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# Determination of Aspartame, Caffeine, Saccharin, and Benzoic Acid in Beverages by High Performance Liquid Chromatography

## An Undergraduate Analytical Chemistry Experiment

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In recent years several articles have been published in *THIS JOURNAL* describing undergraduate laboratory experiments based on liquid chromatography (LC). These have spanned a considerable range, from classical column chromatography (1) to the modern technique of high performance liquid chromatography (2). The experiments have been directed at organic (3), biochemistry (4), and physical-analytical (5) courses. Publications have described inexpensive LC equipment (6) including gradient controllers (7), as well as the simulation of chromatography on microcomputers (8). A recent report in *THIS JOURNAL* described a simple introductory LC experiment which can be performed with nontoxic solvents and samples using prepackaged, disposable cartridges packed with silica or chemically modified silica (9). One manufacturer of LC instrumentation has also prepared a useful compendium of introductory LC experiments for the university teaching laboratory (10).

A variety of sample types have been used for these experiments, including the biologically important Vitamin A (11), commercial analgesics (12, 13), food dyes (9, 14), and the toxic nitroanilines (15). The determination of caffeine in beverages has been accomplished in various ways, with the most popular methods being those which involve the use of reversed-phase liquid chromatography (16–20). These methods have been used to determine caffeine levels in coffee (16, 17, 19), tea (18), and soft drinks (19, 20), with most methods including the simultaneous separation and analysis of saccharin and sodium benzoate, with minimal sample preparation required.

In this paper, we describe a simple and reliable new quantitative analysis experiment using liquid chromatography for the determination of caffeine, saccharin, and sodium benzoate in beverages. In addition, the experimental conditions can be modified slightly to allow the simultaneous analysis of aspartame, which is increasingly being used to sweeten dietary beverages. Both separations can be applied to the analysis of soft drinks, artificial sweeteners, fruit juices, and coffee. Since these commercial products pervade undergraduate life, they seem ideally suited for study in the laboratory. With the recent introduction of a number of caffeine-free soft drinks, the experiment has taken on new dimensions. The experiment described below is based on methods previously developed (21, 22) for possible use in quality control monitoring of soft drinks. A related report describes a method for the determination of aspartame from breakfast cereals (23).

### Experimental

#### Apparatus

The chromatographic system was a Waters Associates, Inc. (Milford, MA) Model 204 ALC which included a WISP automatic injector, a Model 6000A solvent delivery system, and a Model 440 absorbance detector operating at 254 nm. Automated runs were directed by a

Model 720 System Controller (Waters). The analog outputs of the UV absorbance detector were recorded with a Model 730 Data Module (printer, plotter, integrator) (Waters). The automated components are clearly not necessary for use in a teaching laboratory. A simpler LC instrument consisting of a solvent delivery system, UV absorbance detector, manual injector (Model U6K, Waters) and chart recorder (Model SE 120, Waters) can be used very effectively for this separation. In addition, an integrated LC instrument (Model 340-QA-1 Analyzer, Waters) has been used to perform this experiment with excellent results (21–23).

A  $\mu$ BONDAPAK C18 Radial-PAK cartridge (8-mm i.d.  $\times$  10 cm) (Waters) contained in a Z-Module radial compression separation system (Waters) was used at ambient temperature. The column plate count was measured according to the instructions supplied in the manufacturer's care and use manual.

#### Reagents

All water used was distilled and then processed through a water purification system (Milli-Q, Millipore, Bedford, MA). The acetonitrile was HPLC grade (Waters). The caffeine (Eastman USP), sodium saccharin (Mallinckrodt USP), benzoic acid (Fisher ACS), ammonium acetate (Fisher ACS), aspartame (Sigma), and glacial acetic acid (Mallinckrodt AR) were used as received.

#### Chromatographic Eluants

The eluant used for the isocratic separation of caffeine, saccharin, and benzoic acid consisted of 7% (v/v) acetonitrile and 93% of an aqueous solution containing 0.5% (w/v) ammonium acetate and 2% (v/v) glacial acetic acid. For the simultaneous separation of aspartame from these three components, an eluant containing 15% (v/v) acetonitrile and 85% of the same aqueous component was found to be effective (21).

#### Standard Solutions

A mixed standard solution was prepared by dissolving about 40 mg of each standard in the eluant to yield a 100-mL solution. For analyses involving aspartame, a concentration of about 2 mg/mL in the standard mixture was used.

#### Procedure

The detector, operating at 254 nm, was typically set to 0.1 AUFS. A flow rate of 4 mL/min was used for all separations. At the start of each laboratory period, the column was equilibrated with 25–50 mL of eluant, until a stable baseline was obtained. 20  $\mu$ L injections of the standard solution of caffeine, saccharin and benzoic acid (and four serial dilutions) were used in preparing the calibration lines.

For the analysis of beverages, each sample was degassed in an ultrasonic bath and 7-mL volumes of each were diluted to 10 mL with acetonitrile. This dilution to 30% acetonitrile was found to work reliably, with no apparent problem of early elution of any of the standards. Each solution was filtered through a Millex SR filter (Millipore, Bedford, MA) prior to injection. It should be emphasized that filtration of the samples is important for insuring the longest possible column lifetime. In addition to sample filtration, we typically include an in-line 2  $\mu$ m filter (Waters) between the injector and column for all of our work.

## Results and Discussion

We have used the LC separation of beverage components for several years as an experiment for a quantitative analysis course as well as a freshman-level 6-year-medical-student course. Portions of the data presented in this paper were, in fact, generated by two of the authors (DSG and PCK) who were summer high school students. Until the past year, however, the procedure we have used (20) involved a 30-min separation in which saccharin and caffeine eluted early with only marginal resolution, while the retention of benzoic acid was excessive and led to a long overall analysis time.

Due to time and equipment limitations it is usually necessary for us to divide the students into groups of two or three. Each student is required to analyze independently and report their own data. Switching to the present chromatographic conditions allows reduction of the analysis time to 10 min with the consequent advantage that a single isocratic instrument can be used more effectively in the teaching lab. The improvement offered by the present separation is due both to the use of an optimized eluant and to the versatility of the radial compression system for operation at high eluant flow rates (24).

### Separation

A chromatogram for the three-component mixed standard is shown in Figure 1A and for a typical soft drink sample in Figure 1B. A separation which offers good resolution of all three components is obtained in under 10 min. A number of unidentified components of the soft drink sample were observed to elute near the void volume, but these were adequately resolved from the saccharin peak so that no interference with the quantitation of this component was observed.

Figure 2 shows a chromatogram for the separation of a standard mixture containing aspartame in addition to saccharin, caffeine and benzoic acid. Base-line resolution is obtained for all components in under 8 min.

### Sensitivity and Linearity

The responses for saccharin, caffeine, and sodium benzoate showed excellent linearity ( $R = 0.9999$  or better) over approximately three decades of concentration (50 ng to 10  $\mu$ g injected for each component). Based on the noise observed for a high detector sensitivity the detection limit for each compound was estimated to be 5 ng. In a 20- $\mu$ L sample volume, this corresponds to a concentration of about 250 ppb in the original samples. Larger sample volumes could have been used to obtain lower detection limits.

### Analysis of Soft Drink Samples

The caffeine content measured in nine soft drinks is displayed in a bargraph (Fig. 3). The levels found are in agreement with expectation in that the three caffeine-free beverages showed no measurable amount of caffeine, while the other beverages were found to contain somewhat less than the level specified on the label. Since label claims are generally upper limits (25), this finding is not surprising. No significant interferences were seen in any of the chromatograms for the soft drinks listed in Figure 3.

## Conclusion

The current procedure is preferred over the method we previously used (20) since it gives a smoother base line, better resolution and greatly improved peak shape. This procedure is also more reliable for an undergraduate teaching laboratory since we occasionally had problems with the resolution of saccharin and caffeine when using the previous method. The experiment has been well received by our students because the experiment works every time and because we give the students flexibility to choose their own samples and to formulate a specific project for study.

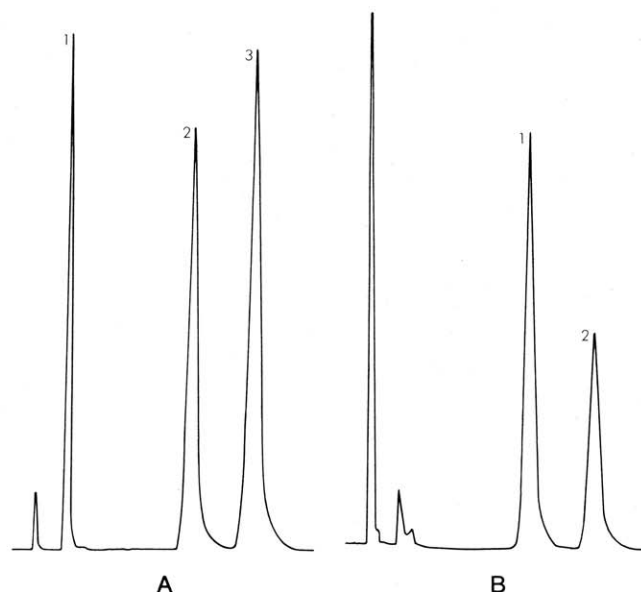


Figure 1. A, separation of the three standards: 1) saccharin, 2) caffeine, 3) benzoic acid. B, separation of a Mountain Dew® soft drink sample: 1) caffeine, 2) benzoic acid.

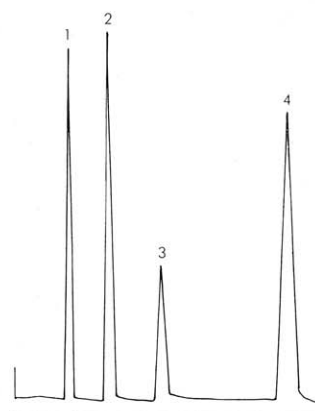


Figure 2. Separation of the four standards: 1) saccharin, 2) caffeine, 3) aspartame, 4) benzoic acid.

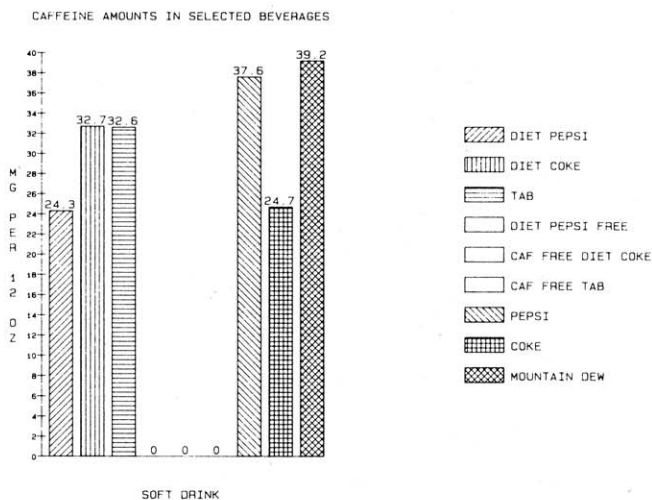


Figure 3. Bar graph presentation of the results of caffeine determinations for selected soft drinks. Concentrations reported are in units of mg/12 oz. can.

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## Literature Cited

- (1) Rubinson, J. F., and Rubinson, K. A., *J. CHEM. EDUC.*, **57**, 909 (1980).
- (2) Kagel, R. A., and Farwell, S. O., *J. CHEM. EDUC.*, **60**, 163 (1983).
- (3) Sacks, J., Greenley, E., Leo, G., Willey, P., Gallis, D., and Mangravite, J. A., *J. CHEM. EDUC.*, **60**, 434 (1983).
- (4) Jackman, L. E., *J. CHEM. EDUC.*, **58**, 77 (1981).
- (5) Lochmuller, C. H., Wilder, D. R., and Gutknecht, W. F., *J. CHEM. EDUC.*, **57**, 381 (1980).
- (6) McCamish, M., Canell, G. R., and Bretherton, L., *J. CHEM. EDUC.*, **59**, 249 (1982).
- (7) Brady, J. E., and Carr, P. W., *J. CHEM. EDUC.*, **60**, 83 (1983).
- (8) DiNunzio, J. E., *J. CHEM. EDUC.*, **59**, 778 (1982).
- (9) Bidlingmeyer, B. A., and Warren, F. V., *J. CHEM. EDUC.*, **61**, 716 (1984).
- (10) WALCEP, Waters Associates, Milford, MA 01757.
- (11) Bohman, O., Engdahl, K.-A., and Johnsson, H., *J. CHEM. EDUC.*, **59**, 251 (1982).
- (12) Haddad, P., Hutchings, S., and Tuffy, M., *J. CHEM. EDUC.*, **60**, 166 (1983).
- (13) Byrd, J. E., *J. CHEM. EDUC.*, **56**, 809 (1979).
- (14) McKone, H. T., and Ivie, K., *J. CHEM. EDUC.*, **57**, 321 (1980).
- (15) Cantwell, F. F., and Brown, D. W., *J. CHEM. EDUC.*, **58**, 820 (1981).
- (16) Madison, B. L., Kozarek, W. J., and Damo, C. P., *J. Assoc. Offic. Anal. Chem.*, **59**, 1258 (1976).
- (17) Attina, M., and Ciranni, G., *Farm. Ed. Prac.*, **31**, 650 (1976), through *Anal. Abs.*, **33**, 1F36 (1977).
- (18) Hoeffler, A. C., and Coggon, P., *J. Chrom.*, **129**, 460 (1976).
- (19) Woodward, B. B., Heffelfinger, G. P., and Ruggles, D. I., *J. Assoc. Offic. Anal. Chem.*, **62**, 1011 (1979).
- (20) Smyly, D. S., Woodward, B. B., and Conrad, E. C., *J. Assoc. Offic. Anal. Chem.*, **59**, 14 (1976).
- (21) Waters QA-1 Analyzer Application Brief No. 6004, Waters Associates, Milford, MA 01757.
- (22) Waters QA-1 Analyzer Application Brief No. 6010, Waters Associates, Milford, MA 01757.
- (23) Waters QA-1 Analyzer Application Brief No. 6005, Waters Associates, Milford, MA 01757.
- (24) Hillier, E. J., and Tisdale, V., paper presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Atlantic City, NJ, (no. 776), 1982.
- (25) Consumer Information Center Pamphlet, Coca-Cola USA, July, 1983.