GAS CHROMATOGRAPHY (GC)

Pre-Lab Questions

The answers are due at the beginning of each experiment (the questions are for credit and some may appear on your final exam). Refer to Skoog et al., 2007, Principles of Instrumental Analysis, 6th edition.

1). The GC (GLC) will do a chromatographic separation of mixed compounds. Describe the main instrumental constituents involved in the separation and detection of the components of the mixture and explain their principal of operation. Your answer should include:
   a. Injection (split/splitless,) principal(s)
   b. Separation (column differences, principal of separation)
   c. Detection (types and principals)
   d. Acquisition
   e. Instrument control

Objectives:

- To get acquainted with a general setup of a GC system.
- To develop methods for optimization of separating and determining the concentration of known or unknown mixtures.
- To investigate the effects of oven temperature, carrier gas flow rates, and column type on retention times and separation efficiency of an analyte.
- To look at the effect of chain length, molecular conformation and volatility on chromatography separation and column retention times.
- To investigate the limits of detection (LOD) and quantitation (LOQ) of an FID detector.

Introduction: Gas chromatography (GC) is a separation-based analytical tool used extensively for analyses of volatile organic compounds. This could include environmental pollutants, pesticides, petrochemical products and common impurities in pharmaceutical products. Depending on the type of detector used, the analytical range varies from parts per million (ppm) to parts per trillion (ppt).

Instrumental parameters such as oven temperature, carrier gas flow rate, column length, and column stationary phase have a profound effect on the separation efficiency and analysis times. Each one of these parameters can be optimized in order to obtain the best separation of analytes in sample mixtures in the least amount of time.

Factors that affect GC separations: Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below:

- **Volatility of compound**: Low boiling (volatile) components will travel faster through the column than will high boiling components
- **Polarity of compounds**: Polar compounds will move more slowly, especially if the column is polar.
- **Column temperature**: Raising the column temperature speeds up all the compounds in a mixture.
- **Column packing polarity**: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.

- **Flow rate of the gas** through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
- **Length of the column**: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

Flame ionization detectors (FID), electron capture detectors (ECD), and mass spectrometers (MS) are commonly used detectors for GC-based analyses. Each one has its own advantages and limitations in terms of sensitivity, versatility of use and associated costs.

Experiment: In the following experiment you are to chromatograph a number of different analytes. You will assess the effects of various instrumental conditions on the analysis. You will construct standard curves of concentration versus peak area for the FID detector and use these curves to determine the concentrations of an unknown solution.

Instrument and Accessories: You will be using a Varian 3800 Gas Chromatograph in the experiment. The instrument is equipped with a Varian 1177 split/splitless injector, and a FID detector. and a Silicone based column in the instrument oven. The instrument is equipped with a Varian CP-8400 auto sampler which allows automated high-throughput analyses.

Procedure Week 1.

Make a mixture of 100 ul of each of the straight chain alcohols C1 to C8 plus 2-butanol, butanone, Br-benzene, dichloromethane and isooctane. Use this mixture to determine the best separation technique using the conditions in Table 1.

Obtain a sample of each alcohol C1 to C8 and each of 2-butanol, butanone, Br-benzene, dichloromethane and isooctane. These are in auto sampler vials already in the auto sampler rack. Run the individual samples to determine the separation and retention times once you have determined the best method of separation.

Setting up methods:
Method: Open *Compass* software from the Windows desktop. In the login window, enter “student” under *User Identification*, “3590” under *Group*, and “GC 3800” under *Project* and GC3800 under *password*. Go to *File, New*, and open *New Method*. Select Varian 3800 system and click on *Next*. Name your group, date and method, for example T2-2017-method A.

At the bottom-left of the screen, click on the *Systems* tab. Check off Varian 3800 option at the top-left of the screen. A system status schematic should appear in the right-hand portion of the screen displaying current system conditions. The 1177 Injector Rear and Middle (FID) should be checked.
Go back to the Data tab (bottom-left of the screen). Click on *control* and then the *Instrument* Icon. You should now be in the “CP 3800 – Control Method” screen. This is where you make changes to various parameters of the instrument components. You can click on the *autosampler* option and make sure that the autosampler is enabled. Make sure there is wash solvent in the solvent reservoir. The washing syringe parameters do not have to be changed. Record the parameters and be aware of what each parameter means. Move to *injectors*.

Injectors: The injector (Varian 1177 Split/Splitless) is to the CP-Sil 8 (50 m x 0.20ID x.33u film). The 1177 injector temperature is set at 260 ºC, and the time is set to 0. The split state is off, with a split ratio of 100 (which means that 1/100th of sample enters column) for 1 min. and then 20 after 1 min. The other injector(s) (which are not used) are off.

Column Oven Method Controls: The oven program can be set at constant temperature (isothermal) or the temperature increased over time (temperature gradient). The operating temperature (either isothermal or temperature gradient) and time are set to produce the best separation of the various constituents in the sample (see Table 1). Stabilization time can set at 0.50 min.

Column pneumatics: Set the column flow rates for position "front" at 0.1 ml/min with time at 0, position "middle" at a constant flow rate of 0.1 ml/min with 0 time and position "rear" at 1 ml/min with time 0.

Detector: The middle (attached to CP-Sil 8 column) detector is to be used. The heater is set to *On*, The detector temperature is set to 275ºC, electronics are *on*, range is *12*, autozero is set to *Yes*. The gas flows are Nitrogen 25, H_2 30 and Air 300 ml/min. The rear ECD detector, heater is on at 100 C with a make-up nitrogen gas at 0.5 ml per min.

Note: The method must be saved after any change.

Experiment:

Optimization: The goal of the optimization is to get a good separation of the mixture 1 within a reasonable time. You can optimize the GC program for the separation of the alcohols, etc. (mix 1) by varying the temperature, and column flow. A number of parameters that can be tested are shown in table 1. The table compares an isocratic method to a temperature gradient at different flow rates.

Prepare mixture and run the analysis in Table 1 to get the best method.

Table 1. Effect of temperature programming and helium gas flow rate:

Method*	Start col. temp.	Hold time start min	Temp. ramp rate. ºC/min	Final Col. Temp.	Hold time at final temp. (min.)	Total oven time (min.)	Column flow rate (ml/min)
Isothemal	150 ºC	N/A	N/A	150 ºC	N/A	12	1.0
Gradient	35 ºC	1	20	210 ºC	3	12.7	0.5
Gradient	35 ºC	1	20	210 ºC	1	10.7	1.0
Gradient	35 ºC	1	10	210 ºC	3	20	1.0

When you find your best method you can use this method to run the separation of mixture to determine their retention time and identification of the individual alcohols, and other material in the mixture. Inject 0.20 ul of the mixture under the four above methods. Determine the best method and the separation of the mixture. You want best baseline separation in shortest time.

GC retention time: A mixture will be separated using the GC 3800 with a method determined from Table 1. Determine the retention time of each individual component by running the individual components using your best method. The components in the mixture can then be identified by their retention times using the same separation method. This can be done using a sequence table (see below). Label the separated samples in your chromatogram by using the peak ID window.

Setting up a sequence. You can inject samples one at a time or you can set up a sequential run to inject samples one after another automatically. It is best to set up a sequence to run your samples since you can easily keep track of the samples you ran. You can inject samples using your selected method and the injections will be done automatically by the auto sampler. To make a sequence click on *file, new, sequence*. Fill in *method, run name (prefix), run ID (sufix), run time, no. of injections, vial #, injection*

vol., sample type (unknown). Upon clicking on *Start* the instrument will load your method, activate the changes, equilibrate, and will make the injection.

Week 2:

Determine concentration of unknown: You can identify (by retention time) and quantitate (using standard curve) the concentration of an unknown(s) (supplied by instructor). Once you have determined the identity of the "unknown" from the retention time, make standard solutions of this material(s) by diluting to the range of your unknown(s) sample. Mix stock with ethanol (or other low boiling solvent as directed) using the ratios in Table 2. Run the standard solutions (inject 1 ul) and the unknown(s) using your optimized method (Table 1). Calculate the concentration of your unknown from the standard curve (see **Standard Curve** below). You should not have to dilute the unknown(s). This procedure can be run overnight if you are running short of time.

Table 2. Volumes of unknown and diluting solution (ethanol) to make standard curve to determine the concentration of the sample you identified by retention time(s).

Volume of stock standard (unknowns)	Volume solvent (ethanol)	Final volume (Inject 1 ul.)	Total dilution
0.00 mls	1.5 mls.	1.5 mls	blank
0.50	1.0	1.5	1 in 3
0.30	1.2	1.5	1 in 5
0.20	1.3	1.5	1 in 7.5
0.10	1.4	1.5	1 in 15
0.05	1.45	1.5	1 in 30

Standard Curve: To set up a standard curve you must determine the retention times of the standards and samples. Make up your standards (as above) and run standards and unknown using the sequence table. After the samples and standards are run the chromatograms are opened. In the original method, *Peak Identification* table identify standard samples as calibration standards and unknown samples as unknowns. Set the retention times and the window of allowed variation in retention time (0.1 min). Under *control* open *calibration* window. Set calibration as *external standard*. This will bring the samples identified as calibrations to the calibration screen. For each standard set the level of standard represented by the designated chromatogram. Name the standard curve. For the unknowns under calibration insert the standards levels and name of curve. Save the new chromatographic method

After all standards are identified and concentration set in the method open reprocess (cog under reprocess at top of screen) and reprocess the data using the new modified method. Each standard and sample must be reprocessed individually. A list of standards and samples are in the chromatogram window. Sample type is standard or unknown. If sample is standard the level corresponds to the level of each concentration in the calibration window of each chromatogram. The first sample you need to clear previous points and after that clear each level. Click *reprocess* after entering information for each standard. This should produce a standard curve with the name given in calibration window of your method. The standard curves can be opened under *File, Standard Curve*, and your designated name. You can also reprocess the unknowns. The concentrations of the standards and the unknowns will appear in the results content section of each chromatogram.

Viewing of the acquired chromatograms: In order to view collected data, go to *File*, *Open*, and *Open Chromatogram*. Select an appropriate data folder (these are created automatically by date). Find your file. Your file name appears in the top-left portion of the screen (identified by a vial icon), with three

components: *Data, Method, and Results*. The workspace icon (a red spectrum with a magnifying glass) at the top-left portion of the screen allows you to use full screen, overlay, stack or compare spectrums.

Identification of compounds: You are able to have the GC identify your compound by name by reprocessing your data. These names can then be added to your method so that further runs would have the name as well as retention times. In your opened chromatogram, under *Method* click on *Peak Identification*. In the right-hand bottom portion of screen, right-click and select *Add*. This adds a line to the compound identification table. Under Peak Name enter the name of the alcohols and the retention time for each. Under *Abs. Window* enter 0.1 min, and leave the rest as is. When the table is complete, click on the integrate icon at the top of the screen (a yellow lightning bolt over a red chromatogram) or hit F5. Next, click on *Results* under your FID chromatogram name. A table containing peak information of your chromatogram should appear in the right portion of the screen.

Results and Discussion (do separately): In your report draw graphs and explain and compare the results. Plot the retention time versus the boiling point and verses the carbon number of the alcohols and explain what is observed. Plot area versus concentration of each alcohol and explain. Explain and discuss retention times versus boiling point, carbon number, chemical formulation, bonding and column and sample polarity. Relate separation efficiency to flow rate, column packing and temperature, and discuss separation based on Van Deemter equations. There are many interaction you need to think about and bring into your results and discussion. Does 1- butanol give similar retention to 2- butanol? The ingredients should be named by the program. The chromatograms from the columns should be in your laboratory write-up with name, retention times, etc..

CLOSING QUESTIONS:

1. What molecular properties (trends) affect boiling point and therefore elution order from the GC column.
2. Why does 2-butanol have a lower boiling point (and elutes from the column quicker) than 1-butanol?
3. The 3800 GC also has an ECD detector. What is this detector specific for?
4. What are the properties of the column used in this experiment.