Chemistry 231 – Spring 2013
Analysis of flower volatiles by Solid Phase Micro Extraction and Gas Chromatography
Developed by Dr. Brad Baker

Overview

The purpose of this lab is to introduce you to the use of solid phase microextraction (SPME). Specifically, SPME will be used to concentrate four flower volatiles dissolved in hexane. The method will then be used to analyze flower volatiles from a flower that you will provide. Analysis will be accomplished by gas chromatography (GC).

Introduction

For this experiment, you will be using a Supelco polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fiber. The fiber is housed in a holder that allows the user to expose the fiber for sampling. Sampling may be done by contacting the fiber with either liquids or the gas phase. In this lab, you will be sampling the gas phase. The partition coefficient is dependent on both the properties of the fiber and the analyte. In general, the fiber works much like the stationary phase of a GC column. After collecting the sample on the fiber it is desorbed by exposing the fiber to high temperature inside the GC injector. Calibrations should be carried out using the internal standard of your choice.

Experiment

You will receive a solution that contains two known flower volatiles. The compounds will be dissolved in hexane. It is your job to determine the identity and concentration of these two compounds by GC. The possible volatiles are:

Ocimene (note: 70% of the analyte concentration of this solution is ocimene, so other background peaks will also be present. Ocimene will be the peak with the greatest area. The 70% has been accounted for in the reported concentration of the stock solution.)
myrcene
linalool
S-limonene

You will be provided with stock solutions (in the 2000 µg/mL range) that can be used to make standards. These stock solutions are made with hexane.

Solid Phase Microextraction

Our lab only has one SPME fiber holder, so we will have to share. The fiber in the holder can be used for several hundred injections before it needs to be replaced. If you notice a degradation of analyses during the experiment, please let the instructor know. Even though the fibers are 'disposable', they are quite expensive and we don't want to break one before its life is over. **Please be very careful with the SPME apparatus while the fiber is exposed. The fiber should not be touched, or mechanically disturbed. Do not use chlorinated solvents with the SPME apparatus, as these can dissolve the epoxy that holds the fiber in place. Be careful not to bend the fiber holder's needle.** The fiber contained in the holder should have been conditioned previously and will be ready for sampling. To insure that a low background is

obtained, it is always a good idea to expose the fiber inside a hot (250^{o}C) GC injector for several minutes with the split vent open before beginning your lab session.

Sampling with the SPME device

The SPME fiber can absorb molecules from either the liquid or the gas phase. In this lab, we will be sampling the headspace above a liquid. This means that you want to avoid submerging the SPME fiber directly into your sample. Place a fixed volume of liquid sample into a vial that has a cap with a septum. The volume will depend on how deep the SPME needle protrudes into the vial; you don't want the fiber to touch the liquid when it is extended. Once the volume is determined, use the same volume for all of your samples. With the SPME fiber fully retracted, pierce the septum of the sample vial with the SPME holder's needle and continue to insert the needle until the holder body is against the top of the sample vial. Expose the fiber by pushing in the spring-loaded plunger of the SPME holder, and leave the fiber exposed by locking the plunger into position. Leave the fiber exposed for a consistent time period (2-3 minutes is usually enough). While sampling, be sure to support the SPME holder's body, so the assembly does not tip over. After the set sample time has passed, fully retract the SPME fiber back into the needle. Do not depend on the spring mechanism of the holder to do this; the fiber will not fully retract. With the fiber fully retracted, pull the needle out of the septum. You should analyze the sample as soon as possible. Because it is difficult to consistently collect the same amount of sample each time you use the SPME device, you should include an internal standard with all of your solutions. You need to choose an internal standard that is compatible with the solvent that you are using, separates well from the analytes that you are interested in during chromatography, and that can be detected well by a flame ionization detector.

There is the possibility that we will have a second SPME apparatus to use on a second GC or GC-MS instrument.

Analyzing the SPME sample

You should use a splitless injection for analyzing samples on the SPME fiber. This means that the split vent should be closed when the initial injection occurs, and then opened about 0.5 minutes into the analysis. You cannot use the SPME apparatus with a GC that is set up for an on-column injection (the Buck GCs for example). With the SPME fiber fully retracted insert the SPME holder's needle into the injection port of the GC until the holder body is against the top of the injector. Expose the fiber to the inside of the injector in the same manner as you exposed the fiber to your sample. The injector temperature should be high (above 200^{o}C, but do not exceed 250^{o}C) to insure that the sample is quickly desorbed. Leave the fiber exposed at least until the split valve opens. Be sure that the fiber is fully retracted before removing the needle from the injector. As long as the entire sample has been desorbed from the fiber, it is ready to be used again for sample collection.

Gas Chromatograph

For this experiment you will be using a HP 5890 GC with a flame ionization detector. The instrument is controlled by an old version of the Chemstation software; similar to the software that controls the HPLCs that we use in this class (except much simpler). The instructor will give you a quick run-down of this instrument before you get started. You may also be able to use the Agilent 7890A GC with a mass selective detector (MSD) or possibly a different Agilent 6890 GC (we are working to see what is available for use). Also, this instrument is

managed by the organic chemistry faculty, and it must be operated according to their protocols (the book is on the table next to the instrument).

Analysis of Unknowns

Be sure to analyze your unknown solution at least three times, and more if time permits so that you may report uncertainty.

Quantification by GC

You must use an internal standard for this lab. Be sure to choose a compound as your standard that can easily be separated from the analytes in your sample, and is completely soluble in the solvent you are using. The following lists the steps necessary to use an internal standard for quantitative analysis.

1. Let X = the unknown, and S = the standard.
2. $\frac{A_X}{A_S} = F\frac{C_X}{C_S}$, where A is the area, C is the concentration, and *F* is a 'response factor' between X and S.
3. A solution is made that contains known amounts of X and S, this solution is used to determine *F*.
4. Add a known amount of S to any solution that contains an unknown amount of X. Realize that this will dilute the solution, and this must be accounted for when calculating the final concentration of X.
5. After obtaining a chromatogram of the solution, use the equation in '2' above, to determine C_X. Be sure to account for the dilution that occurred when adding a known amount of S.

Flower Volatiles

The idea to analyze flower volatiles comes from the following reference:

Flamini et al., (2003), *Journal of Chromatography A*, **998,** pp 229-233.

It is recommended that you locate and read this article. After you have identified and quantified the compounds in your sample, you will analyze some compounds from actual flowers. Bring in several fresh flowers (of any type, but all of the same type, and know the name of the flower) for analysis. Bring the flowers in on the day that you plan to do the analysis so that they are still fresh. You should try to analyze the volatiles from at least: the petals, stamens, and pollen. Place the flower parts into a vial with septum. Be sure that the SPME fiber will not touch any of the flower parts during sampling. Let the samples sit for at least 30 minutes so that the volatiles have a chance to diffuse from the flower parts. You may want to use a longer sampling time for this part of the experiment. Analyze the sample just as you did the samples derived from solution. Since it is not possible to use an internal standard, we are not concerned with quantifying the absolute amount of each compound emitted by the flower parts. Instead, make an evaluation of the relative amounts of each compound. If a compound cannot be identified, simply identify it by number, and only be concerned with compounds that make up more than 5% of total emissions.

Report

Your report should be double spaced, with 12 pt Times New Roman font and 1 inch margins. Include your name, a title, a 2-3-sentence abstract, a short introduction, experiment description, results and discussion, and a short conclusion. In the experiment description, be sure to include (in addition to everything else) a description of the SPME and the conditions that were used to analyze your samples. The results and discussion should include a discussion of both your unknown and flower analysis. Be sure to conform to the 'guidelines for Chem 231 lab reports' before handing in the report.