Analysis of Currently Available Analgesic Tablets by Modern Liquid Chromatography

An Undergraduate Laboratory Introduction to HPLC

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The array of techniques deserving attention in an undergraduate instrumental analysis course is expanding so rapidly that the choice of laboratory experiments must be updated frequently and should be based on pertinence (both present and long term), broadness of scope, ability to stimulate interest, practicality, and financial considerations. Modern liquid chromatography (LC) has, through many improvements in the 1970's, matured into a widely applied technique (*1–3*). Due to the versatile nature of LC analysis and a broadening LC market, it will continue to be an area of major importance in the 1980's (*4*). Therefore, a practical student experiment demonstrating the actual use and overall utility of modern HPLC would be an asset to most undergraduate instrumental analysis courses.

The versatile nature of LC stems from the many ways in which the chemistry of the separation can be varied and from the methodology allowing accurate quantitation (*5*). In this experiment these two qualities are revealed as students develop an LC separation for an extract of a nonprescription analgesic tablet containing aspirin, caffeine, acetaminophen, and an internal standard, salicylic acid; and then determine the quantity of aspirin and caffeine in the tablet. For reference ease, Figure 1 shows the structures of aspirin, caffeine, acetaminophen, and salicylic acid.

Presently, the most popular column packings for modern LC are those with surface-reacted (or chemically-bonded) organic stationary phases (*6*). Although the term LC implies the availability of several separation modes, many laboratories currently report that over three-fourths of their LC separations are performed on bonded phase columns. These chemically-bonded phases usually have a carbon chain length of eight or eighteen (C_{18} being presently most popular) and are operated in the so-called reverse phase mode (*7*). In our search for a practical student experiment using reverse phase LC, we found that one of the few sources for this type of experiment was the application literature available from the various manufacturers of HPLC instrumentation. However, these application experiments are often either somewhat out-of-date (using inefficient dry packed columns or normal phase separations) or depend on special equipment (*e.g.*, fluorescence detection or ternary gradient). Nevertheless, our idea to base an LC experiment on the analysis of analgesic tablets came from two such application experiments, one from Gow Mac Instrument Co. (*8*) which uses ion-exchange LC and the other from Waters Associates Inc. (*9*) which uses reverse phase LC.

The experiment described in this paper is an improved, modified version of the experiment Waters Associates Inc. published in their WALCEP series. One major revision from the original experiment is the addition of salicylic acid as an internal standard. This internal standard allows accurate quantitation even if sample loss or injection errors occur during the procedure. Another experimental improvement is the use of a high efficiency C_8 bonded phase column which yields approximately 14,000 plates for aspirin relative to 800 plates obtained for the column in the original WALCEP experiment. A further problem with the WALCEP experiment and its up-dated version (*10*) is that the analyte is APC tablets

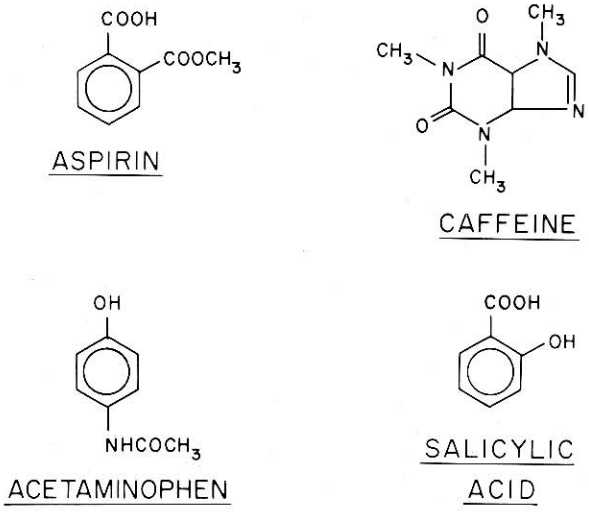

Figure 1. Structures of Vanquish® tablet components and internal standard.

which are no longer available due to decreased use of phenacetin formulations. Our revised experiment uses the readily available acetaminophen formula (*e.g.*, Vanquish®), thereby making this experiment practical for anyone with basic HPLC equipment and the correct column (refer to Discussion section for comments on columns other than the Dupont Zorbax C_8 and tablets other than Vanquish®).

The described LC separation takes place in a reverse phase-ion suppression mode (*11*) using a simple 1% acetic acid buffer to suppress ionization of the acids (aspirin and salicylic acid) and consequently promoting their retention relative to the bases (acetaminophen and caffeine). Mobile phase composition is varied by adjusting the ratio of methanol/buffer until the proper isocratic conditions are obtained. Once separated, the analyte components are detected by a UV absorption detector at 254 nm. Peak height ratios are calculated by comparing the peak heights of several standards and a real sample with peak heights of the internal standard. Analytical curves are plotted for aspirin and caffeine and are used to obtain the unknown values. These experimental quantitative values are compared to the corresponding weight of component per tablet values on the label of the commercial product.

The chromatographic conditions described in this experiment yield high efficiency (narrow) and well resolved peaks for the four component sample mixture in less than 10 min. This is a good example of how useful modern reverse phase LC is in the separation of polar organic compounds. The quantitative procedure employed in this experiment not only demonstrates the principle and value of the internal standard method but also illustrates quite vividly the idea of slope sensitivity.

Materials and Methods

HPLC equipment requirements include isocratic pumping at 1–2 mL/min with up to 200 atm of back pressure, 1 μL or

10 μL injection volume and UV absorbance detection at 254 nm using either 0.08 AUFS or 0.8 AUFS sensitivity (depending on injection volume). Also required is a high efficiency (microparticulate) prepacked reverse phase column capable of resolving the sample components (see Discussion section on the use of various columns).

We used a Model 5000 (Varian, Palo Alto, CA 94303) liquid chromatograph equipped with a Model U6K (Waters Associates Inc., Milford, MA 01757) injector and a Model 502 (Waters Associates Inc.) absorbance detector. The reverse phase column we used in the analysis was a Zorbax C_8 (25 cm × 4.6 mm i.d.) (Dupont Co., Wilmington, DE 19898). Other columns tested were a μBondapak C_{18} (30 cm × 4 mm i.d.) (Waters Associate Inc.) and a Ultrasphere-Octyl (25 cm × 4.6 mm i.d.) (Alex Scientific Inc., Berkeley, CA 94710).

The column was eluted with methanol/1% acetic acid (40/60 by vol) at a flow rate of 1 mL/min and ambient temperature. The column effluent was monitored at 254 nm and with a 1 μL injection volume a detector sensitivity of 0.08 AUFS was appropriate.

The mobile phase components, methanol (UV grade, Burdick and Jackson, Muskegon, MI 49442) and buffer (1%, by vol, acetic acid) Super-Q water (Millipore Corp., El Paso, TX 79998) were filtered/degassed using a 0.45 μm filter (Millipore). The standards, acetylsalicylic acid, caffeine, 4-acetamidophenol (acetaminophen), and salicylic acid were purchased from Aldrich Chemical Co., Milwaukee, WI 53201. The sample in this experiment is Vanquish® (Glenbrook Laboratories, Div. of Sterling Drug Inc., New York, NY 10016).

Student Experimental Procedure

The first part of this experiment consists of the students preparing the standard solutions and the tablet extract for HPLC analysis. This preparative portion should take about an hour to complete, after which the instructor should demonstrate the HPLC equipment. The instructor may well choose to perform all the injections personally since certain HPLC injection systems are delicate. Students may work in small groups and the entire experiment can be completed by a group in approximately 3 hr.

Preparation of Standard Solutions

Rinse four clean 250-mL Erlenmeyer flasks with methanol and label them No. 1, 2, 3, and I.S. To flask No. 1 add 200 mg aspirin and 20 mg caffeine. Add 250 mg aspirin and 40 mg caffeine to flask No. 2 and 300 mg aspirin and 60 mg caffeine to flask No. 3. Add as accurately as possible, using a graduated cylinder, 100 mL methanol to each flask and swirl to dissolve.

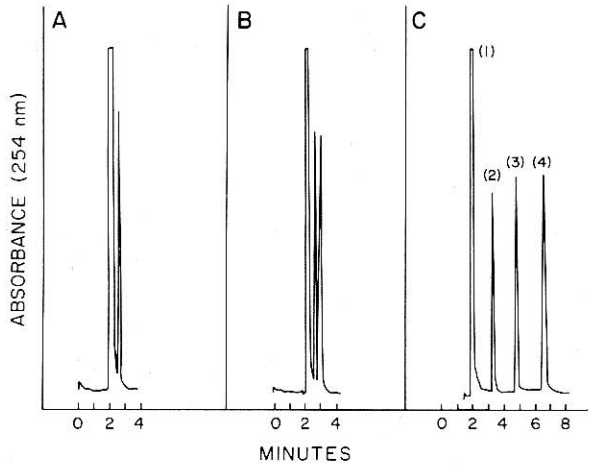

Figure 2. Relationship between mobile phase composition and retention and resolution of acetaminophen (1), caffeine (2), aspirin (3), and internal standard, salicylic acid (4). A = 80% methanol/buffer B = 60% methanol/buffer and C = 40% methanol/buffer.

Preparation of Internal Standard Solution

To flask "I.S." add 3 g salicylic acid and 100 mL methanol and swirl to dissolve.

Sample Preparation

Rinse a clean 250-mL Erlenmeyer flask with methanol and dry in an oven. Label "V". Put one Vanquish® tablet in flask "V" and crush it with a clean glass stirring rod. Add 100 mL methanol and finish crushing chunks as small as possible.

Addition of the Internal Standard

Add 10 mL, *via* pipet, of solution "I.S." to solutions No. 1, 2, 3, and "V". Add a magnetic stirring bar to solution "V" and stir for 5 min. Gravity filter about 1 mL of solution "V" through qualitative 2 filter paper to yield the final sample.

HPLC Analysis

Beginning with a mobile phase composition of 80% methanol/20% buffer and a flow rate of 1.5 mL/min, inject an aliquot (1 μL or 10 μL) of solution "V". Obtain sample component resolution by going to first 60% then 40% methanol. At 40% methanol/60% buffer (1% acetic acid), or at optimum conditions, inject samples No. 1, 2, 3, and "V".

Quantitation

Construct a standard curve for aspirin and caffeine showing peak height (standard of interest)/peak height (internal standard) *versus* mg (standard of interest)/tablet. From this calibration curve, report mg/tablet aspirin and caffeine for the Vanquish® tablet.

Results

Figure 2 illustrates the change in retention and resolution expected from a 20% change in organic modifier concentration. We found the optimum concentration to be about 40% MeOH/60% buffer; hence, all subsequent chromatograms were obtained with this mobile phase composition. The optimized chromatogram obtained from the Vanquish® tablet extract is shown in Figure 2C. Due to the high molar absorptivity at 254 nm and the early elution time of acetaminophen, this particular peak usually occurs off scale on an analog strip chart recorder. However, the peaks for the components of interest are well resolved and are approximately the same size as the internal standard peak.

The three point curves obtained for milligrams aspirin and caffeine per tablet *versus* peak height ratio are quite sufficient to produce accurate results since the calibration points are close to the expected sample values. Typical standard curves (Fig. 3) show a coefficient of variance of 0.999 for both aspirin and caffeine.

The actual instrumental analysis class which performed this experiment had six students; a three student group ran the experiment one afternoon,the other group of three later that

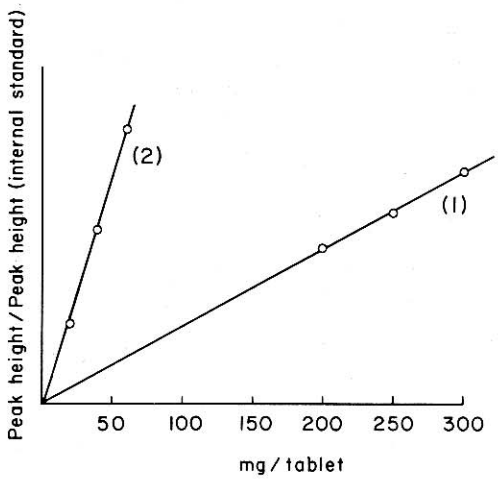

Figure 3. Typical standard curve obtained by students for aspirin (1) and caffeine (2) at 254 nm.

Table 1. Comparison of Student Results with Manufacturer's mg/Tablet. (Values from Packaging Label.)

	Aspirin	Caffeine
Vanquish® Label	227 mg	33 mg
Group I (average)	222 mg	32.6 mg
Student 1	226 mg	32.2 mg
Student 2	225 mg	32.7 mg
Student 3	215 mg	32.0 mg
Group II (average)	232 mg	34.1 mg
Student 1	240 mg	34.0 mg
Student 2	240 mg	33.9 mg
Student 3	217 mg	34.4 mg

week. Each group prepared a set of standards and a sample, and shared the data for their individual lab reports. As is apparent from Table 1, the overall quantitative agreement between the experimental data and the corresponding component values on the manufacturer's label is excellent. Small differences between these comparative values are expected because of small tablet-to-tablet variations.

Discussion

For this experiment to be a guaranteed success, the instructor should first verify that the HPLC system is operating correctly by obtaining a chromatogram of the manufacturer's column test mixture and by comparing it to the chromatogram received with the column. Next, the appropriate injection volume and detector sensitivity must be selected. We found either 1 μL injection volume for 0.08 AUFS or 10 μL for 0.8 AUFS worked well. As usual, the entire experiment should be given a trial run to verify proper column selectivity and detector sensitivity. We have found aspirin to decompose rather quickly in solution and, therefore, all solutions must be used soon after they are prepared. For example, the aspirin in a Vanquish® tablet extract, after storage for one month, gave only 15% of its original peak height ratio.

Undoubtedly, every laboratory does not have a Zorbax C_8 column and consequently many instructors would rather use a reverse phase column they already have in the laboratory. Of the three different columns we tried, the Dupont Zorbax C_8 was found to be the best for this particular separation. The other two columns we tried were the Waters μBondapak C_{18} and the Altex Ultrasphere Octyl. All three columns had similar retention relative to mobile phase strength, *i.e.*, all compounds eluted between $k' = 2$ to 10 at around 45% methanol/55% buffer. For these compounds, the μBondapak C_{18} and Ultrasphere Octyl columns showed very similar efficiency whereas the Zorbax C_8 had a significantly greater number of plates for all four compounds (see Table 2). The greatest difference between the three columns is seen, however, when one compares the selectivity (α) between the four components. Basically what is outlined in Table 2 is that for caffeine and aspirin, and aspirin and salicylic acid, the selectivity of the Waters column is just sufficient to give approximate baseline resolution. The Dupont column's selectivity is ideal (see Fig. 1-C) and the Altex column has almost too much selectivity to allow complete resolution of the first two components ($\alpha = 1.2$) without having excessive retention of the last component ($k' = 8.4$). Although the Waters and Altex columns gave inferior separations compared to the Dupont column, either could be used with the sacrifice of some resolution. The separations are completely adequate to perform accurate quantitation and undoubtedly, there are other reverse phase LC columns which would be satisfactory. Once again, it is important to verify that the column is performing according to manufacturers specifications before evaluating a particular column for its ability to separate the components in a Vanquish® tablet.

The analgesic tablets which will work in this experiment can contain acetaminophen, caffeine, aspirin, and buffer excipients (aluminum hydroxide, magnesium hydroxide). Therefore, Vanquish®, Bufferin® (aspirin and buffer), Anacin® (aspirin and caffeine), Bayer® (aspirin), and Tylenol® (acetaminophen) tablets are all satisfactory. Tablets which contain salicylamide (e.g., Excedrin® and Excedrin P.M.®) are not recommended, at least not with a Zorbax C_8 column because of coelution of caffeine and salicylamide.

Table 2. Efficiency (N), Capacity Factor (k'), and Selectivity (α) of Analgesic Tablet Components on Three Different Columns. (Separations are Optimized.)

	Acetaminophen		Caffeine		Aspirin		Salicylic Acid
Waters C-18 (45% MeOH)							
N	980		4,500		7,000		2,570
k'	4.0		5.6		6.3		7.7
α		1.4		1.3		1.4	
Dupont C-8 (40% MeOH)							
N	1,950		11,890		14,030		9,220
k'	3.3		6.1		7.7		10.7
α		1.9		1.3		1.4	
Altex C-8 (45% MeOH)							
N	970		5,780		7,950		3,020
k'	2.2		2.6		5.2		8.4
α		1.2		2.0		1.6	

Rather than a simple introduction to modern LC, this experiment might also be used as either the first part of a more extensive two-week experiment or as a training session for a prospective user of HPLC. Subsequent experiments toward these latter two goals are numerous. One obvious experiment would be to have the students obtain information similar to that in Table 2 by using several different reverse phase columns. The fact that various commercial LC columns display different selectivity is a great advantage to the practicing liquid chromatographer since this fact gives him/her a simple way to vary the separation. These additional experiments should also be designed to give the student an introduction to column fittings and tubing in addition to column care and installation. Substituting acetonitrile, tetrahydrofuran, or isopropanol for methanol in the mobile phase would be a simple experiment illustrating the effect of various organic modifiers and also demonstrating how LC solvents are prepared and changed in the instrument. Since the wavelength absorption profiles for the four components are quite different in the range 230 nm to 330 nm, several interesting experiments dealing with detector selectivity and sensitivity are possible if a variable or multiple wavelength detector is available.

Conclusions

Student interest in this experiment was very high with many mentioning weeks later how much they "got out of" the experiment. They all agreed that it was the "best" experiment of the course and several said they enjoyed being able to compare their results with the values on the product label. Students are always encouraged by experiments which work well and give them correct answers. Although this approach gives the student an unrealistic view of analysis, it is more important in an initial LC experiment to encourage learning than to discourage it by reality.

This experiment is simple to prepare, requires little glassware, minimal sample manipulation by the students, and applies modern HPLC to a determination for which it is well suited, pharmaceutical product analysis.

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