Determination of the Scoville Heat Value for Hot Sauces and Chilies: An HPLC Experiment

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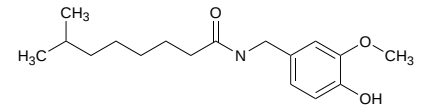
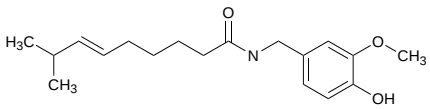
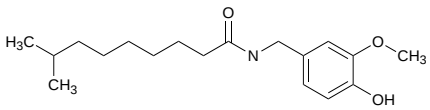
Capsainoids are the group of compounds that give chili peppers and hot sauces their pungent taste (see structures below). Wilbur Scoville first quantified the pungency of foods in 1912 using an organoleptic method (*1*). In this test, a panel of trained people tasted serial dilutions of a sample until it was no longer detected. The resulting dilution factor was called the Scoville heat value (SHV) of the sample. In 1977, Todd et al. (*2*) removed the subjectivity commonly found in taste tests by correlating the organoleptic method to a novel gas chromatographic (GC) method. A Scoville heat value was assigned to each of the capsainoids on the basis of the organoleptic tests. A calculation that involved the GC results and the individual SHVs was used to determine the total heat value for the sample.



Nordihydrocapsaicin



Capsaicin



Dihydrocapsaicin

Since 1977, many groups have determined the capsainoids by either gas chromatography (GC) (*3*) or high-performance liquid chromatography (HPLC) (*4–12*). GC methods typically require derivatization of the compounds to make them sufficiently volatile for determination. Reversed-phased HPLC analysis is the most widely used by researchers and commercial laboratories. HPLC does not require special sample pretreatment and its sensitivity is adequate for the capsainoid concentrations commonly found in food products. The Association of Official Analytical Chemists (AOAC) Method 995.03 describes a commonly used technique for capsainoid determinations. Each of these referenced methods was used as a guideline for development of the procedure described in this manuscript.

While all the HPLC methods described in the literature successfully determine capsaicin (CP), nordihydrocapsaicin (NC), and dihyrocapsaicin (DC), none of them considers the time and money constraints of a junior- or senior-level analytical chemistry teaching laboratory. The goal of this experiment is to determine the three capsainoids (CP, NC, DC) in off-the-shelf products, at a reasonable cost, within a four-hour time frame. The experiment will demonstrate simple extraction techniques, refine standard-making and sample-handling skills, and introduce liquid chromatography. Commercial products (hot sauces and chilies) are analyzed for their capsainoid content. Students must use chromatographic data (integrated absorbance) to calculate concentrations. These are then used to calculate the Scoville heat value for the sample. The students can relate the calculated heat value with actual sample tasting. The relationship between amount of organic compounds determined by analytical chemistry and the heat tasted will give the students a real-world understanding of analytical chemistry and its everyday use.

Materials and Methods

Chemicals

Ethanol (95%) is used as an extraction solvent. The mobile phase consists of acetonitrile (ACN), water, and phosphoric acid (0.1%). Synthetic capsaicin (97%) is needed to prepare working standards. Capsaicin was purchased from Pfaltz and Bauer Inc. (CAT# C02640).

Equipment

This experiment requires an HPLC system with a UV–vis absorption detector. Detection is most sensitive at 205 nm, but 280 nm is adequate for samples with high levels of capsaicin (254 nm does not exhibit sufficient sensitivity). A 25-cm C18 analytical column with a similar guard column is required. Gradient elution is preferred (40–90% ACN in 15 min), but isocratic elution (50% ACN for 20 min) will work with reduced sensitivity. A flow rate of 1 mL/min is typical, with an injection volume of 20 μL. Separation at room temperature is adequate, but improved resolution is observed if the column is thermostated at 50 °C.

Experimental Procedure

Prior to the laboratory period, the HPLC system is allowed to equilibrate for 30 min at the starting conditions and a 1000-ppm stock solution is prepared by dissolving 0.1 g of capsaicin in a 100-mL volumetric flask with 95% ethanol. Individual students mass 15 g of hot sauce or 2–3 g of dried ground spice in a weigh-boat. The mass is recorded, and the contents are washed into a 125-mL Erlenmeyer flask. Ethanol is added to bring the liquid level to the 50-mL graduation on the flask. A Teflon stir-bar is placed in the flask, and the flask is placed on a hot-plate (no open flame). The solution is held at a slow boil for 30 min with constant stirring. This allows a volume reduction for the subsequent quantitative transfer. The flask is removed and allowed to cool, and the stir bar is removed. The extract is filtered into a 100-mL volumetric flask. The material in the flask is brought to volume with ethanol. A 5-mL aliquot is removed and filtered

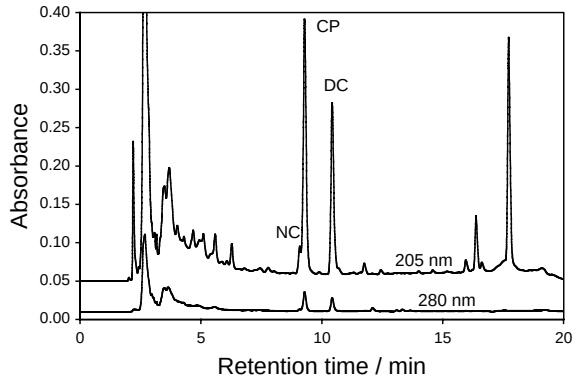


Figure 1. Chromatograms of ground cayenne pepper extract using gradient elution. The baseline for the 205 nm chromatogram has been offset for clarity.

through a 0.45-μm pore size syringe filter cartridge. Twenty microliters of the filtered sample is injected onto the column.

A calibration curve is generated using serial dilutions of the capsaicin stock solution (typical range 1–50 ppm) and the area (or height) of the capsaicin peak in the standard chromatogram. The elution order (Fig. 1) observed for the sample extracts is NC, CP, then DC, as reported in the literature (*5, 6, 8–11*). The concentration of each of the three capsainoids is calculated from the single capsaicin calibration curve. The high cost and limited availability of purified DC and NC prohibit individual calibration of these compounds. According to the literature, the relative responses of DC and NC are within 5% of that for capsaicin (*6*). The final concentration of each capsainoid is expressed as grams of compound per gram of sample. The Scoville heat value for each capsainoid in the sample is calculated by multiplying the concentration by the SHV for the pure compound: NC, 9.3 million; CP, 16.1 million; DC, 16.1 million (*2*). The three calculated SHVs are then added to give the total Scoville heat value for the sample.

Results and Discussion

Various commercial hot sauces (e.g. Tabasco, Texas Pete), ground chilies (habanero, cayenne, jalapeño), and capsaicin-based arthritis pain relief creams can be successfully analyzed. The sauces typically have SHVs in the range 400–3000. The chilies have values in the 8,000–300,000 range. Arthritis creams typically contain about 0.075% capsaicin.

The accuracy of the method may be affected by sensitivity. A 0.5-ppm standard solution analyzed by the gradient elution method was determined with a 10% error, whereas the same standard solution analyzed by the isocratic method returned a 50% error (at the 205-nm wavelength). At the 280-nm wavelength with isocratic elution, the 0.5-ppm standard solution could not be distinguished from the background noise. If the isocratic method must be employed using the 280-nm band, the instructor should consider only those samples with significant amounts of capsaicin (ground chilies, capsaicin creams).

The SHV for individual types of ground chilies can vary widely depending upon soil and weather conditions (*2*), so values reported in the literature can only be used as a guideline. For example, habanero chilies were determined to have an SHV of 150,000 in the laboratory, but the literature suggests a value in the range 200,000–300,000 (*3, 13*). Cayenne peppers were determined to have an SHV similar to the literature value of 41,000 (*3, 13*). Anaheim peppers were determined to have an SHV of 8,000, which is much higher than the literature value of about 3,000 (*3, 13*). The hot sauces have no previously reported Scoville heat value, so taste tests should be performed by the student to confirm which sauces are indeed hotter (taste tests of the raw peppers are not recommended!).

As an additional cost-cutting measure, an over-the-counter arthritis pain relief cream may be used to prepare the capsaicin standard solutions. The cream is easy to handle and dissolves in warm ethanol. Only syringe filtering is needed prior to injection. A small amount of purified capsaicin could be used to standardize a large tube of cream, or the capsaicin concentration on the tube label may be taken literally. The cream analyzed in this experiment was found to contain more capsaicin than was printed on the label. This suggests that the cream should be standardized with pure capsaicin before it is used as a working standard.

Acknowledgments

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[W]Supplemental Material

See this issue of *JCE Online* for detailed instructions for students and notes for the instructor, including trouble-shooting, lab forms, and sample results and spectra.

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