Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine

Glenda K. Ferguson
Department of Chemistry and Physics, Wesleyan College, Macon, GA 31210

Modern instrumental laboratory experiments that make use of consumer products can be most instructive. Through these investigations, students can gain hands-on experience with vital, state-of-the-art analytical tools and link scientific subject material to their own lives. As this leads to heightened interest, students are bound to understand and retain the course material more efficiently. Realizing this, educators have developed a number of such experiments. Several that make use of high-performance liquid chromatography (HPLC) (*1–12*) have appeared in this *Journal*. Beverages (*1–6*), food products (*2, 6, 7, 8*), vegetable oils (*9*), sunscreen products (*10*), and medications (*11, 12*) have been analyzed quantitatively in these endeavors.

In 1983, Kagel and Farwell (*11*) described an interesting quantitative experiment for the separation of the components of Vanquish tablets: the analgesics acetaminophen (4-acetamidophenol) and aspirin (2-acetoxybenzoic acid), and the central nervous system stimulant caffeine (1,3,7-trimethylxanthine). The structures of these compounds are shown in Figure 1. With salicylic acid as an internal standard, Kagel and Farwell (*11*) reported a separation time of approximately 8 min on a Zorbax C8 (25 cm × 4.6 mm i.d.) column, with a methanol/acetic acid/water mobile phase. Their experiment entailed mobile phase optimization and quantitative determinations of caffeine and aspirin in these tablets.

While Kagel and Farwell's experiment provided undergraduates with an interesting and practical hands-on introduction to HPLC, it is not without limitations in today's instrumental analysis laboratory. The Kagel experiment used an internal standard to minimize errors, but replicate injections of the three standard solutions and the analgesic sample were not performed. Since the mean of a series of replicates is generally more accurate than any single value owing to indeterminate error cancellations, replicate analyses should lead to a more accurate caffeine determination. These are more feasible in the undergraduate lab today, owing to the advent of smaller, more efficient columns capable of much shorter analysis times. Other HPLC experiments that involve analgesic mixtures have appeared in this *Journal* (*13, 14*), but these do not incorporate quantitative analyses. Clearly, an updated quantitative experiment for this drug combination is warranted.

This paper describes a modern experiment for the quantitative assay of caffeine in a commercially available medication that also includes acetaminophen and aspirin as active ingredients, Goody's Extra Strength Headache Powders. It should be noted, however, that our liquid chromatographic separation scheme is not limited to this particular brand of medication, nor is it limited to caffeine as the analyte. Other name brands such as Excedrin, Vanquish, or generic versions of these three-component mixtures could be analyzed with the HPLC method described herein. Since our method resolves all three components, only minor procedural modifications would be necessary for determinations of other components or even the simultaneous assays of all active components in these mixtures. This separation scheme is also suitable for the analysis of caffeine and/or acetaminophen in caffeine/acetaminophen combinations such as Aspirin-Free Excedrin.

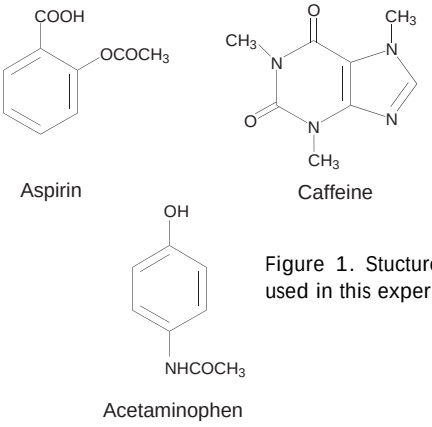

Figure 1. Stuctures of compounds used in this experiment.

Our separation scheme is approximately 2.5 times faster than that reported by Kagel and Farwell (*11*) and has afforded time for quadruplicate injections as well as expansion to four standard solutions. We used a shorter analytical column and developed an HPLC protocol by which components are resolved in only 3 min. The experiment detailed herein makes use of a straightforward HPLC apparatus with a C18 column, an isocratic mobile phase, UV detection at 254 nm, and a basic integrator; an expensive, sophisticated system is not required. Figure 2 shows a block diagram of our experimental setup. The separation is both repeatable and rapid. Moreover, the experiment can be completed in a single three-hour period. The experiment is appropriate for any chemistry student who has completed a minimum of one year of general chemistry and is ideal for an analytical or instrumental analysis course.

Prior to the development of this quantitative exercise, the retention properties of these compounds and also salicylic acid, the major degradation product of aspirin, on a C18 column with acetonitrile:water:acetic acid:triethylamine mobile phases were studied in our laboratory (*15*).

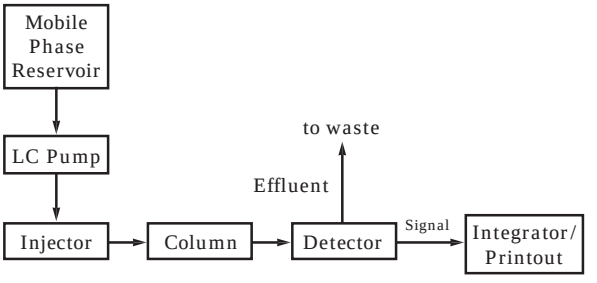

Figure 2. Block diagram of HPLC experimental setup.

Overview

Students in our quantitative analytical chemistry course perform this experiment in pairs or groups of 3. We typically have 6–8 students in the course. All of these students have taken a year of general chemistry, and the majority have completed a year of organic chemistry.

The mobile phase for the chromatographic analyses and stock solutions of acetaminophen and aspirin are provided for students. The mobile phase is also used by students as the solvent in solution preparation. Students are expected to read the experiment and perform calculations pertaining to caffeine standard preparation by serial dilution before coming to lab. After the calculations are checked by the instructor, groups prepare and filter their solutions. This usually requires 30–45 min. Students then proceed with their HPLC analyses.

Approximately 1 hour provides sufficient time for a group's actual chromatographic analyses. The instructor equilibrates the system for at least 30 min before the first injection. Groups take turns using the chromatograph, performing quadruplicate injections of all four caffeine standard solutions as well as of the analgesic-mixture solution of unknown caffeine concentration. Each group also runs one injection each of acetaminophen and aspirin solutions for retention-time assessments. Group members are encouraged to alternate the duties of injecting and operating the integrator.

In the analysis of the data, students average the caffeine peak areas for each solution and calculate the relative peak areas by dividing each by the smallest. Each group generates a graph of relative peak area versus caffeine concentration and the relationship's best-fit equation. From this, students determine the concentration of caffeine in their injected unknown and ultimately the concentration of caffeine in the original analgesic powder. Each group then calculates the absolute standard deviation of the mean result. Students write standard laboratory reports complete with sections on purpose, procedure, results, and conclusions.

Experimental Procedure

Reagents

Caffeine was purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Acetonitrile, triethylamine, and glacial acetic acid, all of HPLC-grade, were purchased from Fisher Scientific (Pittsburgh, PA). E-Pure-grade water was produced in our laboratory. Goody's Extra Strength Headache Powders (Goody's Pharmaceuticals, Memphis, TN) were purchased locally.

Apparatus and Operating Conditions

An Acuflow Series II (Scientific Systems, State College, PA) isocratic pump is used to deliver the eluent at a constant flow rate of 1.5 mL/min. Solutions are injected to fully fill a 10-μL loop on a Rheodyne (Cotati, CA) 7010 injector. Analyses are carried out on a Hewlett-Packard (Avondale, PA) 10 cm × 2.1 mm i.d. (5-μm particle size) ODS-Hypersil analytical column with no guard column. An AcuCon 500 (Scientific Systems) variable-wavelength UV–vis detector is set at 254 nm to monitor absorbance of the effluent. The detector is set for 10-mV output at 1.0 AU. A Hewlett-Packard Model 3395 integrator is operated at a chart speed of 1 cm/min to record chromatograms and calculate peak areas.

Student Procedure

Provided Solutions: Mobile Phase and Analgesic Standards

The mobile phase used in this experiment is a mixture of 94.1:5.5:0.2:0.2 (v/v/v/v) water:acetonitrile:triethylamine: acetic acid. It was prepared by placing 110.0 mL of acetonitrile, 4.0 mL of triethylamine, and 4.0 mL of acetic acid into a 2-L volumetric flask and diluting to volume with E-Pure water. The mobile phase was mixed thoroughly, filtered through a Magna 0.45-μm nylon filter membrane (Micron Separations, Westboro, MA), and degassed for 5 min with helium before use. This mobile phase will be used as the solvent in the preparation of the solutions described below. In addition, filtered solutions of acetaminophen and aspirin at concentrations of 1.00×10^{-5} g/mLare provided.

Preparation of Caffeine Standard Solutions

Weigh 1.0000 g of caffeine and transfer it to a 100-mL volumetric flask. Add approximately 50 mL of solvent, and swirl and heat gently on a hot-plate. Once dilution is complete, dilute to volume to give rise to a 1.00×10^{-2}-g/mL stock solution. Perform serial dilutions of this solution to prepare solutions of the following concentrations (in g/mL): 1.00×10^{-3}, 1.00×10^{-4}, 5.00×10^{-5}, 1.00×10^{-5}, 5.00×10^{-6}. The last four of these will be used as standards in the acquisition of the calibration curve data. Filter approximately 3 mL of each solution with the use of Cameo 0.22-μm nylon syringe filters (MSI, Westboro, MA) and place in labeled vials.

Preparation of Analgesic Mixture Solution

Grind and mix the entire contents of one Goody's analgesic powder with a mortar and pestle to insure homogeneity. Weigh approximately 0.5 g of the sample to the nearest 0.1 mg and transfer to a 100-mL volumetric flask. Add approximately 50 mL of solvent and swirl to aid dissolution. Dilute to volume with solvent. Perform a 1:10 dilution to prepare 10 mL of the solution to be injected. Filter approximately 3 mL of this solution as described for the caffeine standards.

Determination of Retention Times of Aspirin, Acetaminophen, and Caffeine

Inject 10 μL (the loop volume) of the 1.00×10^{-5}-g/mL caffeine solution. Repeat with the provided solutions of aspirin and acetaminophen at the same concentrations. Record the retention times of these three compounds.

Calibration Curve: Collection and Treatment of Data

Perform quadruplicate 10-μL injections with each of the caffeine standard solutions. During each run, record the area of the caffeine peak from the integrator. Calculate the average area at each concentration and the relative areas by dividing each average area by the smallest. Plot a calibration curve of relative peak area against concentration (g/mL) with the use of CA-Cricket Graph III (Computer Associates, Malvern, PA).

Quantitative Analysis of Analgesic Powder

Analyze the analgesic sample solution in quadruplicate 10-μL injections. Divide each caffeine peak area value by the smallest average area of the standards from the previous step. For each injection, determine the concentration of caffeine in the solution. With these data, determine the total mass of caffeine in the original 100-mL Goody's powder solution and calculate the percent by mass of caffeine in the powder sample

for each trial. Finally, calculate the average percent caffeine by mass and the absolute standard deviation of the mean.

Results and Discussion

Students must know the retention times of the components of the analgesic/caffeine formulation in order to assess their separations. We have chosen to provide students with stock solutions of acetaminophen and aspirin as described above. Alternately, retention times of these compounds for the chromatographic system at hand may be given. Students could, on the other hand, prepare all required solutions including the acetaminophen and aspirin samples as well as the mobile phase/solvent. This would, however, necessitate approximately 1.5–2 h of additional laboratory time. If this route is chosen, the instructor may wish to have students prepare their solutions in one lab period and perform the chromatography in the next, especially in cases of limited solvent filtration apparati and/or chromatographic equipment. When solutions are kept for several days, care should be taken to store them in amber vials to protect against decomposition.

A typical chromatogram of the Goody's powder component separation is shown in Figure 3a. That representing a caffeine standard is shown in Figure 3b. The acetaminophen and aspirin peaks occur at 0.7 min and 1.4 min, respectively. Caffeine shows a retention time of 2.2 min. In previous studies, we have found salicylic acid to elute at 1.3 min. Figure 3a shows no such peak in our analgesic sample. While a salicylic acid peak may be present in analyses of older samples, this should not interfere in the quantitation of caffeine.

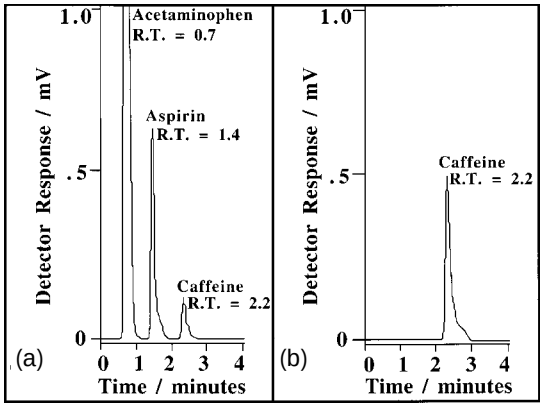

Figure 3. Chromatograms obtained from (a) an analgesic powder sample solution and (b) a caffeine standard solution of concentration 5.0×10^{-5} g/mL. Units of retention times are min. Conditions are given within the text.

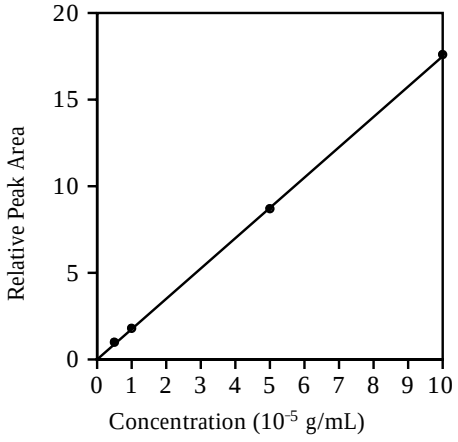

Figure 4. Caffeine calibration curve.

Figure 4 shows a typical peak area versus concentration graph indicating the linear relationship over the concentration range of 1.00×10^{-4} g/mL to 5.00×10^{-6} g/mL. Student data from our three groups in the spring of 1997 gave rise to excellent correlation coefficients of .999 and greater. The percent by mass of caffeine in the Goody's Extra Strength powder was determined by students to be in the range of 3.05–3.10%, with absolute standard deviations of the mean in the range of 0.01–0.05%.

Numerous interesting modifications can be made to the procedure. Each group could be given a different analgesic preparation in order to compare various brands. A comparison between name brand products and their generic counterparts could be carried out. Alternately, students could perform quantitative analyses on all of the active ingredients of these medications by incorporating a set of standards composed of all three analytes.

The separation scheme detailed herein involves the use of relatively simple and inexpensive chemicals and equipment. With proper care, the C18 column has a relatively long lifetime. This concise exercise demonstrates both the qualitative and the quantitative power of liquid chromatography in a single experiment. Moreover, students can "relate" to the valuable skills they acquire in this lab.

Acknowledgments

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