How Hot is Your Sauce? Determination of Capsaicinoids in Hot Pepper Sauces using HPLC.

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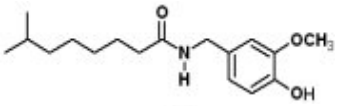
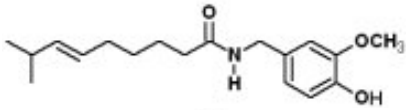
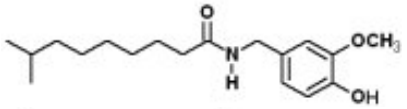
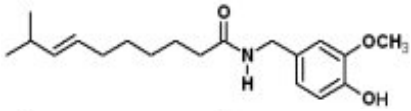
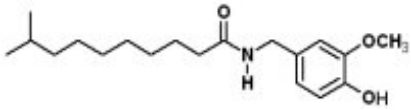
Introduction:

How hot is hot? This may sound like a very subjective question, but for nearly one hundred years, work has been done to attempt to quantify the bite that peppers can deliver. In the early 1900's, pharmacist Wilbur Scoville developed a method by which the "hotness" of a material is quantified in terms of a Scoville Heat Index. Scoville's method was semi-quantitative at best and resulted from using a panel of tasters and determining the dilution needed to remove the hotness from a sauce or food. Using this scale, hotness varied from zero units for bell peppers to upwards of 300,000 Scoville heat units for habenero peppers.

In this experiment, we will take a more quantitative approach to determining the "hotness" of a pepper-based sauce. The compounds responsible for the heat of a pepper are small organic molecules called capsaicinoids. The most common capsaicinoids are shown in Table 1 and vary only by the length and terminal structure of the hydrocarbon side chain.

Structure	Name	Scoville Heat Units (millions per gram)
	Nordihydrocapsaicin (NDC)	9.3
	Capsaicin (C)	16.1
	Dihydrocapsaicin (DC)	16.1
	Homocapsaicin (HC)	6.9
	Homodihydrocapsaicin (HDC)	8.1

Objective:

Your task in this experiment is to extract the capsaicinoids from a sample of hot sauce and quantify their concentrations using HPLC with UV absorbance detection. In the process, you will explore means for developing and optimizing an HPLC separation, choosing a detection wavelength, and preparing standards for an analysis. You will not be given detailed instructions for the separation, but will be expected to develop a protocol to allow you to do the necessary determination.

Procedural Guidelines:

Required Chemicals and Solutions:

1. A real-world capsaicin-containing sample, such as a hot sauce or hot pepper.
2. HPLC Grade methanol (for mobile phase)
3. 18 MΩ Resistive Water
4. Capsaicin standard to for optimization of separation (labeled Capsaicin A)
5. Capsaicin stock standard solution. (labeled Capsaicin B)
6. 4 Standard capsaicin solutions.

Extraction and Sample Prep:

Accurately weigh 10-15 grams of hot sauce into a 125 mL Erlenmeyer flask. Add approximately 50 mL ethanol and a magnetic stirbar and heat the mixture at a low boil for ~30 min (take care NOT to boil off all of the solvent!). While you are waiting for the solution to boil, use the time to collect a UV-Vis spectrum of the capsaicin standard solution. After boiling, allow the mixture to cool to room temperature and filter the mixture into a 50 mL volumetric flask. Dilute the extract to the mark with ethanol. Prior to injection into the HPLC, perform a final filtration by passing ~2 mL of the diluted mixture through a 0.45 micron diameter pore size syringe filter, collecting the solution in a small plastic vial.

HPLC Setup:

You will be doing a reverse-phase separation using a C_{18} bonded-phase resin as the stationary phase and a methanol/water mixture as the stationary phase. Using the standard capsaicin A that has been prepared for you, you will need to determine a set of optimum conditions to use for your analysis. These conditions include: mobile phase composition, flow rate, and detection wavelength. Using a UV-Vis spectrophotometer, collect a spectrum of the capsaicin standard and determine the optimum wavelength for the LC detector. You should see two peaks dominating the chromatograms of standards A, the first peak corresponds to capsaicin (C), and the second to dihydrocapsaicin (DC). In the standard, the two are present at a 65:35 ratio (C:DC).

Quantitative Analysis:

In order to quantify the amount of capsaicinoid present in your sauce, you will need to prepare the appropriate standards and develop a calibration curve. Use the relative sizes of the capasaicin peaks in the stock solution and in your sample to determine the appropriate dilutions needed to prepare four standards whose concentrations bracket your sample. Use standard B (which is pure C) to prepare your standard solutions. Once the solutions are prepared, run injections of your standards and sample, as well as a blank to prepare your calibration curve. Run at least three injections of your sample to determine the precision of the method. Prepare a running calibration curve to be sure that things are proceeding as expected. The chromatograms of your sample may also include a very large, early eluting peak corresponding to other components of the sauce. It may be necessary to rescale the y-axis of your chromatogram to see the peaks of interest.

Results:

1. Plot a calibration curve and determine the least-squares line for your calibration data. Report the slope and intercept, as well as their standard deviations. Using calibration curves created by both peak areas and peak height, determine the total capsaicinoid concentration in your original sample (in both concentration units and in Scoville Heat Units). Compare this result to what you expect.

2. It is also crucial to report the precision of the analysis and ultimately the confidence limits. If possible, you should report the % error by comparison of your calculated analyte concentration to that expected for your sample. Finally, with knowledge of your precision and the calibration curve you obtained, you should be able to determine an approximate limit of detection for the method of analysis, under the experimental conditions you used for the measurement.

3. What determinate errors might have played a role in impacting the accuracy of your results? What factors might have been the greatest contributors to the precision you obtained during this analysis.