Determination of the Scoville Heat Value of Hot Sauces and Chilies:

An HPLC Experiment

Purpose:

The purpose of this lab is to determine the amount of capsainoid compounds in various hot sauces and chilies. The concentrations will be used to determine the Scoville Heat Value for the sample.

Instrumentation:

An integrated HPLC system (HP 1090) will be used for this experiment. The T.A. will instruct you regarding the operation procedures.

Experimental Procedure:

(1) Preparation of solutions:

Using the 1000 mg/L standard capsaicin solution (in 95% Ethanol) provided by the T.A., prepare a series of 4 dilutions in the range of 1 to 50 mg/L. Use 95% ethanol as the solvent.

Caution: Wear Gloves. Do not get concentrated capsaicin on your skin and wash hands thoroughly when complete. Capsaicin is a skin irritant. Getting capsaicin in your eyes can cause serious harm and is easy to do if any of the material gets on your hands or clothes.

(2) Preparation of the samples:

Weigh 15 g hot sauce or 3 g dried, ground chili in a weigh-boat. Record the mass and transfer its contents to a **labeled** 125-mL Erlenmeyer flask. Use 95% ethanol to wash the sauces or dried chili from the weigh-boats. Bring the total volume to the 50-mL graduation on the flask with ethanol.

Some sauces will require considerable washing to completely remove them from the weigh-boat.

Place a Teflon stir bar in the flask, place the flask on a hotplate/stirrer.

Do not use open flame for heating!
Perform Extraction under Chemical Hood!

Heat with stirring until a slow boil is obtained. Boil for 30 minutes. Remove from heat and allow to cool for a few minutes. Remove the stir bar and wash with ethanol, collecting washes in extraction flask.

While the flask is cooling, prepare the filter apparatus. Fold one (1) disc of Whatman's No. 41 filter paper and place it in a glass funnel (plastic will work as well). Wash the paper three (3) times with ethanol catching the wash in a waste container.

Once the flask has cooled and the filter paper has drained, filter the sample into a **labeled** 100-mL volumetric flask. Be sure to get all of the contents out of the extraction flask. Wash the filtrate three (3) times with ethanol, but <u>be careful not to overfill the volumetric flask</u>. Once filtered, bring the solution to volume with ethanol, cap, and mix well.

Be sure to wash hands after transferring liquids. The capsaicin could be strong enough to irritate the eyes.

Instrumental Analysis:

Once the standards and samples are prepared, the solutions must be filtered with a 0.45-micron pore size syringe filter cartridge to remove minute particles. The solutions are filtered into **labeled** 2-mL auto-sampler vials. These vials are capped, placed in the auto-sampler, and the instrument is instructed to begin analyzing them using the gradient conditions outlined in Table I.
The T.A. will instruct you on using the instrument.

Data Analysis:

The instrument software will automatically print out the chromatogram with each peak labeled and its area and height reported in mAU-sec and mAU, respectively. The area value should be used for calculation. The width of each peak at half-max is required for the questions.

Table I. Experimental Conditions

Gradient			
Time (min)	% Acetonitrile	% Acid Solution[a]	% Water
0	40	10	50
15	90	10	0
16	90	10	0
18	40	10	50
21	40	10	50
Flow		1 mL/min	
Wavelengths		205 nm, 280 nm	
Column Temperature		50°C	
Injection volume		20 µL	

a. 1% Phosphoric Acid Solution

Table II. Example Calculation (ground cayenne peppers)

Compound[a]	Concentration (g compound/g sample)	SHV for pure capsainoids[b] (millions)	Calculated SHV
NC	0.000135	9.3	1260
CP	0.00147	16.1	23700
DC	0.000984	16.1	15800
		Total SHV	**40860**

a. NC = Nordihydrocapsaicin; CP = Capsaicin; DC = Dihydrocapsaicin

b. Todd, Jr., P. H.; Bensinger, M. G.; Biftu, T. J. *J. Food Sci.*, **1977**, *42*, 660-665, 680.

Results and Discussion:

1. Calculate the following chromatographic quantities:

A. The retention time (t_r) for each analyte.

Read from chromatogram

B. The number of theoretical plates in the column using the capsaicin peak and the dihydrocapsaicin peak. ($w_{1/2}$ is the width of the peak at ½ height)

$$N = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2$$

C. The Resolution between: 1. Nordihydrocapsaicin and capsaicin (if both are present), 2. Capsaicin and dihydrocapsaicin. (k_1' for peak eluting first in the pair, k_2' for last peak in pair)

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right)$$

$$k' = \frac{t_r - t_{void}}{t_{void}} \qquad \alpha = \frac{k_2'}{k_1'}$$

D. The height of the theoretical plate (L is column length in cm).

$$H = \frac{L}{N}$$

2. Prepare a calibration curve (integrated absorbance Vs. concentration) for capsaicin and report the calibration sensitivity (slope of line). If two wavelengths are examined prepare a calibration plot for both. Why are the sensitivities different? Which curve is better to use for further calculations? Why?

3. Determine the Scoville Heat Value (SHV) for your sample. This is done by multiplying the concentration of each capsainoid (g/g) by the pure compound's SHV, then adding each of these values to get the sample's total SHV. See Table II for an example. If the sample was analyzed more than once, report the SHV with 95% confidence limits.

   $\{[\text{capsaicin(g/g)}]*(16.1*10^6)\} + \{[\text{dihydrocap(g/g)}]*(16.1*10^6)\} +$

   $\{[\text{nodihydrocap(g/g)}]*(9.3*10^6)\} = \text{Scoville Heat Value}$

4. How does your calculated SHV compare to the taste of the hot sauce? Does the hot sauce with the higher SHV taste "hotter"?

5. What color was the extracted sample? Is there a relationship between the color and the SHV? Why or why not? Be careful!!

Instructor Notes

Instrumentation:

Pump system: Programmable gradient system or single pump for isochratic

Guard column/Working Column: C-18, 5 μm particle size, 25 cm X 4.6 mm i.d.

Detector:
1) UV-Vis Diode array with a range of at least 200 nm – 400 nm
2) Variable Wavelength Detector with a range of at least 200 nm – 400 nm
3) Single Wavelength Detector at either 280 nm or 205 nm
(Capsaicin will not absorb appreciably at 254 nm, refer to Fig. 1)

Chromatographic conditions are summarized in Table III.

Chemicals:

1. Ethanol, USP 95% [64-17-5]
2. Acetonitrile, HPLC Grade, [75-05-8]
3. Phosphoric Acid, 85%, [7664-38-2]
4. Water, Distilled, De-ionized
5. Capsaicin, known purity, [404-86-4] (Capsaicin is a skin irritant and is hazardous in other ways. Consult the MSDS for capsaicin before preparing the stock solution. One source of the MSDS is Sigma-Aldrich, whose Web site is at http://www.sigma-aldrich.com/).

Lab Supplies:

1. 125-mL Erlymeyer flasks for extractions
2. Weigh-boats
3. Stir bars and retriever
4. Whatman No. 41 filter paper or equivalent
5. Glass or plastic long-stem funnels
6. Hotplate/ stirrer
7. 100-mL Volumetric flasks and caps
8. 0.45-μm pore size syringe filter cartridges
9. disposable syringes

Table III. Experimental Conditions

Gradient			
Time (min)	% Acetonitrile	% Acid Solution[a]	% Water
0	40	10	50
15	90	10	0
16	90	10	0
18	40	10	50
21	40	10	50
Isochratic			
Time (min)	% Acetonitrile	% Acid Solution[b]	
0	50	50	
20	50	50	
Flow		1 mL/min	
Wavelengths		205 nm, 280 nm	
Column Temperature		50°C	
Injection volume		20 µL	

a. 1% Phosphoric Acid Solution

b. 0.2% Phosphoric Acid Solution

Troubleshooting

Sample preparation:

1. If fresh chilies are used, be sure to dry them first. Drying can be accomplished in a oven set to 60° C and will take several days. Most chilies can be purchased dried and ground.

2. Some sauces are thick. They are difficult to pour into the weigh-boats. Care must be taken to not get sauce on the balance pan.

3. Some sauces will require considerable washing to get them out of the weigh-boat and into the flask. If the total volume, after washing, is much greater than 50 mL (according to the graduation on the flask), heat the solution longer than 30 minutes. Enough solution must be removed from the flask to allow quantitative transfer and washing of the filtrate.

4. The samples will bump if not stirred fast enough. If bumping occurs, have the student increase the stir speed.

5. Be sure to remind students to not go over the 100-mL graduation on the volumetric flask. Starting over or gradually reducing the volume in the flask are options, but are time consuming.

Instrumentation and analysis:

1. Make sure a guard column is installed. There is material present in the samples that might not elute from the analytical column.

2. Be sure to filter the standards and especially the samples with a 0.45 μm pore size filter before injection. **SAMPLES MUST BE FILTERED FOR FINE PARTICLES.**

3. The nordihydrocapsaicin elutes very closely to the capsaicin. In some instances the concentration is negligible compared to capsaicin and dihydrocapsaicin. If this peak is not present, do not be alarmed.

Typical Results

Table IV summarizes results for several hot sauces, 3 chili peppers, and an arthritis pain relief crème. The Scoville Heat Value for each sample as determined at 205 nm and 280 nm using gradient and isochratic elution are presented. Literature values for the chili peppers are given for comparison. A chromatogram of each sample recorded at 205 nm using gradient elution is presented in Figures 2-10. The elution order is Nordihydrocapsaicin (1), Capsaicin (2), and Dihydrocapsaicin (3). The nordihydrocapsaicin is barely noticeable in some of the hot sauces. The Habanero chili peppers were diluted 10 fold to get the capsaicin concentration within the calibration curve. These chromatograms are only given as examples of what is to be expected. Figure 11 depicts structural drawings of the three capsainoids.

Table IV. Results

	SHV by Gradient Elution (205 nm, *280 nm*)	SHV by Isochratic Elution (205 nm, *280 nm*)	Literature SHV[a]	Literature SHV[b]
Yucatan Habanero Sauce	1,900, *1640*	1890, *1090*		
Texas Pete	1,050, *770*	936, *648*		
Hooter's Hot Sauce	1,230, *900*	1190, *ND*		
Chef Han's Hot Sauce	380, *330*	245, *ND*		
Tabasco	2890, *2570*	2830, *1700*		
Ground Habanero	155,000, *157,000*	157,000, *133,000*	300,000	200,000-300,000
Ground Cayenne	40,800, *33,800*	41300, *32500*	41,000	35,000-40,000
Ground Anaheim	8,700, *8,000*	8900, *ND*	3,400	1,000-1,500
	% Capsaicin by Gradient Elution	% Capsaicin by Isochratic Elution	Label value (% capsaicin)	
Arthritis Pain Relief Creme	0.094, *0.095*	0.094, *0.092*	0.075	

a. Thomas, B. V.; Schreiber, A. A.; Weisskopf, C. P. *J. Agric. Food Chem*., **1998**, *46*, 2655-2663.

b. GNS Spice Portfolio, GNS Spices, Walnut, California 1997.

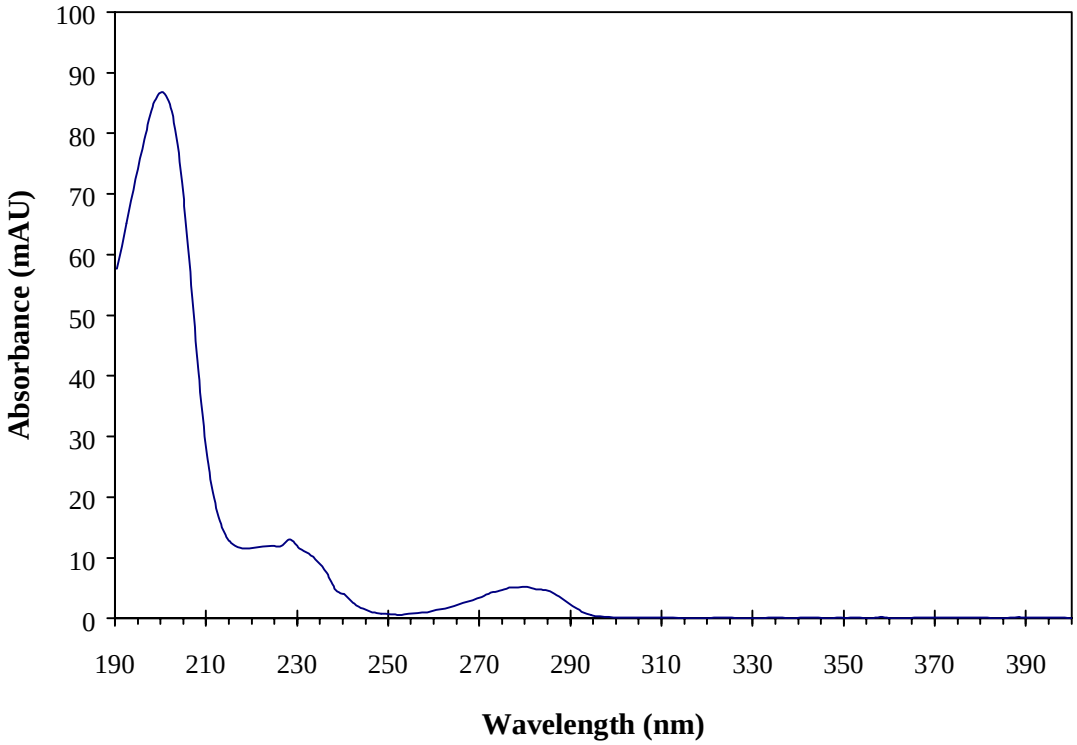


Figure 1. UV Spectrum of Capsaicin.

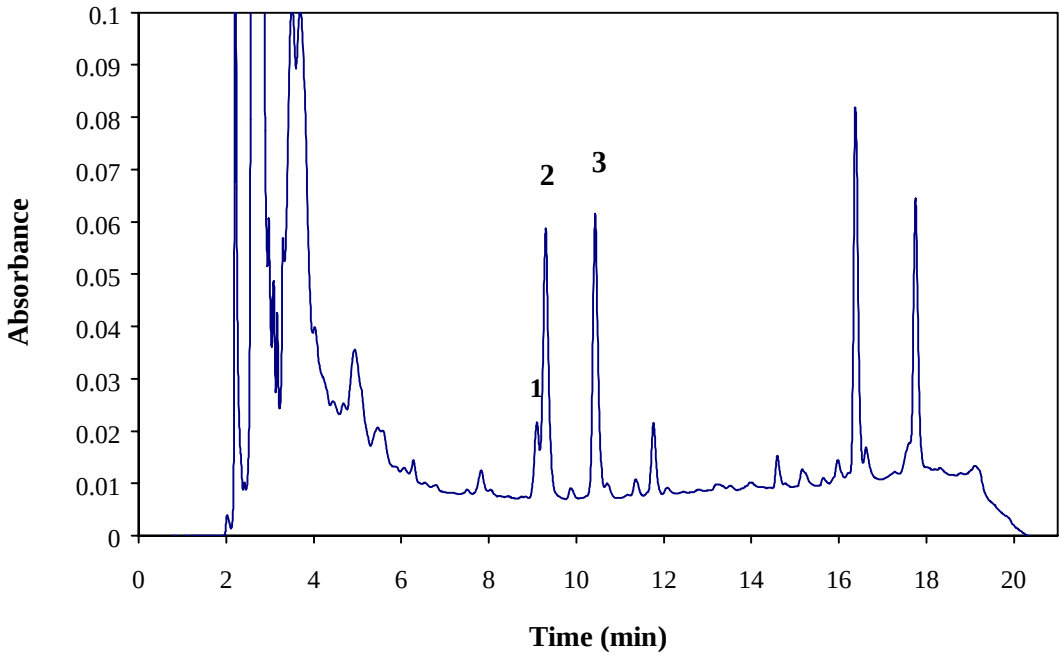


Figure 2. Chromatogram of Anaheim Chili Pepper.

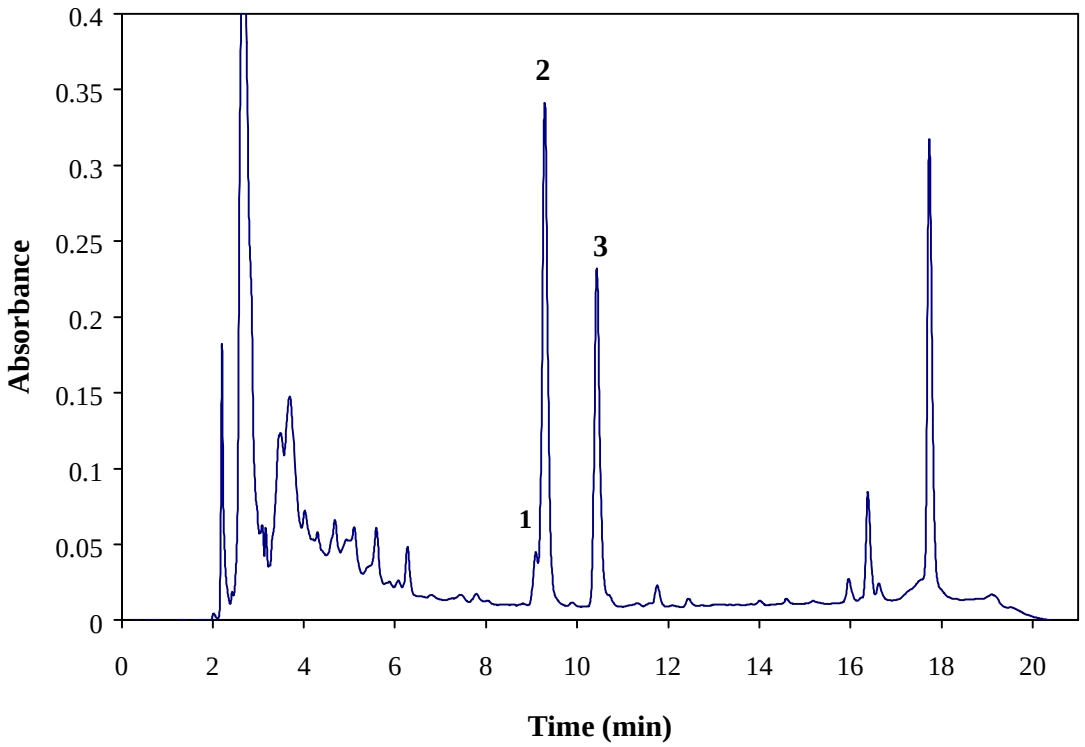


Figure 3. Chromatogram of Cayenne Pepper.

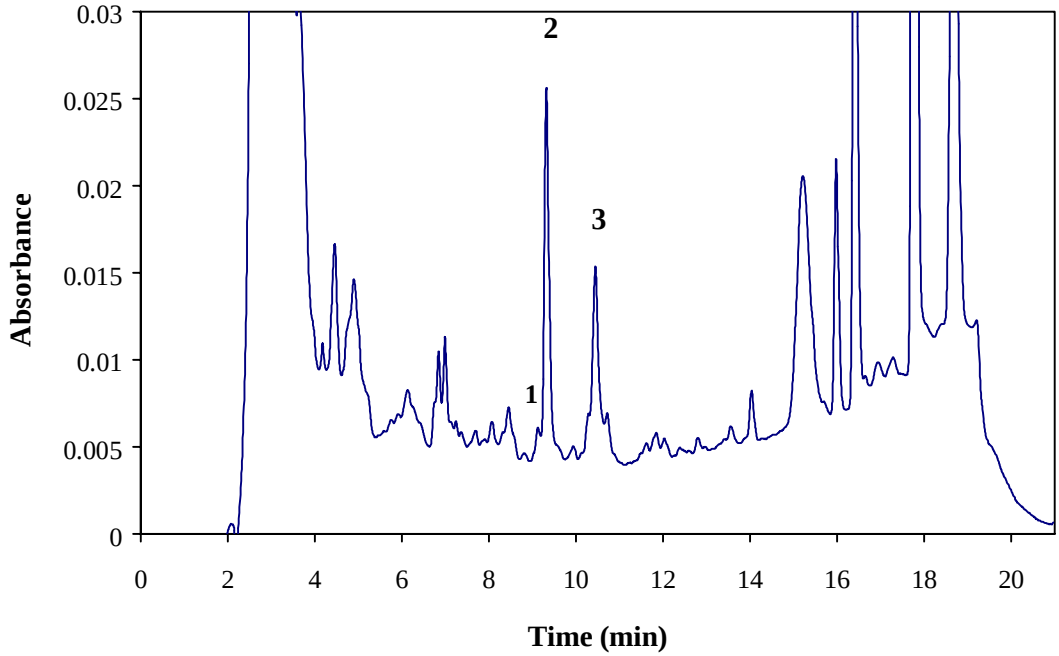


Figure 4. Chromatogram of Chef Han's Hot Sauce.

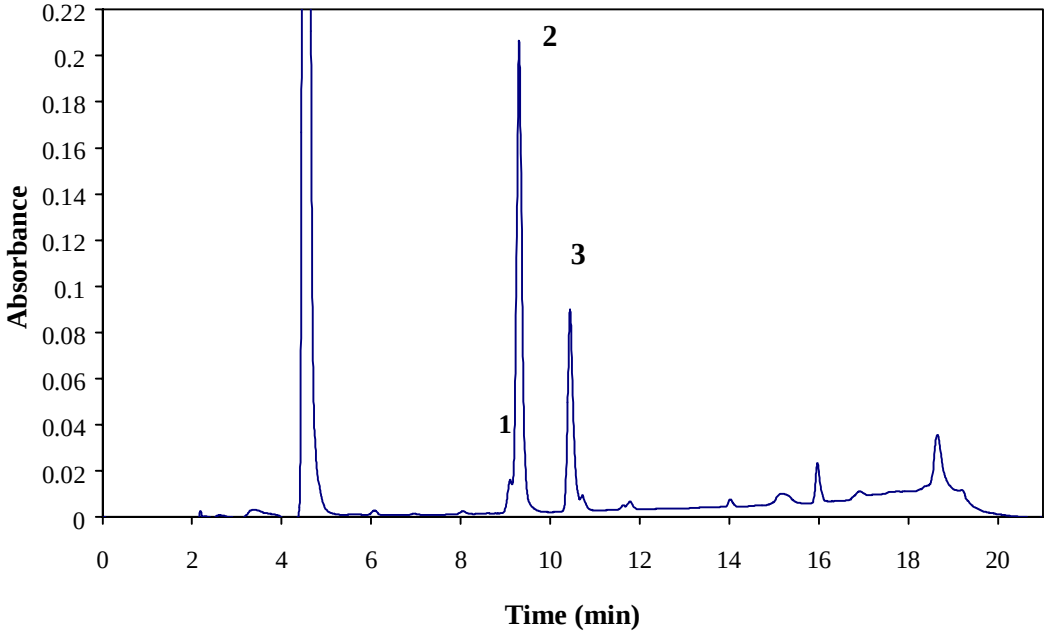


Figure 5. Chromatogram of Arthritis Pain Relief Crème.

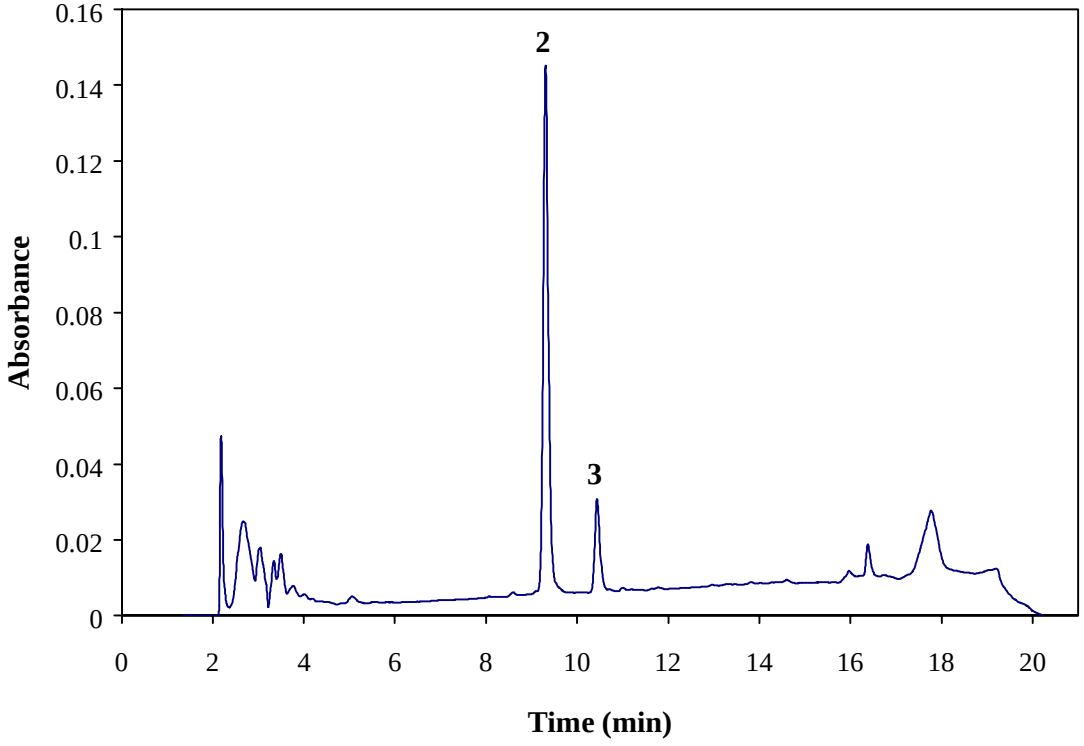


Figure 6. Chromatogram of Habanero Chili Pepper.

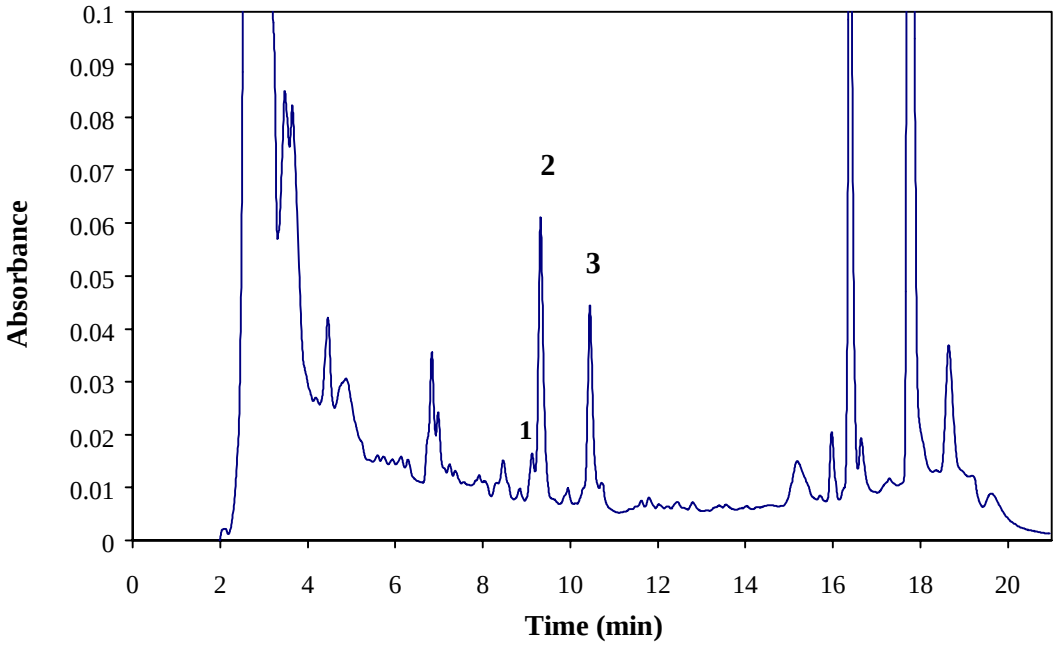


Figure 7. Chromatogram of Hooter's Hot Sauce.

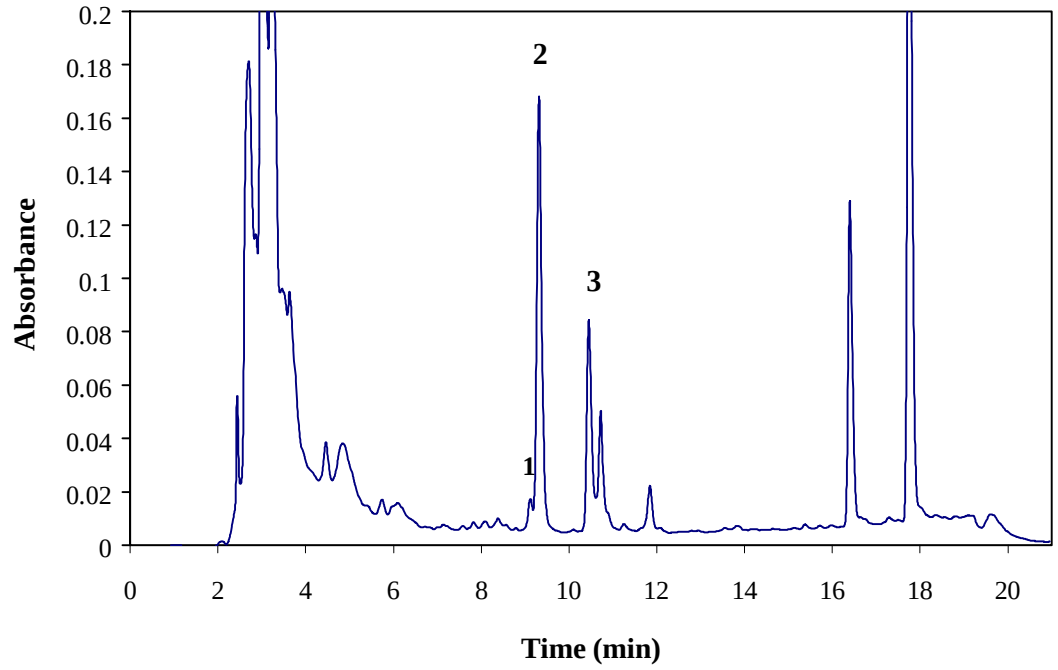


Figure 8. Chromatogram of Tabasco Hot Sauce.

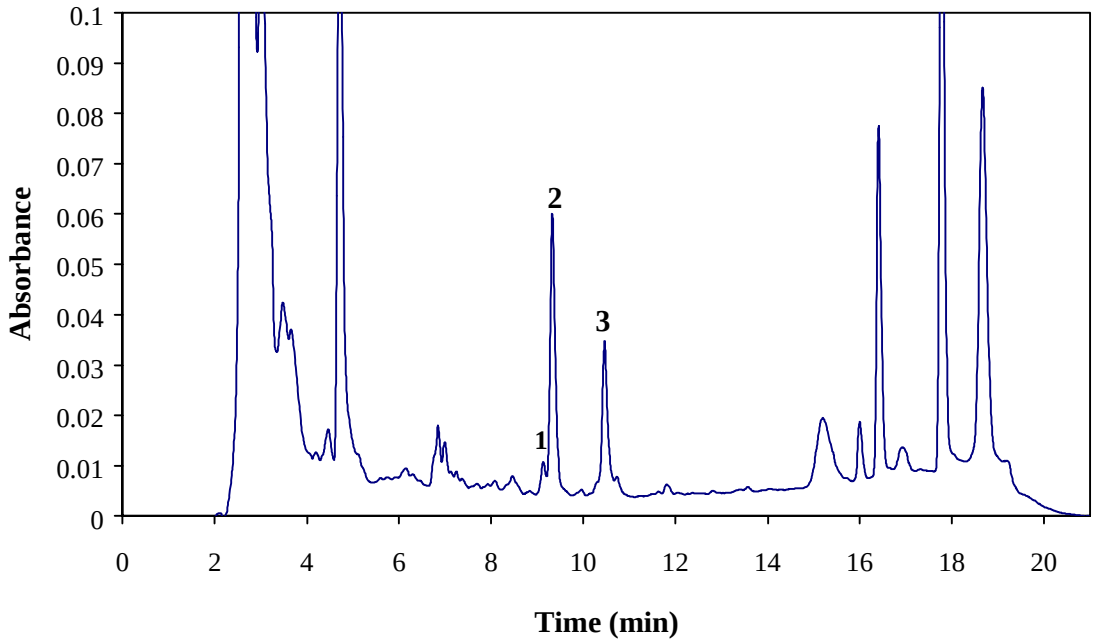


Figure 9. Chromatogram of Texas Pete Hot Sauce.

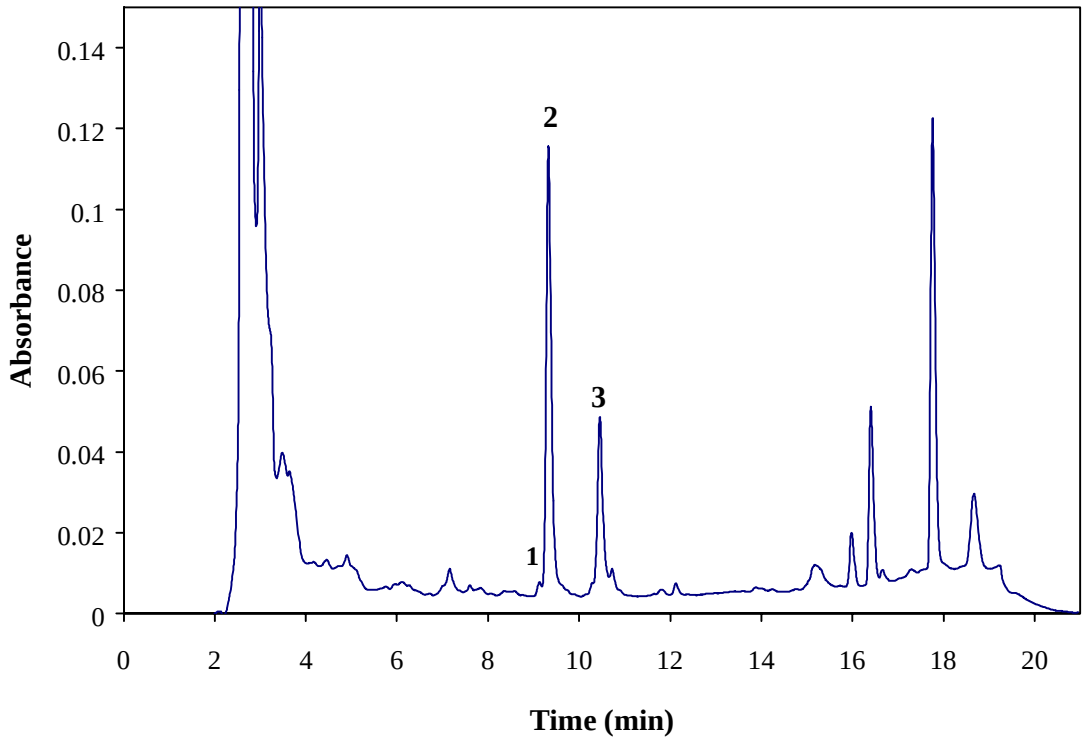
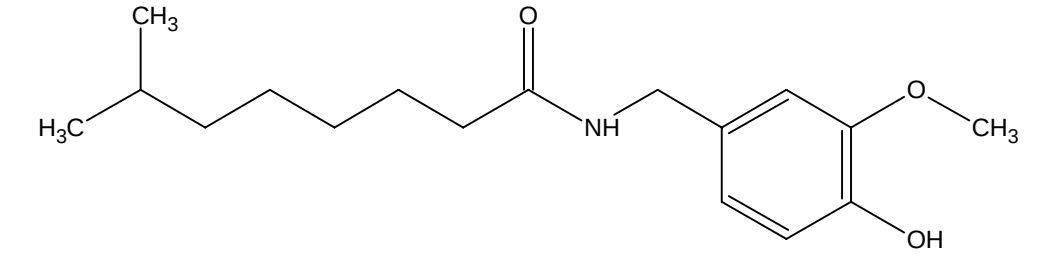
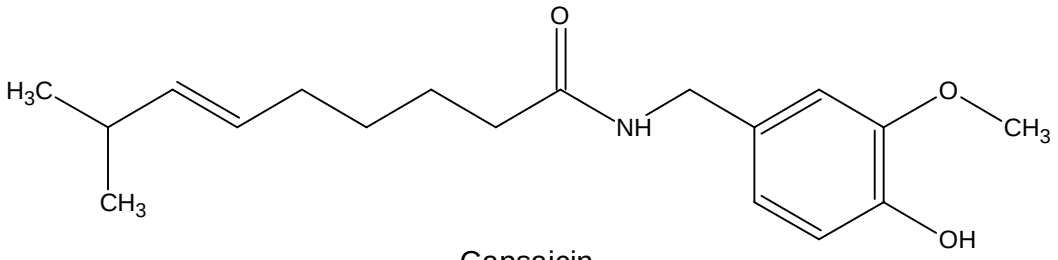


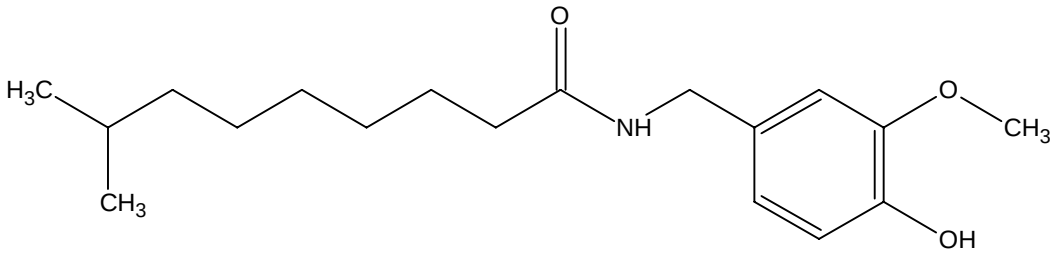
Figure 10. Chromatogram of Yucatan Habanero Hot Sauce.



Nordihydrocapsaicin



Capsaicin



Dihydrocapsaicin

Figure 11. Structural Drawings of the Capsainoids.