

## Project 3: Volatile Fragrances and Flavors

### Qualitative Analysis by GC-MS

#### PRE-LAB ASSIGNMENT

- Read the entire laboratory project and section 27B (pp. 789–792 and 798–800) in Skoog *et al.*<sup>1</sup>
- Prepare, on a typed sheet of paper, the Project Objectives of this lab; on the same sheet, answer the following questions:
  - 1) What are the functions of the "GC" component and the "MS" component of GC–MS?
  - 2) Students will be pre-assigned a flavor from the list below. Prior to the pre-laboratory discussion, you should conduct a literature search (include citations) to find the main compounds which contribute to the characteristic taste and odor of your assigned flavor. Use ChemDraw to draw the chemical structures and note both the systematic and common names of the compounds. Also include molecular weights and boiling points.  
**Flavors: anise, caraway, cinnamon, clove, peppermint, spearmint, wintergreen.**
  - 3) Use the NIST database to find the mass spectrum for two of the compounds and deduce the structure corresponding to the base peak.
  - 4) You will investigate the volatile components of an essential oil and at least two samples of gum or other retail item advertised as having that flavor. Formulate and bring to pre-lab a prediction that can be tested by comparing the essential oil with foods that possess that particular flavor.

#### INTRODUCTION

As we have learned, humans use odor and taste receptors to detect at very low concentrations a considerable number of molecules that give distinctive fragrances and flavors to food. Chemical analysis of the fragrance and flavor molecules is often more difficult; the analysis often requires

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<sup>1</sup> Skoog, Holler, and Crouch, *Principles of Instrumental Analysis*, 6<sup>th</sup> ed., Thomson Brooks/Cole, Belmont, CA, 2007.

extraction and concentration before gas chromatographic – mass spectrometric analysis. A relatively new technique, solid phase microextraction (SPME), has made the analysis of fragrances and flavors considerably easier.

Solid phase microextraction is a simple, solventless extraction procedure in which a phase-coated fused-silica fiber is immersed in a liquid sample, or exposed to the headspace above a liquid or solid sample. Analytes adsorb on the fiber, and then are thermally desorbed during gas chromatographic analysis. The identity of the molecules can be confirmed by mass spectrometry.

In this experiment you will investigate the volatile components of an essential oil and foods that possess that particular flavor. Qualitative analysis or identification of the molecules that give the distinctive odor and taste to these foods is the main focus of this project. You will also look at the variations in the relative amounts of these compounds, but will not determine absolute amounts or concentrations.

## **EXPERIMENTAL**

The fragrance and flavor molecules will be concentrated by headspace SPME. SPME will be done using a 50/30  $\mu\text{m}$  DVB/Carbon/PDMS fiber in 4 mL headspace vials using the SPME sampling stand at elevated temperature. While individual samples may involve adjustment of the extraction procedure, a suggested starting point is 0.2 – 1 mL of oil or 0.5 g of food item (cut into small pieces) and an extraction time of 1 minute for the oil and 3 minutes for food items. The stockroom will supply the essential oils, while students must provide the product samples.

Begin by locating the SPME holder, the black aluminum heating block with the white Teflon SPME guide, and several 4 mL headspace vials. The instructor will demonstrate how to assemble and use the heating block and the SPME holder. (See Figures 1 and 2.) Place your loaded sample vials in the heating block and equilibrate at about 60 °C.

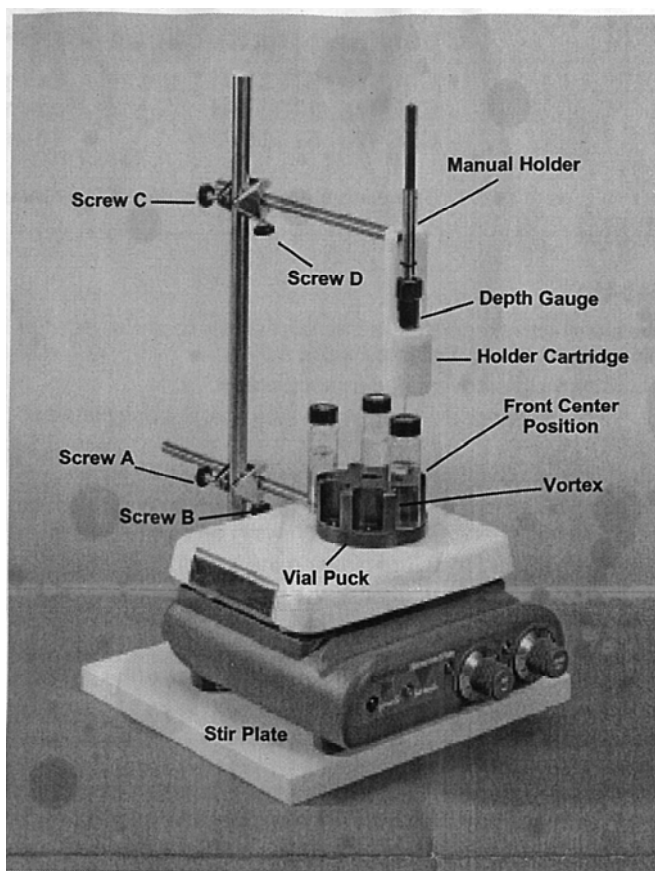


Figure 1. Vial heating block and guide.

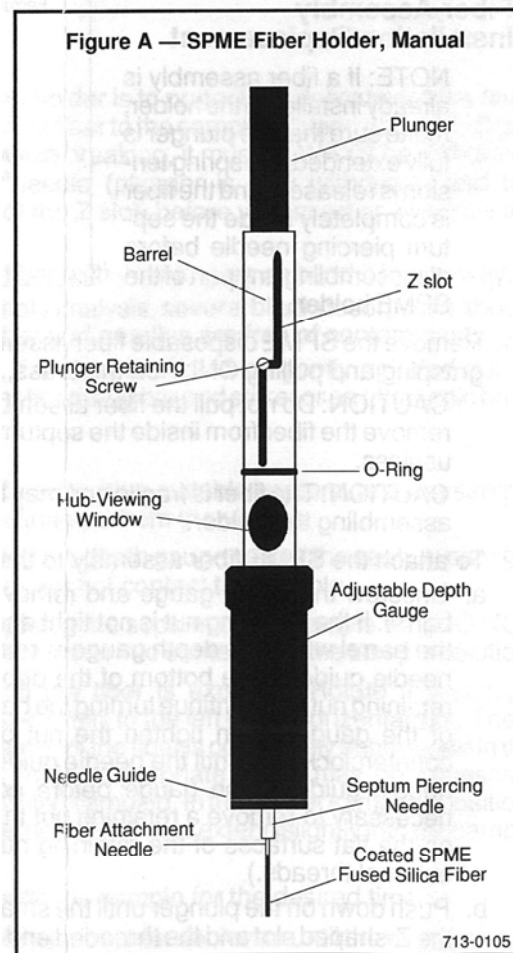


Figure 2. SPME fiber holder.

Configure the instrument for analysis while the samples are equilibrating. Samples will be manually injected into the GC/MS so the auto-injector must be turned off, then carefully removed from the injector port and set aside. Note the exposed golden metal injector port. Put the black metal manual SPME injector guide on top of the gold-colored metal injector port.

The instructor will demonstrate the use of "Methods," which contain the specific conditions and instructions which control the GC/MS while running an analysis. The SPME fiber must be cleaned before each sample analysis, so a suitable cleaning method should be loaded, reviewed, and run. Confirm the path (e.g., C:\HPCHEM\1\DATA\ExpBio##\ ) for the data folder before analysis of a sample. Prepare for sample analysis by loading a suitable method (e.g., *Flavors*). The instructor will review this method with you and help you make any desired changes. You

should print a copy of the method for your laboratory records.

Once equilibrated, samples are collected by exposing the fiber in the headspace of a vial to absorb the volatile compounds. Place the black SPME holder in the white Teflon SPME guide. Be certain that the guide hole is lined up with the vial's septum and press down on the black holder so the needle punctures the septum. Push down on the plunger until it reaches its stop point. The fiber should be in the head space of the vial, above (and not touching) the liquid or solid sample. Retract the fiber after the appropriate extraction time.

After extraction, SPME samples are injected manually into the GC/MS and analyzed using the method constructed for this experiment. Under the Method menu select *Run* and type in operator names, sample name, and the data folder path, and then click the “Run Method” box (lower left). You should soon see a manual injection window. Place the black SPME holder (with fiber still retracted) into the injector port guide. Press “start”, and expose the fiber by pressing the SPME holder plunger down to the stop point. Do NOT override solvent delay; after 2 minutes, retract the fiber and remove the black SPME holder from the injector guide.

Each student will run at least three chromatograms, one oil and at least two food samples. The cleaning method must be run after the sample chromatogram has finished and before the next extraction.

#### **DATA ANALYSIS: IDENTIFICATION OF VOLATILE COMPOUNDS**

The ChemStation software on the GC/MS will allow you to analyze your chromatograms and to compare the chromatograms of the oil and commercial products. You may analyze your results any time after your runs are complete; simply load a data file into a Data Analysis window to view the chromatogram. Integrate the areas under the peaks by selecting *% Report* under the Chromatogram menu; a table will print which lists the area and percent of the total area represented by each peak.

- 1. Include a printout of all chromatograms and % Reports as part of your informal lab report.*

Based on the relative peak areas in the % Report, select the largest peaks for further identification. This selection is somewhat arbitrary; however, a reasonable starting point is to select peaks that contribute at least ten percent of the total peak area. The selection criterion will likely change as you compare chromatograms. You can expand the view and enlarge the chromatogram around a selected peak by left click and drag; if you then right click and drag your cursor across a peak, a window will open below the chromatogram. This window shows the mass spectrum of the molecule responsible for the peak that you selected. If you right double click anywhere in the mass spectrum window, the software will compare your peak's mass spectrum result with those for thousands of pure compounds in its library, and give you a tentative identification of the molecule in your selected peak. Use an independent database (i.e. NIST) to confirm the identity of the compounds giving rise to the peaks.

- 2. Include in the appendix of your informal report a printout of the mass spectrum and tentative identification for each chromatographic peak you investigate.*
- 3. Include in the appendix of your informal report the database mass spectrum used to confirm the identity of the compound. Cite the source of the mass spectrum.*

#### **DATA ANALYSIS: COMPARISON**

Compare the chromatograms of the oil and the food items. What peaks do they have in common? Are the same compounds found in the oil and foods? What are the differences in composition (as indicated by the area % Reports) between the oil and food items? If the chromatograms differ significantly in the number or relative sizes of peaks, you may need to return to a chromatogram for further identification and quantification. What compounds appear to contribute to the taste and odor of the flavor? Are the compounds you found consistent with the components of the flavor reported in the literature?

- 4. Prepare a table that summarizes the information requested above.*
- 5. Discuss the results of the analysis both qualitatively and quantitatively as described above. Compare the compositions of the oil and food items. Compare your experimental*

*results with the literature. Include citations for any literature references.*

*6. Compare your experimental results with your pre-lab prediction. Is it confirmed or refuted?*