Environmental Forensics: Chemical Fingerprinting of Fuels

Goal: Use gas chromatography mass spectrometry (GC-MS) of “fingerprints” of fuel standards to determine the source of a series of fuel spills.

Objectives
1. describe basic GC-MS operating principles and operation
2. describe and predict relationships between chemical structure/mass, boiling point, and chromatographic retention time
3. draw meaningful analogies between chromatography and the fate of chemical contamination in the natural world

1. Background

1.1. Environmental Significance

1.1.1. Petroleum fuels
It is estimated that between 1.7 and 8.8 million tons of petroleum hydrocarbons are released into the marine environment each year, with a best estimate of 3.2 million tons per year. Although major oil spills, like the Exxon-Valdez spill of 1989 or last summer’s Deepwater Horizon spill in the Gulf of Mexico, garner much media and citizen attention, you may be surprised to learn that tanker accidents only account for ~13% of the total petroleum hydrocarbon mass released to the environment. The remaining 87% is primarily released through smaller, less noticeable “spills”, such as washing of tanks and changing of tanker ballast water. The source of these less publicized spills can be difficult to discern. Additionally, approximately 0.3 million tons of petroleum makes its way into marine systems entirely via natural processes, such as natural seeps.

Petroleum doesn’t dissolve in water to a great extent because it is hydrophobic (“water hating”). Hydrophobic compounds – compounds that are generally nonpolar and large – have the potential to accumulate in fatty (hydrophobic) tissues in organisms and in organic fractions of soil and sediment. The chemistry phrase “like dissolves like” explains a lot of environmental chemical behavior! Thus, the contamination can quite literally “stick” around for a long time. Additionally, hydrophobic petroleum compounds can also remain in the environment as “non-aqueous phase liquids” or NAPLs (pronounced “napples”). In other words, NAPL is just undissolved, pure organic liquid, oil or gasoline in this case, that slowly dissolves or evaporates over the course of years. NAPLs generally serve as long-term sources of environmental contamination. Many compounds in petroleum are toxic or carcinogenic to organisms. Moreover, the direct effects, including oil-coated birds and contaminated or severely depleted food supplies can wreak havoc on an ecosystem.

Petroleum hydrocarbons can be degraded somewhat into less harmful compounds through natural processes, such as biodegradation by bacteria that are able to metabolize these chemicals and use them as food sources. Unfortunately, the rate of biodegradation is often too slow to mitigate the negative ecosystem consequences of the high rate of anthropogenic petroleum releases. With human energy consumption increasing at approximately 2.2%/yr and petroleum
products (e.g., coal, oil, gasoline) providing 2/3 of measured energy use worldwide, it is important to understand petroleum chemistry and its environmental implications.

1.1.2. Biodiesel
Due to increased environmental and foreign policy concerns associated with the use of petroleum fuels and consequent tax incentives designed to promote non-petroleum fuels, the use of biodiesel is increasing dramatically. Scientists have found that biodiesel reduces CO₂, SO₂, CO and particulate output per unit of energy relative to petro-diesel, although biodiesel NOₓ emissions are generally higher due to higher combustion temperatures. NOₓ compounds include NO and NO₂, which contribute to the formation of acid rain, photochemical smog, and deliver additional nitrogen to ecosystems, which can increase the potential for eutrophication. Biodiesel is composed of fatty acid methyl esters (FAMEs), which are readily biodegraded and relatively nontoxic. The chemical fingerprint of pure biodiesel (B100) is much simpler than petroleum fingerprints, since only a few different methyl ester compounds are contained in most mixture. However, biodiesel is often blended with petrodiesel, complicating its chemical fingerprint. Biodiesel itself, can be chemically derived from virtually any vegetable oil or animal fat.

1.2. Experimental Approach
We are approaching this lab from the perspective of environmental forensics, a field that seeks to answer questions regarding environmental contamination, such as:

Who caused the contamination? How long ago did it occur? Was it accidental or deliberate?

We will rely on the concept of a chemical fingerprint, which refers to the fact that many contaminants, petroleum or otherwise, often have specific combinations and ratios of chemical constituents that differ among sources of that product. For example, you may think that “crude oil is crude oil is crude oil”, and yet, if you know what to look for, you would see that crude oils derived from different oil fields around the world have distinct chemical fingerprints.

Pattern recognition is a related concept, in which the most important features or patterns of a fingerprint are extracted. Suppose you had 10 samples of fuel and their chemical fingerprints. It would not be helpful to focus on a feature that 9 out of 10 fuel fingerprints had in common. On the other hand, by identifying a set of features that differed among the 10 fuels, you’d have a good chance of recognizing and identifying one of those fuels if it were involved in a spill.

In this lab, I will provide you with chemical fingerprints for four standard fuels. You will use these standard fingerprints to create your own pattern recognition methods and determine which fuel(s) was/were involved in a series of fuel spills.
2. Theory

2.3. Understanding petroleum fuel fingerprints

2.3.1. Primary, secondary, and tertiary factors.
Fossil fuels, including coal and crude oil, are complex mixtures of 100s of individual chemical compounds. Fossil fuels consist largely of hydrocarbons, molecules made up of C and H, although S, O, N, and metals are also present at lower abundances. Chemical fingerprinting of fuels focuses primarily on hydrocarbons. To interpret the hydrocarbon fingerprint of a petroleum fuel spill, and ultimately discover the source of the spill, requires an understanding of factors that can influence and change fingerprint: primary, secondary, and tertiary factors.

Primary factors, also called genetic factors, include the geologic conditions under which the fuel was formed and accumulated in the subsurface. For example, oil derives from marine environments, while coal is of terrestrial origin; these factors produce quite different fingerprints. Secondary factors are anthropogenic changes that the fuel undergoes during extraction or processing. Refining of crude oil into fractions or petroleum distillates, discussed further below, represents a secondary factor in development of a petroleum hydrocarbon fingerprint. Finally, tertiary factors, the changes the fuel undergoes after its release into the environment, include evaporation, dissolution into water, biodegradation, and many other processes.

Figure 1 shows a series of fingerprints for a fuel spill and how it changes over time in the environment; this “environmental weathering” is an example of a tertiary factor. Even if you don’t know much about what these plots actually mean, you can see that the three graphs look different – the chemical fingerprint is changing over time.

Figure 1. Changes in fingerprint over time due to environmental weathering. (Murphy and Morrison, 2002)
2.3.2. Crude oil refining: a secondary factor

Crude oil is not terribly useful in and of itself. In order to increase the usability for various purposes, crude oil is refined. Refining includes separating the complex mixture into groups of chemicals based on their boiling point. They perform this separation by distilling the crude oil. Distillation towers and a schematic of the distillation process are shown in Figures 2 and 3, respectively.

Gases, typically containing 1-4 carbons (C1-4), are drawn off the crude oil at ambient temperatures. As temperature is increased in the distillation column, C5-12 compounds (gasoline) are evaporated and collected. This temperature ramp occurs spatially within the distillation tower and continues until the crude has been separated into several fractions. Table 1 shows some common fractions produced and their physical properties.

Table 1. Common crude oil fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th># C atoms</th>
<th>MW</th>
<th>Boiling range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaseous</td>
<td>1-4</td>
<td>16-58</td>
<td>-126-0</td>
</tr>
<tr>
<td>Gasoline</td>
<td>5-12</td>
<td>72-170</td>
<td>0-204</td>
</tr>
<tr>
<td>Kerosene</td>
<td>10-16</td>
<td>156-226</td>
<td>180-274</td>
</tr>
<tr>
<td>Gas oil</td>
<td>15-22</td>
<td>212-294</td>
<td>260-371</td>
</tr>
<tr>
<td>Lube oil</td>
<td>19-35</td>
<td>268-492</td>
<td>338-468</td>
</tr>
<tr>
<td>Residue</td>
<td>36-90</td>
<td>492-1262</td>
<td>468+</td>
</tr>
</tbody>
</table>

In addition to distilling into separate fractions, other refining processes, including cracking and alkylation, produce additional useful petroleum products. Cracking involves splitting large molecules from the heavier fractions (e.g., kerosene or gas oil) into smaller molecules; this more than doubles the production of gasoline, a desirable outcome because gasoline is among the most useful (and profitable) petroleum fractions. In contrast to cracking, the process of alkylation combines smaller molecules into larger ones. In this way, low-C# gases are combined to form higher-C# compounds – and once again, more gasoline! These refining processes convert much of the aliphatic fraction into aromatic compounds to boost the octane rating of gasoline, one measure of fuel quality. You will see these differences in the chemical fingerprint of gasoline versus diesel fuel. In fact, when we talk about gasoline, we talk almost exclusively about the aromatic “BTEX” compounds (benzene, toluene, ethylbenzene, and xylenes), which are shown in Figure 4 below.
2.4. Petroleum fuel use at Middlebury College

Table 2 shows the Middlebury College’s yearly fuel consumption for individual fuel types up to 2000. In 2006, Middlebury replaced #2 oil, used for heating some residences and off-campus rental units, with a biodiesel blend. The biodiesel blend, B20, consists of 20% biodiesel and 80% petro-diesel. The use of B100 (100% biodiesel) is being explored. B100 has been shown to cut CO$_2$ emissions by 78% relative to petrodiesel, while B20 cuts CO$_2$ emissions by ~15%. Biodiesel blends are useful, because the lower viscosity compared to B100 allows blends to be used directly in petro-engines without modification. B100 generally requires the engine to be started on petro-diesel, which heats up the B100 and lowers its viscosity, and then switched over to the B100; before stopping the engine, the B100 must also be flushed from the lines with petro-diesel so the lines do not get “clogged” with viscous, cold B100. This process requires slight engine modification. Most recently (January 2009), Middlebury College brought a biomass power plant on line, which will burn sustainably harvested wood products to further reduce the College’s consumption of fossil fuel.

**TABLE 2: Yearly Fossil Fuel Consumption**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Coal (Ton)</th>
<th>Gasoline (gal)</th>
<th>Diesel (gal)</th>
<th>#2 Oil (gal)</th>
<th>#6 Oil (gal)</th>
<th>Propane (gal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>630</td>
<td>21,934</td>
<td>21,387</td>
<td>73,171</td>
<td>949,651</td>
<td>No Data</td>
</tr>
<tr>
<td>1991</td>
<td>945</td>
<td>48,657</td>
<td>43,841</td>
<td>233,385</td>
<td>899,169</td>
<td>No Data</td>
</tr>
<tr>
<td>1992</td>
<td>0</td>
<td>48,136</td>
<td>35,428</td>
<td>254,949</td>
<td>1,242,467</td>
<td>No Data</td>
</tr>
<tr>
<td>1993</td>
<td>507</td>
<td>47,840</td>
<td>50,876</td>
<td>326,360</td>
<td>1,371,846</td>
<td>30,551</td>
</tr>
<tr>
<td>1994</td>
<td>0</td>
<td>51,549</td>
<td>29,019</td>
<td>306,803</td>
<td>1,274,363</td>
<td>27,879</td>
</tr>
<tr>
<td>1995</td>
<td>0</td>
<td>55,520</td>
<td>47,625</td>
<td>316,161</td>
<td>1,269,161</td>
<td>34,617</td>
</tr>
<tr>
<td>1996</td>
<td>0</td>
<td>55,618</td>
<td>36,120</td>
<td>368,439</td>
<td>1,419,290</td>
<td>32,033</td>
</tr>
<tr>
<td>1997</td>
<td>0</td>
<td>55,534</td>
<td>45,972</td>
<td>343,478</td>
<td>1,253,018</td>
<td>39,632</td>
</tr>
<tr>
<td>1998</td>
<td>0</td>
<td>61,136</td>
<td>38,184</td>
<td>322,191</td>
<td>1,295,487</td>
<td>38,440</td>
</tr>
<tr>
<td>1999</td>
<td>0</td>
<td>64,606</td>
<td>44,006</td>
<td>403,199</td>
<td>1,481,972</td>
<td>39,745</td>
</tr>
<tr>
<td>2000</td>
<td>0</td>
<td>70,221</td>
<td>71,520</td>
<td>390,599</td>
<td>1,694,233</td>
<td>40,759</td>
</tr>
</tbody>
</table>


In this lab, you will investigate a series of (hypothetical) fuel spills at Middlebury College to determine exactly which fuels were involved (gasoline, petrodiesel, B20, or B100).
2.5. Obtaining a chemical fingerprint

2.5.1. Separating the Mixture
In order to examine the chemical fingerprints of fuels, the complex mixture needs to be separated into its individual chemical components. Gas chromatography (GC) allows us to perform this separation. Figure 5 shows the major components of a GC system. A carrier gas (helium, in our case) is passed through the GC column and carries the injected sample (solvent + analytes) through the column. The analytes in the sample are separated in the column based on their differing affinities for a stationary phase that coats the inside of the GC column. As each analyte elutes from the column, it is detected and its signal recorded.

Reverse-phase chromatography, or chromatography using a hydrophobic stationary phase, was used to obtain the chemical fingerprints for a series of pure fuels. Because “like dissolves like”, hydrophobic compounds will “dissolve in” the hydrophobic stationary phase and be retained in the GC column longer than less hydrophobic compounds. Thus, separation of the components in the fuel is achieved due to different affinities between the column stationary phase and each analyte. A temperature ramp is also used to elute the analytes from the column. By starting at room temperature, the heavier compounds remain at the front of the column; only the more volatile compounds get transported with the carrier gas through the column. As the temperature increases, so too does the volatility of the heavier compounds, ultimately allowing them to travel through the column to the detector. These processes separate the mixture and result in a chromatogram, a plot of the detector signal versus time. An example chromatogram for diesel fuel is shown in Figure 6.

**Diesel Fuel**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6H14</td>
<td>Decane</td>
</tr>
<tr>
<td>C8H18</td>
<td>Dodecane</td>
</tr>
<tr>
<td>C10H22</td>
<td>Tetradecane</td>
</tr>
<tr>
<td>C12H26</td>
<td>Hexadecane</td>
</tr>
<tr>
<td>C14H30</td>
<td>Octadecane</td>
</tr>
<tr>
<td>C16H34</td>
<td>Octacosane</td>
</tr>
<tr>
<td>C20H42</td>
<td>Hexacosane</td>
</tr>
</tbody>
</table>

![Figure 5. Major components of a GC system.](http://en.wikipedia.org/wiki/Gas_chromatography (1/07)](http://en.wikipedia.org/wiki/Gas_chromatography)

![Figure 6. Diesel fuel chromatogram.](http://www.chem.agilent.com/temp/rad43E85/00028588.pdf)
2.5.2. Identifying the components of the mixture

Once the analytes in the fuel mixture have been separated, we need a means of detecting and identifying them. In this lab, we will use mass spectrometry (MS) to achieve these tasks. In MS, the GC effluent (carrier gas + analyte) is bombarded with electrons, causing the molecules to break into characteristic fragment ions. The relative abundance of different size (mass) molecular fragments provides a mass spectrum by which individual peaks in a chromatogram can be identified as a specific compound (Figure 7). For example, consider the molecule decane, a chain of 10 carbon atoms linked together with all the requisite hydrogens in place. When it is bombarded by the electrons, it breaks apart into a characteristic mass spectrum (Figure 8). You notice that there is a small “molecular ion” – an intact decane molecule – at 142 m/z (m/z = mass-to-charge). Since the charge on most ions is +1, the m/z ratio essentially represents the mass of the ion, and, in fact, the molecular weight of decane is 142. In addition to the molecular ion, you see a series of fragment ions, each differing by 14 mass units.

What has a mass of 14?

A -CH₂- unit, termed a methylene group! Thus, you can see that when the decane molecule is bombarded with electrons, it characteristically breaks off various numbers of methylene groups. For other molecules, the molecular ion is observed at much higher abundance, due to higher stability of the molecule. This is the case, for example, with benzene. Thus, most molecules, even those with the same molecular weight, will have different mass spectral fingerprints. The uniqueness of the fingerprint is tied to both which masses are present, as well as the relative abundances of different masses.

We will identify the individual compounds represented in the fuel standard chromatograms by matching mass spectra, such as that for decane in Figure 8, to standard spectra contained in an electronic MS library. This is much like matching a fingerprint obtained at a crime scene to fingerprints in an electronic database (e.g., AFIS). You will use a combination of GC retention times and MS fingerprints from the fuel standard chromatograms to identify peaks in your unknown spill sample.

2.6. Pattern Recognition

Once we have collected a GC chemical fingerprint (e.g., for diesel fuel in Figure 6) and identified the individual mixture components using MS (e.g., Figure 8), you will need to identify what combined features of the fingerprint make each fuel “look” different from other fuels. The fundamental approach used for distinguishing fuels involves monitoring for the presence/absence
of “tell-tale” compounds; comparing peak area ratios is also a common technique. An example of the former: FAMEs are only present in biodiesel and not in petroleum products. Examples of using peak area ratios include comparing ratios of

- high MW compounds to low MW compounds,
- aromatic compounds to aliphatics compounds,
- straight-chain to branched aliphatics, and
- two closely spaced compounds (e.g., C16 to C17).

Often there is not a single feature that can be used in isolation to identify an unknown petroleum product; the mixtures are simply too complex and have many features in common. Thus, it is important to appreciate the idea of a “pattern”, or multiple features that taken together seem to identify the compound. You will work in pairs/threes and use the GC-MS data I provide for gasoline, diesel, B20, and B100 standards to create your own pattern recognition method. You will then individually use this method to identify the unknown fuels in a series of fuel spills.

3. Schedule

Week 1

Before lab
1. Assimilate information in lab handout.
2. In your lab notebook in your own words
   - State the purpose of this lab and the approach of the lab (2-3 sentences)
   - Answer the pre-lab questions (last page of this handout)

During lab (meet in MBH 4th floor fishbowl)
3. Learn basic operation of GC-MS and use of the Chemstation software
4. Work collaboratively to
   - Interpret GCMS data and develop fuel recognition methods for the standard fuels using the GCMS data I provide
   - apply your methods to identify the fuel(s) present in the spills (for which I will provide data)

After lab
5. Prepare notebook (results and conclusions) for next week (details to be handed out in lab)

Week 2 (meet in MBH 4th floor fishbowl)
6. Discuss with group: present your methods, results, and conclusions, and be prepared to answer questions.
7. In-class chromatography/fuel chemistry activity.
4. Laboratory Methods

The data for the four fuel standards were collected on the same instrument and under the same conditions that the unknown spill samples were run. This allows you to compare the retention times of peaks in the unknowns directly to the fuel standard data.

**Instrumental parameters:** Agilent GC5890/MS5975 with DB5-MS column (5% phenyl methylsiloxane) (30 m × 0.25mm × 0.25-µL film thickness); 2-µL sample injection; helium carrier at 1 mL/min; splitless injection; solvent delay 3 min; injector 280 °C; transfer line 300 °C; oven temperature program 30 °C for 5 min, ramp to 280 °C at 6 °C/min, ramp to 300 °C at 25 °C/min and hold for 10 min.)

5. References


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**Pre-lab Questions**

1. What change would you expect to see in your chromatogram if you injected a sample too early (while it was still too hot from the previous run)? Be specific.

2. Briefly describe the most obvious change you notice in the chromatograms of the fresh vs. severely weathered gasoline (Figure 1). Use information in this lab handout about the elution order of compounds from the GC column (what comes out first?) to explain the difference you noted. In other words, how is the environment like our GC column?

3. Note the labeled peaks in the diesel chromatogram (Figure 6). Look up two of the labeled compounds on the internet: write their names and draw their chemical structures.