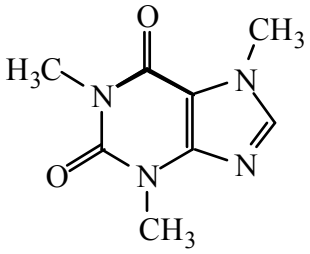
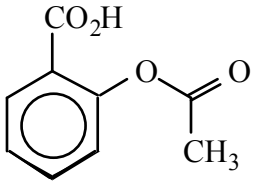
31. Analysis of Analgesic Tablets by High-Performance Liquid Chromatography[1]

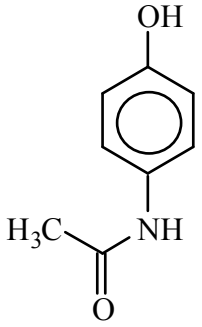
Nonprescription headache medications such as Excedrin or Vanquish contain mixtures of acetaminophen and aspirin for relief and caffeine as a stimulant. This experiment describes conditions for separating and measuring the components by high-performance liquid chromatography (HPLC). Instructions are given for measuring caffeine, but any and all of the components could be measured.



Caffeine



Aspirin
(acetylsalicylic acid)



Acetaminophen

REAGENTS

HPLC solvent: Organic solvents should be handled in a fume hood. All solvents in this experiment should be HPLC-grade. Mix 110 mL of acetonitrile, 4.0 mL of triethylamine, and 4.0 mL of acetic acid in a 2-L volumetric flask and dilute to the mark with HPLC-grade water. Filter through a 0.45-µm filter and store in a tightly capped amber bottle.

Caffeine stock solution (100 µg/mL): Dissolve 1.000 g of caffeine in 50 mL of HPLC solvent in a 100-mL volumetric flask with gentle heating (in the hood). Cool to room temperature and dilute to the mark with HPLC solvent. Dilute 10.00 mL to 100 mL with HPLC solvent in a volumetric flask to obtain 1 000 µg/mL. Dilute once again to obtain 100 µg/mL.

Acetaminophen and aspirin samples: Prepare two solutions, each containing one of the analytes at a concentration of ~50 µg/mL in HPLC solvent. Filter through 0.22 µm nylon syringe filters and store in capped amber bottles.

PROCEDURE

1. *Caffeine quantitative analysis standards:* Dilute the 100 µg/mL stock solution down to 50, 10, and 5 µg/mL with HPLC solvent. Filter ~3 mL of each solution through a 0.22-µm syringe filter into a capped vial. Filter ~3 mL of the 100 µg/mL solution into a fourth vial.

2. *Sample preparation:* Grind the analgesic tablet into a fine powder with a clean mortar and pestle. Dissolve ~0.5 g (weighed accurately) in 50 mL of HPLC solvent with gentle heating. Cool to room temperature and dilute to volume with HPLC solvent. Dilute 10.00 mL of this solution to 100 mL with HPLC solvent in a volumetric flask. Filter ~3 mL of the dilute solution through a 0.22-μm syringe filter into a capped vial.

3. *Chromatography conditions:* Use a 2.1-mm-diameter ×10-cm-long C_{18}-silica column with 5-μm particle size and ultraviolet detection at 254 nm. With a flow rate of 1.5 mL/min, each run is complete in 4 min.

4. *Calibration curve:* Inject 10 μL of each of the caffeine standards (5, 10, 50, and 100 μg/mL) into the HPLC and measure the peak area. Repeat this process twice more and use the average areas from the three runs to construct a calibration curve of area versus concentration. Compute the least-squares slope and intercept for the line through points.

5. *Qualitative analysis:* Record a chromatogram of 10 μL of the analgesic tablet solution. Then mix 2 drops of the tablet solution with 2 drops of 50 μg/mL caffeine solution in a test tube or vial. Inject 10 μL of the mixture into the chromatograph and observe which peak grows. Repeat the process again by adding 50 μg/mL acetaminophen and 50 μg/mL aspirin and identify which peaks in the analgesic are acetaminophen and aspirin.

6. *Quantitative analysis:* Inject 10 μL of the analgesic tablet solution and measure the area of the caffeine peak. Repeat this process twice more and take the average from three injections. Using your calibration graph, determine the concentration of caffeine in the solution and the weight percent of caffeine in the original tablet.

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1. G. K. Ferguson, *J. Chem. Ed*. **1998**, *75*, 467.