

Project 5: Scoville Heat Value of Foods

HPLC Analysis of Capsaicinoids

LABORATORY REPORT: Formal Writing Exercises

PRE-LAB ASSIGNMENT

- Read the entire laboratory project and section 28C (pp. 818–825) in Skoog *et al.*¹
- Prepare, on a typed sheet of paper, the Project Objectives of this lab; on the same sheet, complete the assignment below:
 - 1) Find the chemical formulas, structures, and molecular weights of the three capsaicinoids. Use ChemDraw to draw the structures. Read the SDS for capsaicin from Sigma Aldrich and record the HMIS Classification and health hazards.
 - 2) Based on the structure and solubilities of the capsaicinoids, do you expect water or whole milk to be better at tempering the effect of the capsaicinoids? Why?
 - 3) Write a short description of the original Scoville method from a general search on the term. What is the basis for the Scoville Heat Index? Why do the capsaicinoids have different weighting factors?
 - 4) Find a reference with typical Scoville Heat Values of peppers and note the range.
 - 5) Formulate and bring to pre-lab a prediction that can be tested by comparing the SHVs of food products.

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

INTRODUCTION

In the previous project, you tested tastant binding to the sweet receptor. Our sense of temperature derives from hot and cold receptors found in the skin. The cold receptor (TRPM8) is a cation channel that is gated open at $T < 20\text{ }^{\circ}\text{C}$. This channel is also called the “cold and menthol receptor” (CMR), because it is activated by the agonist menthol. Menthol applied to the skin feels cold because it activates the same neural receptor that low temperature does. The hot receptor (TRPV1) cation channel is gated open at $T > 43\text{ }^{\circ}\text{C}$, and is also activated by the agonist capsaicin. In fact, the pungent flavor and “heat” of chili peppers is caused by the family of molecules called capsaicinoids. The most common member of the family is capsaicin, which is also used as the active ingredient of “pepper” sprays. In this experiment you will analyze ground chili peppers, hot peppers, or hot sauces for three capsaicinoids: capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. Once the concentrations of the capsaicinoids are known, the Scoville Heat Value (SHV) of the food can be calculated.

The SHV was originally developed as an organoleptic test to indicate the relative potency of hot peppers, with higher SHVs for hotter peppers. The SHV has since been quantified and can be calculated from the measured concentrations and SHV of the pure capsaicinoid compounds. The resulting SHV of foods may range over two orders of magnitude from mild to extremely hot. In this project you will determine the concentrations of capsaicinoids by high performance liquid chromatography (HPLC) using UV detection and gradient elution. HPLC is a suitable technique for this analysis because the capsaicinoids are non-volatile and absorb strongly in the UV. Students will work as groups to compare the SHV of various food products.

EXPERIMENTAL: DAILY PLAN

Prior to beginning laboratory work, students should have in their notebook a daily plan based on pre-lab discussions. Have the instructor check your plan before you begin work. Specifically, lay out the details of the class plan for generating a calibration curve and testing a prediction by analyzing food samples. Explicitly state the prediction and include the assignment of responsibilities for both the calibration curve and food samples. The plan of autosampler positions should also be included. Bring your assigned sample to lab. At least one student from each lab section should taste (outside of the lab) ALL of the foods, assign a qualitative “hotness”

value, and share the qualitative assessment with the section.

EXPERIMENTAL: CALIBRATION CURVE

A standard solution containing approximately 1000 ppm ($\mu\text{g}/\text{mL}$) capsaicin in HPLC grade ethanol will be available in the laboratory. Using *volumetric* pipets and flasks, the class will prepare five dilutions of the standard in the range 5 ppm to 100 ppm for creating a calibration curve. Triplicate chromatograms should be run for each standard, and the HPLC autosampler uses 1.0 mL vials, so plan the volumes of your standard dilutions accordingly. Note the exact concentration of the stock solution and calculate the exact concentrations of the diluted standards. The resulting calibration curve will be used by the entire lab section.

Caution: Capsaicin is a skin irritant. Do not get concentrated capsaicin solutions on your skin or in your eyes. Wear gloves and eye protection at all times when handling capsaicin solutions.

EXPERIMENTAL: SAMPLE PREPARATION

In a plastic weigh boat, accurately weigh 10 g of hot sauce, 5 g of fresh chili peppers (blended), or 2 g of dried ground chili. Quantitatively transfer the sample to a 50 mL Erlenmeyer flask, using HPLC grade 95% ethanol to rinse the weigh boat. Add 95% ethanol to the flask to bring the volume to 15 mL. Cover the flask with a watch glass.

Extract the samples on a hotplate/stirrer in the hood. Heat the flasks with constant stirring until a slow boil is obtained. Heat the samples for an additional 30 minutes being careful not to evaporate away the ethanol. After cooling, quantitatively transfer the extract into a 25 mL volumetric flask: Decant the liquid and gravity filter through qualitative filter paper; rinse three times with small volumes of ethanol and dilute to volume with ethanol. Store the extracts in labeled sample vials in the refrigerator.

EXPERIMENTAL: TASTE TEST

At least one student from each lab section should taste (outside of the lab) ALL of the foods and assign a qualitative “hotness” value to each. Post these results for the class in the Google Docs spreadsheet.

EXPERIMENTAL: HPLC WITH AUTOSAMPLER

Transfer approximately 1 mL of each standard or sample into sample vials with built-in 0.45 µm filters. Be sure the vials are clearly labeled. You will use the autosampler to run triplicate chromatograms on each of the samples. Use the attached figure to help identify the peaks and retention times corresponding to the three capsaicinoids. The order of elution is nordihydrocapsaicin, capsaicin, then dihydrocapsaicin. The sample chromatograms are likely to have additional peaks which you may ignore.

The HPLC will be run using the following conditions for all standards and samples. All standards and samples must be filtered through a 0.45 µm filter before injecting onto the HPLC column. These conditions are stored as the method Capsaicin Auto 2012B.

Column:	150 mm x 4.6 mm C-18
Mobile Phase B:	0.5% (v/v) phosphoric acid
C:	acetonitrile (HPLC grade)
Gradient Elution:	50 – 80 % acetonitrile in 12 minutes
Detector :	UV at 205 nm

The instructor will demonstrate the use of the HPLC with autosampler. Note the name of the folder in which data will be saved (e.g., Exp Biochem S2014). Samples contained in 2-mL filter vials are placed in their assigned positions in the autosampler. The analysis is automated through the use of a "Sequence" (e.g., Capsaicin Lab 2014) which specifies the Method to be used, the samples to be analyzed, and the number of replicate injections for each sample. Each section will fill in the Sequence Table for all samples and standards prior to running the sequence. The analyses will continue automatically until all the samples are run. The data will be stored under the Sequence name in the data folder.

DATA ANALYSIS

In HPLC, analyte concentration is proportional to the integrated area under the analyte chromatographic peak. You must therefore use the HPLC's software to determine the integrated

peak area under each peak of interest. Post the exact concentration of your standard and the average peak area (\pm standard deviation) for the class. The calibration curve is a plot of average peak area of the capsaicin standard versus the concentration in ppm. The concentrations of the three capsaicinoids in sample solutions can be calculated using the relevant integrated peak areas and the calibration curve, assuming equal detector response for the various capsaicinoids. Remember the uncertainty in each calculated capsaicinoid concentration is given by the standard deviation in concentration equation (Equation A-20, p. A14).

1. *Include in your informal report a data table with the exact concentrations and average peak areas (\pm standard deviation) for the capsaicin standards.*
2. *Include in your informal report the capsaicin calibration curve with absorbance error bars and best-fit equation. Note in the caption if the error bars are too small to be seen.*
3. *Include, for your samples only, a data table with the concentrations (and uncertainties given by the standard deviation in concentration equation) of the three capsaicinoids in each sample solution.*

Use the concentrations of capsaicinoids determined above ($\text{ppm} = \mu\text{g/mL}$) and volume of sample solution to calculate the mass of the three capsaicinoids in each sample. Then calculate the concentrations of the capsaicinoids in the food samples in units of μg of capsaicinoid per gram of food ($\mu\text{g/g} = \text{ppm}$). The Scoville Heat Value is related to the μg of capsaicinoids per gram of food and is calculated as follows:

$$\text{SHV} = C + D + N \tag{1}$$

where:

$$N = (\mu\text{g of nordihydrocapsaicin per gram}) \times 9.3$$

$$C = (\mu\text{g of capsaicin per gram}) \times 16.1$$

$$D = (\mu\text{g of dihydrocapsaicin per gram}) \times 16.1$$

The uncertainty in SHV is calculated by propagating the error in equation (1) using the previously calculated errors in concentration and treating the multipliers as constants (see

Statistical Concepts, p. A11).

As soon as you have finished the calculation above, post your data to the Google Docs spreadsheet.

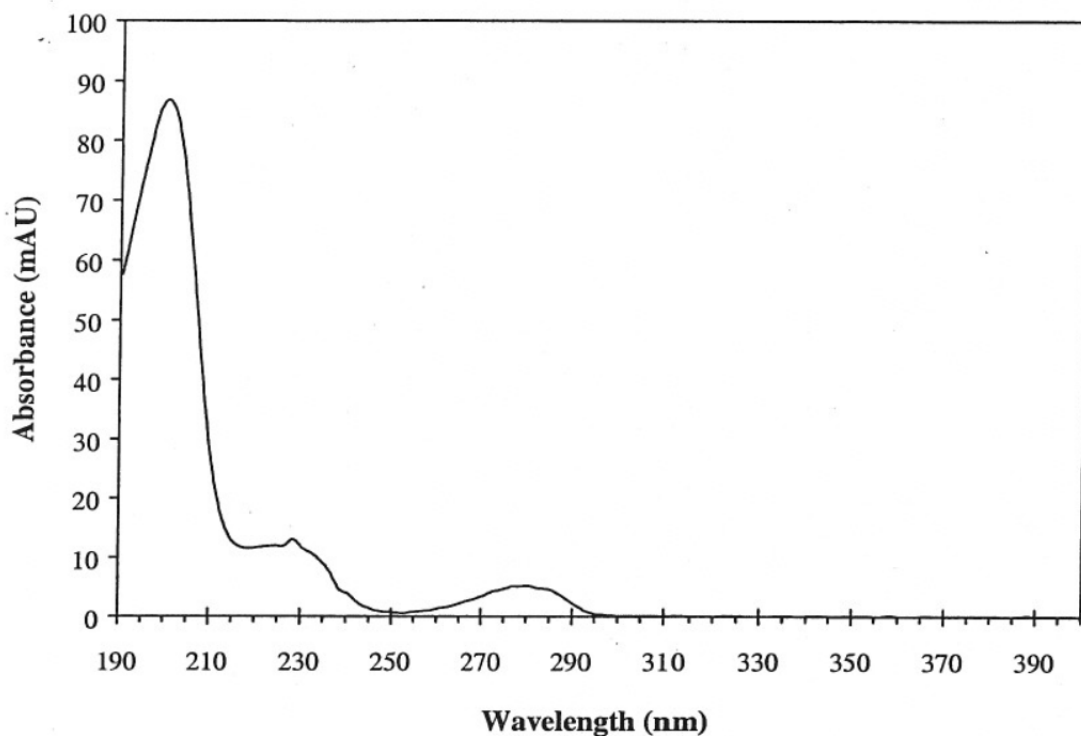
4. *Include in your informal report a data table with the concentrations of the three capsaicinoids in all the foods, and the calculated Scoville Heat Index (with uncertainty). Provide a reference to the exact page in your notebook where a sample calculation can be found.*
5. *Compare the experimental SHVs with those in the literature. Be sure to include citations for any references.*
6. *Does the relative magnitude of the SHVs correspond to your taste experience?*
7. *Discuss any trends observed among the data for your lab section. Is the prediction of the section confirmed or refuted?*

DATA ANALYSIS: SUMMARY

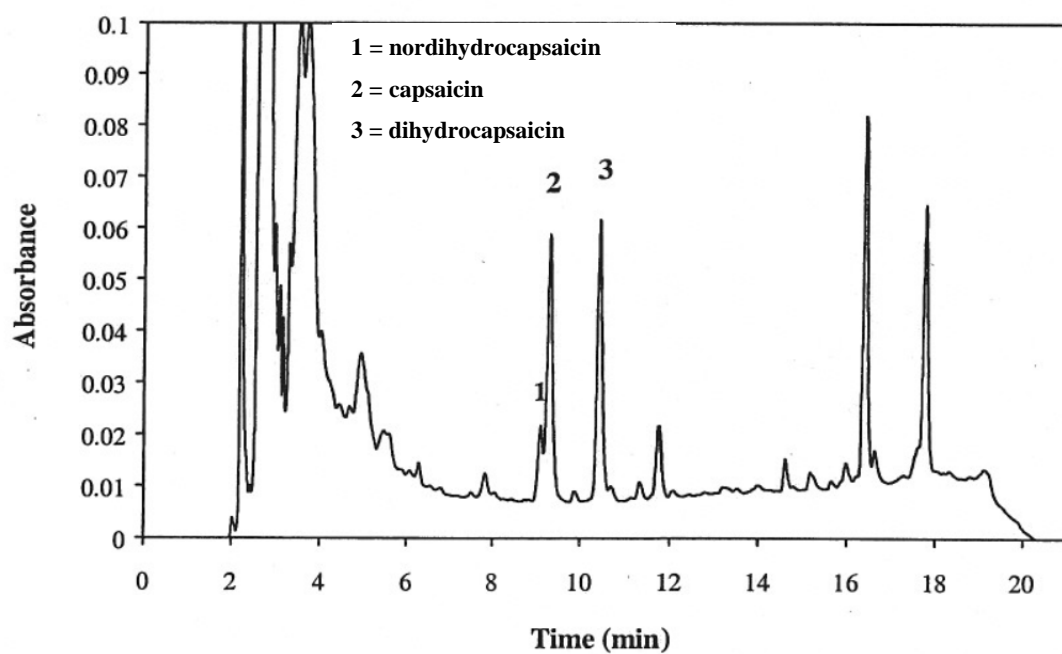
8. *Provide a Summary paragraph as described in "Writing in the Chemistry Laboratory: The Informal Report."*

FURTHER READING

“Determination of the Scoville Heat Value for Hot Sauces and Chilies: An HPLC Experiment”
J. D. Batchelor and B. T. Jones, *J. Chem. Educ.*, **77**(2), 266-267 (2000).



UV Spectrum of Capsaicin.



Chromatogram of Anaheim Chili Pepper.

