

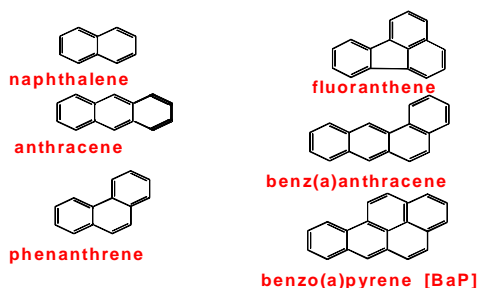
C193 – Instrumental Methods of Analysis
Philadelphia University

Identification of Polycyclic Aromatic Hydrocarbons (PAHs) using Gas Chromatography-Mass Spectrometry and Quantification using the Internal Standard Method

INTRODUCTION: Polycyclic aromatic hydrocarbons (PAHs) are a suite of organic compounds released into the environment as gases or particles during the incomplete combustion of organic material. The compounds have a number of sources including:

- Mobile sources such as cars, buses, trucks, ships and aircraft
- Industrial sources such as power generation, steelworks, coke ovens, aluminum production, cement kilns, oil refining and waste incineration
- Domestic sources such as combustion for heating and cooking especially solid fuel heaters using wood and coal
- Fires and smoke resulting from the burning of vegetation in agricultural processes, bushfires, grilling of food, or tobacco smoke

The smallest member of the PAH group is naphthalene (see below), a two-ring compound. Three- to five-ring PAHs may occur as either gases or particles in air. PAHs with five or more rings (Benzo(a)pyrene or B[a]P) tend to attach themselves to the surface of particles or are soot particles when formed.



Although many different PAHs have been identified, there is still limited published toxicological data each of them. One of the best characterized is benzo(a)pyrene (above), considered to be one of the most potent PAHs from a toxicological point of view. The US EPA has identified 16 priority PAHs based on their potential to be carcinogenic in animals and humans.

GOALS OF LABORATORY: In this session, you will be given a solution containing 1 PAH compound. First, you must identify the PAH by comparing it to a standard calibration standard as well as confirmation by comparison to library spectra. Secondly, you are asked to determine the mass of your PAH in your particular sample using the internal standard method.

We'll go through this in a step-wise formation. The goal really is not to get a value for the concentration of your PAH (though that will be nice) but to have you experience the steps needed in devising a method for analysis as well as getting acquainted with the GC-MS.

PROCEDURE FOR ANALYSIS USING GC-MS:

This following was modified from a Physical Chemistry lab written by Dr. Cheryl Longfellow of Philadelphia University. It will be helpful in getting you acquainted with the instrument.

In this lab you will use GC/MS to identify and quantify polycyclic aromatic hydrocarbons (PAHs). To ensure that each student has some understanding of how to operate the GC/MS, we will do a few short exercises. Please note all of your answers to the following questions in your lab book.

Do not use the instrument unsupervised. The GC/MS is configured so that Injector B is connected to an Elite 5MS column that in turn is connected to the mass spectrometer. **It is important to ensure that the column has a He flow through it whenever the GC oven is on.** A flow of 1 mL/min is typical. There is an ECD (Electron Capture Detector) that would be used with a second column connected to Injector A. Currently, there is no column attached to Injector A. **Do not attempt to inject a sample into Injector A.** Ensure that the ECD is off (see step 7 below). When the instrument is not in use, it should be left with the pumps on and the filament off. If the instrument is idle for longer than 1 week, decrease the inlet line temperature and the source temperature from 180 to 100 °C.

In this laboratory, we will be using the auto-sampler to make injections of unknown solutions (analytes contained in hexane) into column B. A peak in the TIC (total ion count) indicates that a component has eluted from the column and been detected by the mass spectrometer at a particular delay time. A mass spectrum of each TIC peak is then available (right mouse click on the TIC peak to show the mass spectrum of that peak). To begin the identification process of a peak you will compare your mass spectrum to the mass spectra available in the NIST database using the F1 key.

Answer all questions in your labbook.

1. Sign the logbook with name and date.

2. The first thing to check is the pressure. What is the pressure on the Pirani gauge? What is the pressure on the Penning gauge? What is the maximum pressure at which you can proceed with your experiments (Tutorial p.54)?
3. Every time that the instrument is used it is necessary to check the ratios at m/z 4, 18, 28, 32. What does z stand for? What are these masses? Which mass do you expect to be the highest? What should the optimum ratio be? Examine pages 82-83 in the Tutorial manual. If there is an air leak, what masses do you expect to be the highest? Turn on the filament and high voltages (press for operate) and write down these ratios.
4. From the tune page ensure that you are working from the D drive in the IMA Project. Do a "save as" before beginning with today's date. We will now tune the instrument. This does not need to be done every time. Follow the instructions on pages 105-109 in the Tutorial. Make sure that the reference gas is not being pumped out (i.e. Tune page → Gas → "pump out reference gas" should not be checked). Then turn on the reference gas. Be sure to do a maintenance tune and not a full tune. The mass spectrometer is tuned by letting in a small amount of **perfluorotributylamine** ($C_{12}F_{27}N$) gas as a reference. Set the peak editor for $m/z = 69, 131, 219, 502$ (p.72 Hardware manual). What are the fragments at these four values of m/z ? Print out a copy of your tune results. Check that the masses are calibrated, i.e. the middle of the peak should be centered. Sometimes when doing an autotune it is necessary to press stop and then start again if the ramp does not start right away.
5. The GC method determines the injector temperature, carrier flow, and oven temperature (TurboMass page → GC → method editor, load a new method, use instrumentation → instrument control). It is recommended to set injector B at 300 °C, the carrier flow at 1 ml/min, and the initial oven temperature at 90 °C.

Construct a method which incorporates the information from Table 1:

Table 1. Sequence for temperature programming of the gas chromatographs oven.

Initial temp of 90° for 1 min
Ramp at 5°C/min to 196°C, hold at 196°C for 0 min
Ramp at 4°C/min to 260°C, hold at 260°C for 2 min
Ramp at 3°C/min to 300°C, hold at 300°C for 5 min

This should give you a GC method that is approximately 58.5 minutes long. Ensure that the ECD is off and no carrier gas flows are going to column A. Note that you will be doing injections with the autosampler. Make sure the four solvent vials on the autosampler wheel are full of hexane, the solvent used for this particular laboratory.

6. With the aid of your instructor, develop a MS method (Tutorial p 127). You will be injecting 1 μL of liquid sample (in hexane). Set a solvent delay of 6 minutes to ensure that the filament is off during this time. You will acquire both a TIC (total ion count) and a SIR (select ion response). Acquiring the TIC will allow you to observe the mass spectrum for each of the peaks from the resulting chromatogram. For qualitative studies, TIC is essential and allows comparison of your acquired mass spectrum to that of library spectra for identification. Using SIR, only certain masses are scanned. SIR is more useful in quantitative analysis when the compound is known (and therefore its molecular and sometime secondary ions are known) but the mass of the compound within the sample is not known. Using Table 2, also include a SIR scan in your method assuming you have every one of the listed PAHs in your sample, including the internal standard.

Your unknown solution contains one the five PAHs listing in the following table. How will you know which PAH compound you have? Can you name another way in which you could identify your unknown PAH?

Table 2. List of Possible PAH compounds in your unknown and their respective approximate retention times and molecular ions.

PAH Compound	Approximate Retention Time (min)	Molecular Ion
Phenanthrene	18.79	178
Anthracene	19.01	178
Fluoranthene	24.17	202
Benzo(a)anthracene	31.35	228
Benzo(a)pyrene	38.13	252
<i>Fluoranthene-d10 (Used as Internal Standard)</i>	24.10	212

7. In order to quantify the amount of unknown PAH in your sample, you must analyze a 'calibration standard' and you must have internal standard in both your 'calibration standard' and your unknown PAH solution. Prepare a calibration standard and your sample by the following method.

The Calibration Standard ("Std"):

-take 100 μL of the calibration standard solution and place it in an autosampler vial. This solution contains 5 known PAHs (See Table 2) at a concentration of 1000 ng/mL.

-take 100 μL of internal standard solution and place it in an autosampler vial. The internal standard solution contains d-10 Fluoranthene at a concentration of 1000 ng/mL.

Your Sample (“Sam”):

-to the autosampler vial given to you, add 100 μL of internal standard solution. The internal standard solution contains d-10 Fluorene at a concentration of 1000 ng/mL.

8. Once you are satisfied with your methods, create a sample list (Tutorial p143-146). Recall that you are using injector b and make sure you indicate with vial is in which location on the autosampler. Press start and then run. When the GC reads ready (oven should be at 40 °C), the autosampler will inject 1 μL of your sample. Update your chromatogram in real time (Chromatograph→display→ real time update). At what time do you see the first peak? How many peaks total are there over 58 minutes?
9. Now we will examine some of the peaks more closely. Why are there so many peaks in the spectrum? Use the F1 key to compare it to the NIST library. What compound gives you the most hits? Draw its structure in your labbook. Do you think this makes sense? What is the identity of you PAH in your unknown solution?
10. As you likely guessed, the peak from your unknown PAH and from the internal standard PAH in your sample (**Sam**) can be identified by its retention time relative to the retention time of that specific compound in the calibration standard (**Std**).

Quantification of individual PAH compounds will be performed using the internal standard method. This method eliminates errors due to variation in the sample injection, and is independent of the final extract volume.

Use a spreadsheet to calculate the Relative Response Factors (RRF) for each PAH in the **Std** using the following equation and the information provided in Step will be generated as required by instrument calibration criteria:

$$\text{RRF}_{\text{Std}} = \frac{(\text{mass PAH compound} / \text{area PAH compound})_{\text{Std}}}{(\text{mass internal std} / \text{area internal std})_{\text{Std}}}$$

Now, using “Sam” information, calculate the mass of PAH in your “unknown” by using the following equation (where i.s. = internal standard):

$$(\text{Mass PAH compound})_{\text{sam}} = (\text{Peak area of compound})_{\text{sam}} \times \text{RRF}_{\text{Std}} \times \frac{(\text{mass i.s.})}{(\text{area i.s.})_{\text{sam}}}$$

What is the mass of PAH in your unknown?

Let's assume that the mass of the PAH in your autosampling vial was extracted from 10.0 g of wet sediment. The sediment had a percent water value of 60%. Calculate the concentration of your PAH in the sediment sample. Express the concentration as ng of PAH per g of wet sediment and also in the more common form of ng of PAH per g of dry sediment.

The following equation will be helpful:

$$(\text{Conc. PAH compound})_{\text{sam}} = \frac{(\text{Mass PAH compound})_{\text{sam}}}{(\text{vol or mass collected/extracted})}$$