effect on column resolution and efficiency. Gradually increasing the temperature, manually or in a predetermined software program, can greatly increase scan speeds as well as increase resolution between peaks.

Figure 2: Sample chromatogram.



Samples are commonly injected in very small volumes through a septum or diaphragm into the column head to prevent evaporation of the sample. If the sample is a liquid, then it must be vaporized before being sent into the column. The chromatogram can be used for qualitative and quantitative analysis, but a better method is to direct the output of the chromatographic column into a mass spectrometer (or other identification method) which can then analyze each analyte as it elutes off the column.

II: Mass Spectrometry

Mass Spectrometry refers to a group of analytical techniques that precisely measure masses of molecules, atoms and/or ions. Because each species is characterized by a unique mass, mass spectrometry is the most common identification technique used by chemists, biologists, forensic scientists, etc. There are many different types of mass spectrometry based on the various sections of the instrument and the application desired. In most approaches, vaporized samples are ionized (and commonly fragmented), and these ions are separated based on their mass to charge ratios (m/z) and then detected and processed.

1) **Sample Injection:** There are many different methods used to inject a sample into a mass spectrometer depending on the original phase of the sample. The main requirement is that the sample is converted into the gas phase at very low pressures (down to 10^{-10} atm) for the instrument to function properly. In this lab, the sample will be injected as a liquid with a syringe. The injected liquid will then be heated to convert it into a vapor.

2) **Ionization:** Of the numerous ways to ionize the sample, *electron impact* is the most commonly used. There are several methods that combine vaporization and ionization in one step, especially for solid samples. In electron impact ionization, a filament is used to

generate fast moving electrons that strike gas phase sample molecules, knocking off electrons, and thus ionizing them. This must be done in a vacuum environment (otherwise the electrons would strike N_2 and O_2 molecules instead).

Commonly, the molecular ion produced by the collision of the parent molecule with an electron has excess energy and fragments into daughter ions as a result. The fragmentation pattern is used as a qualitative identification method, and many instruments have a library of references for automatic comparison.

Note that ethanol has a molecular weight of approximately 46 g/mol. However, the peak corresponding to m/z = 46 in its electron impact mass spectrum is not the largest peak. This happens because molecules like ethanol often fragment upon electron impact ionization:

$$\text{Molecule} + e^- \rightarrow \text{Molecular ion}^+ + \text{neutral fragment(s)} + 2\ e^-$$

In the case of ethanol, the largest peak appears at m/z = 31 instead, which corresponds to the loss of a CH_3 group from the molecule upon ionization. You can view the electron-impact mass spectra of ethanol as well as the other molecules probed in this lab in the appendix.

3) **Mass Analyzer:** This is the heart of a mass spectrometer and there are several types of mass analyzers used, including Quadrupole, Time of Flight, and Magnetic Sector Analyzers. The most common, and the one used in our instrument, is the Quadrupole Mass Analyzer. How this device separates out ions based on their m/z (mass-to-charge) ratios can be a bit technical, but is summarized below and will be explained further in lab. A 2-part video by Professor Laux on YouTube is also available to be viewed on this topic.

   The quadrupole, as implied by the name, consists of 2 sets of parallel cylindrical rods (4 total). Opposite rods are electrically connected, two being charged negative and the other two positive by a variable dc source. Each set of rods also has variable radio frequency AC potentials applied to them.

   Based on the DC and AC voltages, each set of rods act as a mass filter. The combination of both voltages limits only a particular m/z ratio value through the quadrupole. Ions move through the filter in a spiraling manner (Fig. 3). If the DC and AC voltages are scanned through in an increasing fashion, then the entire range of ion m/z values can be separated and analyzed. This can be done extremely fast, with all m/z values being scanned in a few milliseconds!

4) **Detection and Processing**: The ion signal is converted into an electronic signal using a *transducer*. The most common transducer is the *Electron Multiplier* in which the ions strike the surface of a cathode, emitting a burst of electrons. These electrons are accelerated through a series of dynodes at higher and higher voltages that each emit another burst of electrons when struck. The result is a greatly amplified electron current. The greater the number of ions striking the cathode, the larger the resulting current, and the higher the peak intensity on the mass spectrum.

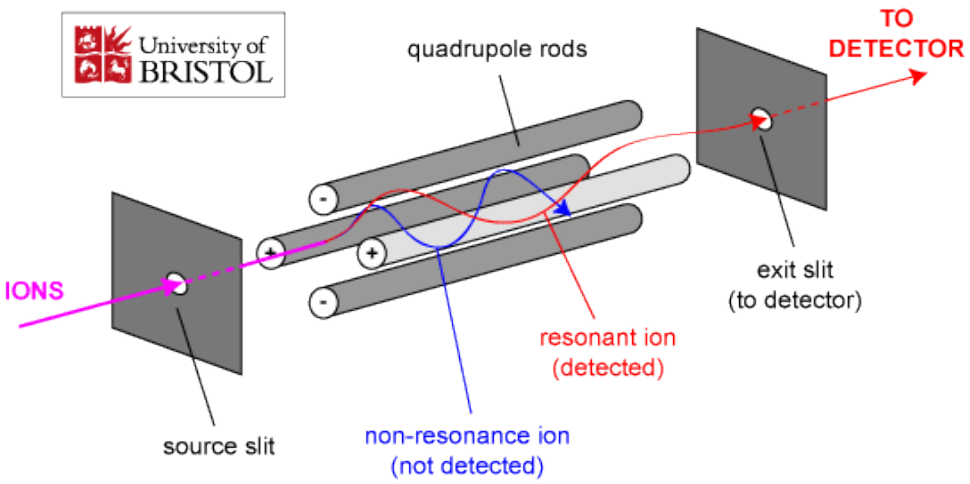


Figure 3: Quadrupole Mass Filter (from the Univ. of Bristol Quadrupole Mass Spectrometry Resource)

EXPERIMENTAL

I: General Suggestions for making solutions

A pure gasoline sample is provided in a brown vial. Make sure this vial is capped at all times to prevent evaporation and inhalation. DO NOT remove the cap unless you run out of gasoline. Use this gasoline to make all the solutions in the experiment.

Brown vials are used for storing the other chemicals used in this experiment (benzene, ethanol, toluene, and o-xylene). Use the hood for preparation of all samples. The brown vials will also be used to store solutions in Parts II & III. The vials MUST be capped as soon as possible to prevent evaporation and inhalation as all chemicals in this experiment are volatile. Other techniques to keep in mind when making solutions:

- Rinse (condition) the 1 mL volumetric flasks at least three times with small amounts of solvent.
- Add the first solute component (gasoline in most cases), then (if applicable) the next component. Dilute with the required amount of solvent and mix thoroughly.
- Syringe needles bend easily when pushing through the septum; be careful when going through a septum by using your gloved fingers as a guide.
- **WARNING**: The possibility of contamination of samples is very high. Please make sure to use the dedicated, labeled syringes for each solution.

II: GC/MS Analysis of Species of Interest

1. On the computer, load the method called **"gas2013.m"**. Your TA will assist you in setting the parameters for the scans.
2. **Teachers should each take a turn making a solution and injecting it into the GC-MS instrument for this lab (4 solutions will be made)**. Make a mixture of ethanol, benzene, toluene, and o-xylene (the four species of interest) in the solvent (1-octanol). Properly condition a 1 mL volumetric flask with the solvent using a disposable glass pipette provided. Using the designated syringes, add 100 μL of ethanol, 10 μL of benzene, 10 μL of toluene, and 10 μL of o-xylene to the flask. Then add enough

solvent to bring the meniscus bottom just onto the line in the neck of the flask. Cap tightly and invert multiple times to mix thoroughly. Transfer this solution to an amber vial and cap.

3. Click on the **One Sample Icon** on the computer (picture of a bottle). Name the file for the sample about to be injected into the GCMS and then click on **Run Method**. Wait for the red "Not Ready" LED on the GCMS to turn off before injecting the sample.
4. Condition the injection syringe with your solution from your amber vial, and then inject **0.05 μL** of the solution into the GCMS through the septum on top of the instrument. Keep the syringe vertical and inject in a quick, repeatable manner. Immediately after the injection, press "start" on the front of the GCMS. On the computer, "*Override the solvent delay (1.00 minutes)?*" will be displayed. **Choose NO**… this is very important so as to not damage the electron impact filament from the large amount of solvent passing through the system.
4. When the run is completed, open the appropriate file and load the TIC (total ion chromatogram. Print the TIC and quickly try to predict which peak corresponds to which analyte based on the retention times and the nonpolar stationary phase. View and print the mass spectrum for each peak by double right clicking on the desired peak. Use the fragmentation patterns to piece together and identify each peak. Confirm your designations by double right clicking on each mass spectrum to perform a library search for the correct analyte.
5. Use these retention times and mass spectra to locate the appropriate peaks in the gasoline samples in the next section.

III: Analysis of Gasoline using the Method of Standard Additions

1. You will measure the ethanol and benzene concentrations in gasoline using the method of standard additions. That is, you will add measured quantities of ethanol and benzene to gasoline and use these as your calibration standards to measure how much ethanol and benzene exist in the original gasoline sample.
2. Table 1 lists the solutions you will be making. Have a new person properly prepare solution A in the hood with the designated syringes (with conditioning!) in a 1 mL volumetric flask. Following the same steps as in Part II, inject **0.05 μL** of the new sample into the GCMS and take its chromatogram. Prepare solution B while you wait for the chromatogram of solution A to finish. In a like fashion, prepare Solution C while Solution B is running. This will save you a lot of time.

Table 1: Composition of mixtures of ethanol, benzene, gasoline and 1-octanol solvent for the Method of Standard Additions.

Solution Number	Volume of **Gasoline (μL)**	Volume of **Ethanol (μL)**	Volume of **Benzene (μL)**	Add **1-octanol** to a **total volume (mL)** of:
A	750	0	0	1
B	750	50	7	1
C	750	150	15	1

3. For the three samples, plot the single ion chromatograms for the ions at m/z = 31, 78, 91 and 106, corresponding to major ions characteristic of ethanol, benzene, toluene and o-xylene respectively. This technique is called "single ion monitoring", or SIM. This can be done by bringing up each file name in turn and clicking **"Chromatogram"→ "Extract Ion Chromatogram".** Type in the ion m/z values of interest listed above and click OK. Click on **"Chromatogram"→ "Percent Area Report" → "Signal to Screen**" to get the peak areas displayed on the computer screen, then locate the specific retention times and peak areas for ethanol, benzene, toluene and o-xylene. Record these peak areas.
4. Calculate the ratios of the peak areas corresponding to ethanol/toluene, benzene/toluene, ethanol/o-xylene and benzene/o-xylene. Using the ratios to toluene and o-xylene, in effect, uses these as internal standards to correct for any differences in injection volumes.

Data Table

Solution	Ethanol / toluene	Benzene / toluene	Ethanol / *o*-xylene	Benzene / *o*-xylene
A				
B				
C				

5. Plot the ratio of ethanol to toluene and ethanol to o-xylene (i.e. two separate lines on one graph) against the added volume of ethanol on Microsoft Excel (as taught on Day 1). See the Appendix for plotting instructions on Excel if necessary. For comparison, to illustrate the advantages of using an internal standard, also plot the absolute peak area of ethanol against the added volume of ethanol in a separate graph.
6. Plot the ratio of benzene to toluene and benzene to o-xylene (again two lines on one graph) against the added volume of benzene.
7. Carry out a least squares analysis for each of the lines (easily done on Microsoft Excel) to obtain the slope (m) and the y-intercept (b). The relationship between the ratio, R, of the ethanol (or benzene) to the internal standard and the volume of ethanol (or benzene) added to the mixture ($V_{ethanol}$) is as follows:

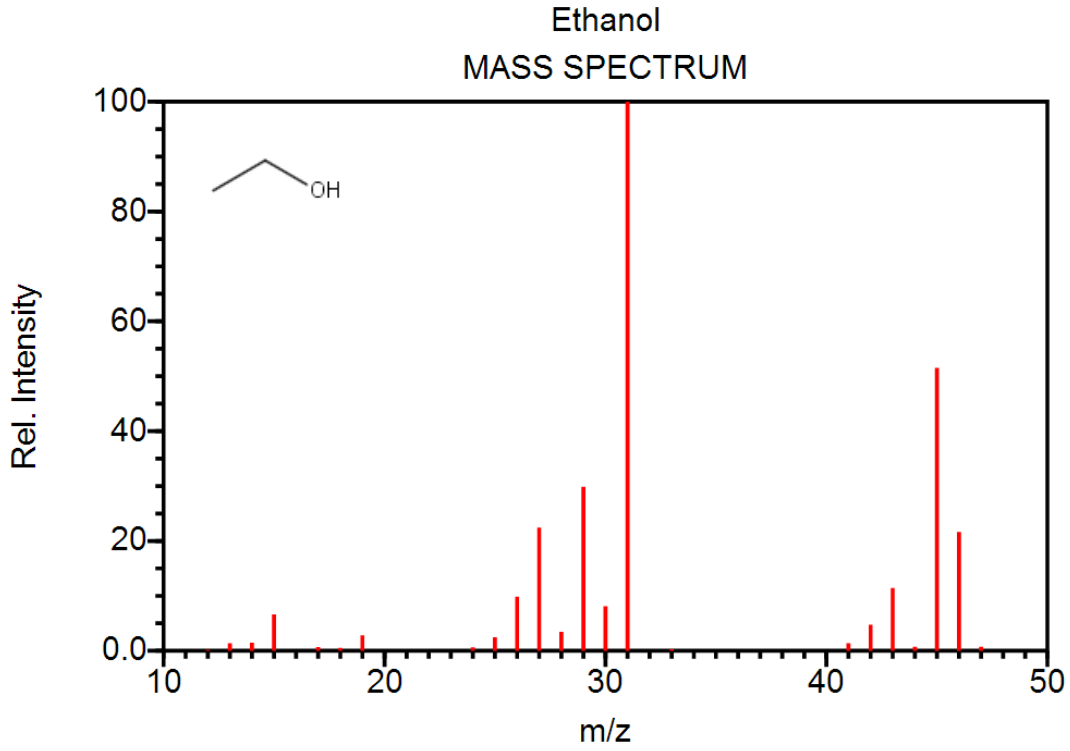
$$\mathbf{R_{ethanol/Std.} = m\,V_{ethanol} + b} \qquad \mathbf{(y = mx + b)} \qquad \mathbf{(I)}$$

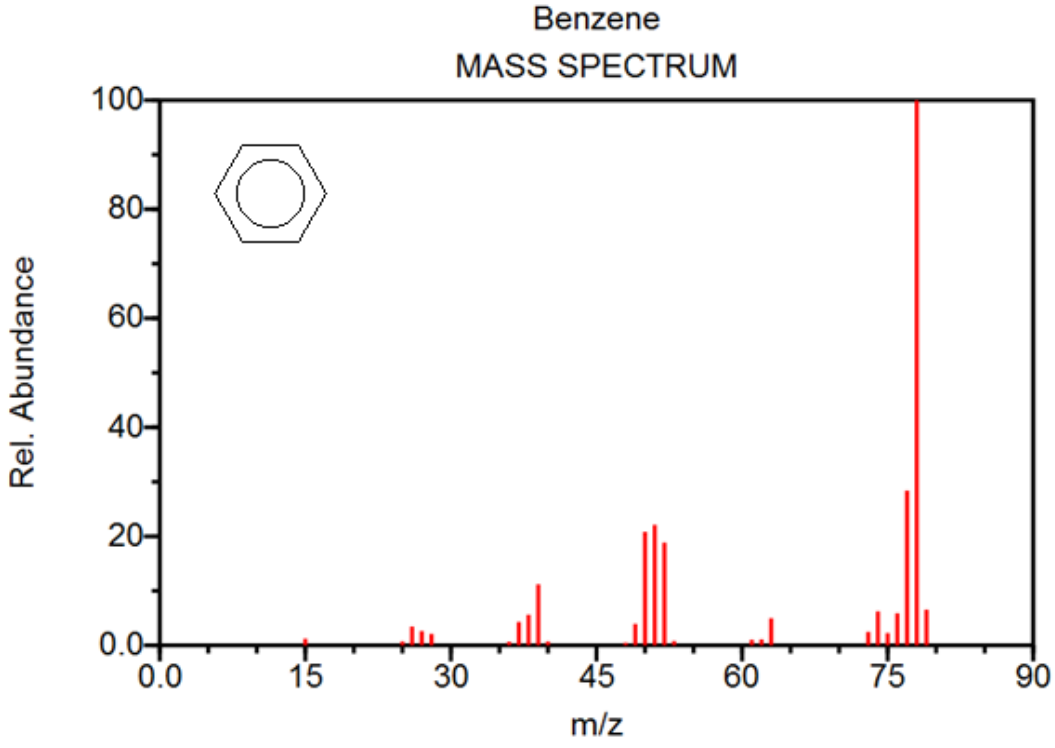
The ratio of the intercept (b) to the slope of the lines (m) is related to the (constant) volume of the gasoline used in each mixture, V_{gas}, and the volume fraction (f) of ethanol (or benzene) in the gasoline, which is the quantity of interest:

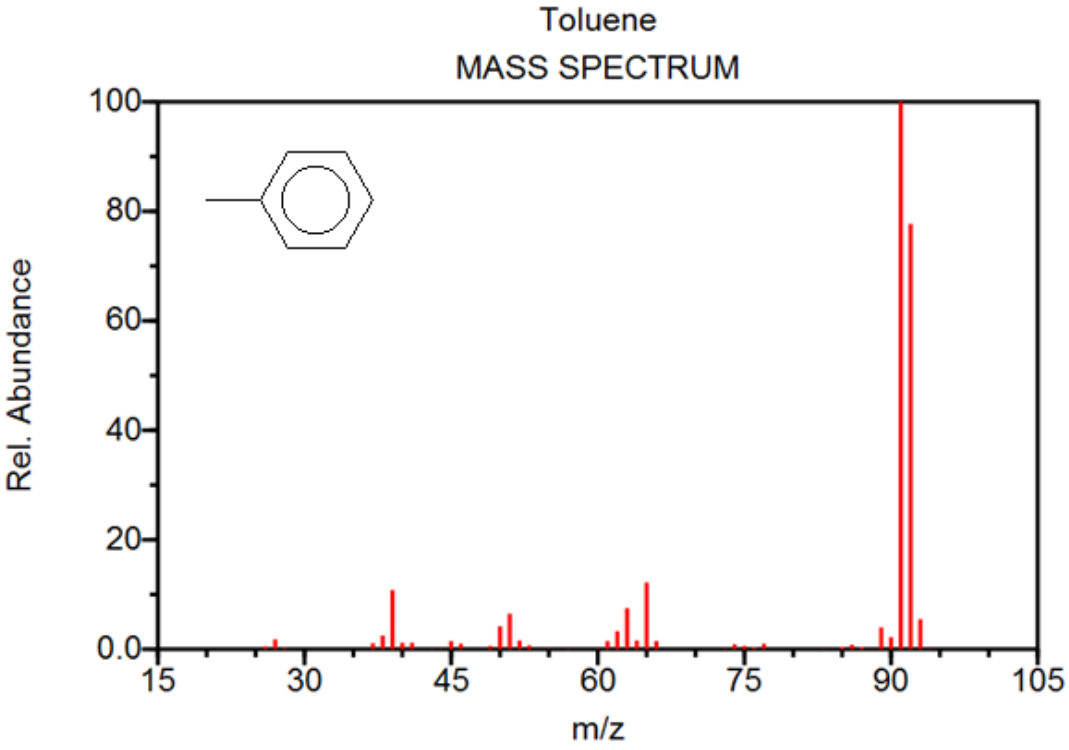
$$\mathbf{b/m = f\,V_{gas}} \qquad \mathbf{(II)}$$

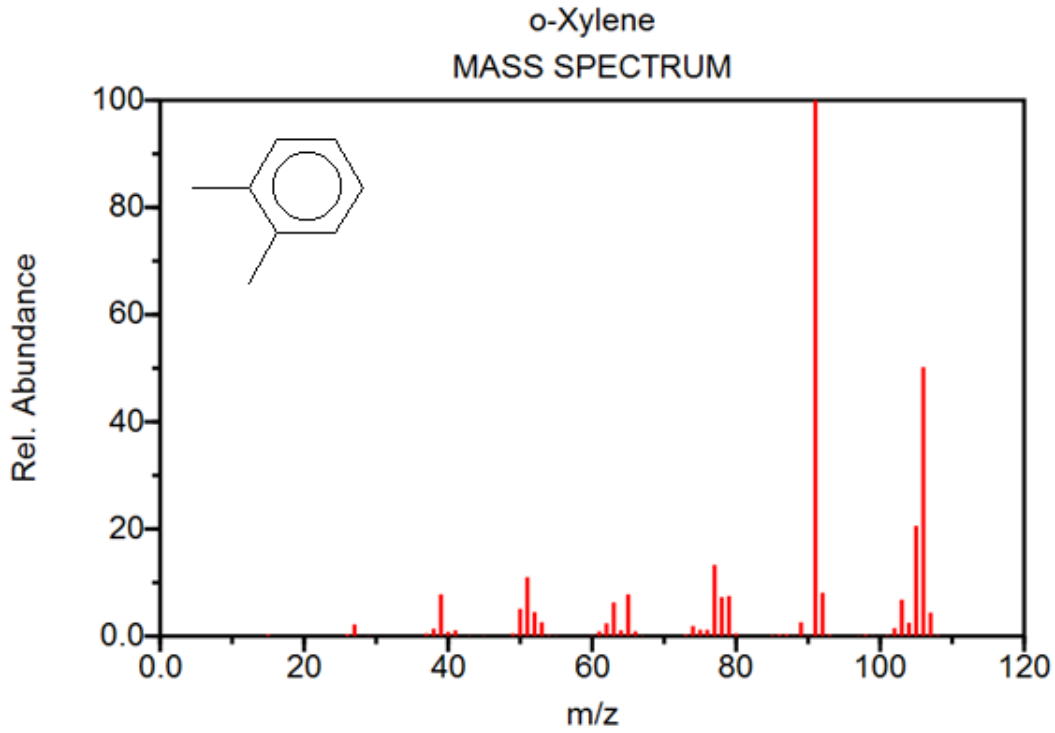
Use the slope and intercept of these plots to calculate the volume fraction of ethanol and benzene in the gasoline. Since you are using two different internal standards, toluene and o-xylene, you will get two different estimates for each compound. Convert these to the volume percentage of ethanol and benzene respectively in gasoline.

<u>Appendix</u>: Electron-impact Mass Spectra of ethanol, benzene, toluene, and o-xylene.






Toluene
MASS SPECTRUM
Rel. Abundance
100
80
60
40
20
0.0
15
30
45
60
75
90
105
m/z


o-Xylene
MASS SPECTRUM
Rel. Abundance
100
80
60
40
20
0.0
0.0
20
40
60
80
100
120
m/z