2011-07-14

Extraction of Caffeine from Energy Drinks

Mulcahy, Seann P.

http://hdl.handle.net/2144/1418

Boston University
Extraction of Caffeine from Energy Drinks

Introduction:

Ever since Wohler’s first synthesis of urea in 1828, the number of organic compounds that chemists have been able to synthesize has grown exponentially. Despite these advances, Nature still remains the most abundant source of organic compounds. Many of these so-called natural products possess a myriad of physical, spectroscopic, and medicinal properties that make their study even more intriguing. However, these natural substances often exist in an aqueous medium and must be extracted from water and separated from other organic components for further characterization.

One of the prime examples of natural product isolation is the substance paclitaxel, a diterpene natural product that is marketed by Bristol-Myers Squibb as Taxol®. Paclitaxel stabilizes microtubules and arrests cell division in overproliferating cells. As such, it has become one of the most widely prescribed anticancer therapies in the world. However, the structure of paclitaxel was once considered much too complex for organic chemists to synthesize from scratch on the scales required for clinical production. Thus throughout the 1960’s and 1970’s, the world’s supply of paclitaxel came from its isolation from the bark of the Pacific Yew tree. After environmental groups raised concern about the destruction of these trees, isolation chemists began looking for an alternate route to its production, eventually leading to a semi-synthesis which remains patented today.

Thus, extraction of natural products is a tool that organic chemists use to discover new structures with diverse properties. In this laboratory, you will mimic natural product isolation by extracting caffeine from an energy drink of your choice in much the same way that coffee becomes decaffeinated (shown below). You will be asked to bring your favorite energy drink to lab (the only time you will be allowed to bring a drink to lab!) to perform the isolation. You will determine the concentration of caffeine in your sample by employing a standard curve and analyze your sample for purity (LC/MS) and identity (1H NMR). Each of these objectives will be met using an isolation technique known as solid phase extraction (SPE).
Solid Phase Extraction

SPE is a technique that allows the isolation of organic components dissolved in an aqueous mixture. In this procedure, the mixture is passed through a solid nonpolar matrix which separates the mixture into its respective components. Analytes which are ionic or polar pass through the column because they have only weak interactions with the matrix. Nonpolar organic components, on the other hand, adsorb (or retained) onto the column by stronger intermolecular forces. By eluting a secondary solvent through the stationary phase, the nonpolar organic component can be released from the stationary phase, collected, and isolated in relatively pure form. A flow chart for the SPE of caffeine from an energy drink is illustrated below.

Consider the following scenario for this experiment:

You are employed by the Food and Drug Administration and have been instructed to conduct a review of all caffeinated beverages to ensure that appropriate labels are affixed to these drinks. You have decided to first target caffeinated energy drinks, due to their popularity on college campuses. You first must isolate the caffeine by solid phase extraction and then analyze the manufacturer’s caffeine content claims by using LC/MS to determine the concentration present in your sample. In addition, you want to confirm that caffeine was isolated and not other nonpolar entities, so you want to confirm its presence by NMR.

References:

Extraction of Caffeine from Energy Drinks

**Equipment:**
- Microscale glassware kit
- 50 mL Erlenmeyer vacuum filtration flask
- Vacuum tube
- 25 and 50 mL beakers
- One C18 Solid Phase Extraction Column
- Neoprene adapters
- 10 mL graduated cylinder
- 1.5 mL HPLC vial
- Pasteur pipets
- Capillary tubes
- TLC plates
- Cotton

**Chemicals:**
- One can of Red Bull™ (AMP™, Monster™, coffee, and 5 Hr Energy™ do not give reliable results and should be avoided)
- Methanol
- Deionized water
- 10% methanol in deionized water
- Ethyl acetate
- Anhydrous sodium sulfate

**Procedure:** adapted from *J. Anal. Toxicol.* 2003, 27, 520.

**Conditioning the SPE Column:** Set up your SPE column as shown in the figure below. Place a number 2 neoprene adapter inside the top of a 25 mL filter flask. Clamp the flask securely to your scaffolding and then apply a vacuum source. Place the SPE column inside the number 2 neoprene adapter and condition the column under vacuum in the following way: add 2 mL of methanol to the column in 1 mL portions. Wait for the methanol to draw through the column completely before adding the second mL. Next, add 2 mL of room temperature deionized (DI) water in 1 mL portions. **Do not draw the water completely through the column this time;** keep the top of it wet by stopping the vacuum before the next step.
SPE of Caffeine: Add a known amount (50-100 mL) of Red Bull in portions to the SPE column while the system is under vacuum, continuously drawing through the solution while applying more to the top of the column (you will have to empty your filter flask at some point). Do this until all 50-100 mL have been applied to the column; allow the column to run dry. Run about 25 mL of DI water through the SPE column in approximately 2 mL portions in the same manner that was done with the energy drink. Allow the column to run dry and continue to pull vacuum through for 2 minutes to remove any excess water that may still be on the column.

Isolating Caffeine off of the SPE Column and analyzing via LC/MS: Turn the vacuum off, remove the SPE column and flask from the clamp, and transfer the solution from the Erlenmeyer flask to a 50mL beaker; do not discard. Rinse the Erlenmeyer flask with water and then acetone into your waste container. Setup the apparatus again as you had at the beginning of the experiment. Run 10 mL of ethyl acetate through the SPE column in the same manner as before. Allow the column to run dry and pull vacuum through for an additional 2 minutes. Breakdown your apparatus as you had previously done. Add about 2 g of anhydrous sodium sulfate to the ethyl acetate solution and mix with a spatula for at least 2 minutes. Filter the dry ethyl acetate solution through a piece of cotton in a Pasteur pipet into a dry 25 mL beaker, leaving the sodium sulfate behind. Transfer 1 drop (1/60th mL) of this solution to a 1.5 mL HPLC vial and dilute it with 1 mL methanol. Submit this sample for LC/MS using the method LCMS_P_CH211 (ask your TF to provide you with the integration results report for your sample). Take the beaker containing the remainder of your ethyl acetate solution and evaporate the ethyl acetate in the fume hood by warming on a hotplate and blowing a slow stream of dry air over the beaker. When the solvent has completely evaporated, redissolve it in 0.7 mL CDCl₃ and take a ¹H NMR. Download the raw NMR data from the Chemistry Server and analyze using MNova LITE.
Pre-Lab Exercise:
1. Consult the label of your energy drink. Name 2 – 3 other organic compounds also dissolved the drink? What are their structures? Based on what you know about polarity, predict whether these components will be retained on the column along with caffeine or eluted with undesirable aqueous material.
2. Produce a standard curve from the data below and provide the equation of a linear trendline:

   - 1 mg/mL of caffeine in water = 3 mAU*s
   - 2 mg/mL of caffeine in water = 6 mAU*s
   - 5 mg/mL of caffeine in water = 15 mAU*s
   - 10 mg/mL of caffeine in water = 30 mAU*s
   - 25 mg/mL of caffeine in water = 75 mAU*s

   If a sample you measured showed a peak height reading of 68 mAU on the LC/MS. How many milligrams of caffeine per milliliter are present in your drink?
3. Caffeine belongs to a family of naturally occurring compounds called xanthines. Two other species of this family, theobromine and theophylline, do not have the same stimulant effects that caffeine possesses. Draw the structures of theobromine and theophylline and describe what pharmaceutical uses they possess.

Post-Lab Assignment: Organic Syntheses Preparation, due in Lab one week after you complete the experiment. Calculate how much caffeine was in your can of Red Bull and compare it to the Nutrition Facts on the label.