Environmentally Friendly Method for the Analysis of Caffeine and Benzoic Acid in Soft Drinks

Abstract

A simple analysis of caffeine and benzoic acid in soft drinks using the compact Axcend Focus LC® is demonstrated. Linear regression of the calibration data gave R^2 values of 0.9993 and 0.9998 for caffeine and benzoic acid, respectively. Analysis of a commercial soft drink gave 228 ppm caffeine (RSD 1.1%) and 184 ppm benzoic acid (RSD 1.8%). Solvent usage was estimated to be approximately 28 µL per analysis.

Introduction

The determination of benzoic acid and caffeine in soft drinks is routinely done to ensure that products are safe and meet label claims as well as health and safety requirements as set by the FDA. The average caffeine content in caffeinated soft drinks is approximately 100-300 ppm, while energy drinks can contain over 600 ppm. Caffeine-free drinks are guaranteed to contain no caffeine. The ability to rapidly analyze caffeine in drinks using a small portable instrument with minimal mobile phase solvent (~ 28 µL) consisting of relatively harmless components (i.e., water, methanol, and a little phosphoric acid) is significant.

Materials and Methods

Preparation of Standard Calibration Solutions

HPLC grade water (Millipore-Sigma, #270733) was used for standard preparation. Caffeine was purchased from Spectrum Chemical, and sodium benzoate was from Sigma-Aldrich (#109169). A standard solution was prepared by dissolving caffeine in HPLC grade water and diluting to a concentration of 10 mg/mL. Likewise, a standard solution of 10 mg/mL of sodium benzoate in water was also prepared (equivalent to 8.47 mg/mL benzoic acid). A stock solution (1000 ppm each) of caffeine and benzoic acid was then prepared by combining 0.300 mL of the caffeine standard solution with 0.354 mL of the sodium benzoate standard solution and diluting to 3.00 mL with 1% H_3PO_4

(Sigma-Aldrich, #49685) in water. The resultant mixture was subsequently diluted to prepare a series of 10, 50, 100, 150, 200, and 250 ppm standard calibration solutions.

Preparation of Soft Drink Samples

Before chromatographic analysis, carbonated samples were degassed by sonicating for approximately 5 min and then filtered through a syringe filter (Kinesis KX Syringe Filter, Regenerated Cellulose, 13 mm dia., 0.22 µm pore size). Each sample was loaded into the injection loop with a 25-µL syringe (Hamilton #1702SN 22'S/2"/AS) after rinsing 3 or 4 times with HPLC grade water.

HPLC Analysis

HPLC analysis was accomplished using an Axcend Focus LC equipped with a cartridge containing a standard 10 cm x 0.150 mm i.d. capillary column packed with 1.7 µm dp C18 stationary phase, an on-column UV-absorption detector with 255 nm LED light source, and an injection valve with 200 nL loop. The binary mobile phase components were (A) 3% methanol + 0.1% formic acid in water and (B) methanol with 0.1% formic acid. The pumps were operated at a combined flow rate of 1.7 µL/min. A 4-min equilibration time at the beginning of each analysis was applied once the instrument reached 5,000 psi during an initial pressurization step. The solvent program was 3% B during the 4-min equilibration followed by a linear gradient to 96% B in 6 min (held constant for another 2 min). A summary of the HPLC analysis conditions is given in Table 1.

Flow Rate	1.7 µL/min
Equilibration Time	4 min
Gradient	3% B to 96% B in 6 min, and held constant for 2 min
Mobile phase A	3% methanol and 0.1 % formic acid in water
Mobile phase B	Methanol with 0.1% formic acid
Column	10 cm x 0.150 mm i.d. packed with 1.7 µm dp CoAnn C18
Detection	UV-absorption at 255 nm
Injection Volume	200 nL

Table 1. Summary of HPLC Analysis Conditions

Results

Calibration Curves

Figure 1 shows a chromatogram of the 50-ppm caffeine and benzoic acid standard calibration solution. Under the analysis conditions, caffeine and benzoic acid eluted at 5.1 and 6.6 min, respectively. The rise in baseline at the end of the chromatogram resulted from a short rinse with 96% acetonitrile to remove any residual compounds in the chromatographic system. Figure 2 shows calibration plots for both caffeine and benzoic acid from HPLC analyses of the standard calibration solutions. Linear regression of the calibration data gave R^2 values of 0.9993 and 0.9998 for caffeine and benzoic acid, respectively.

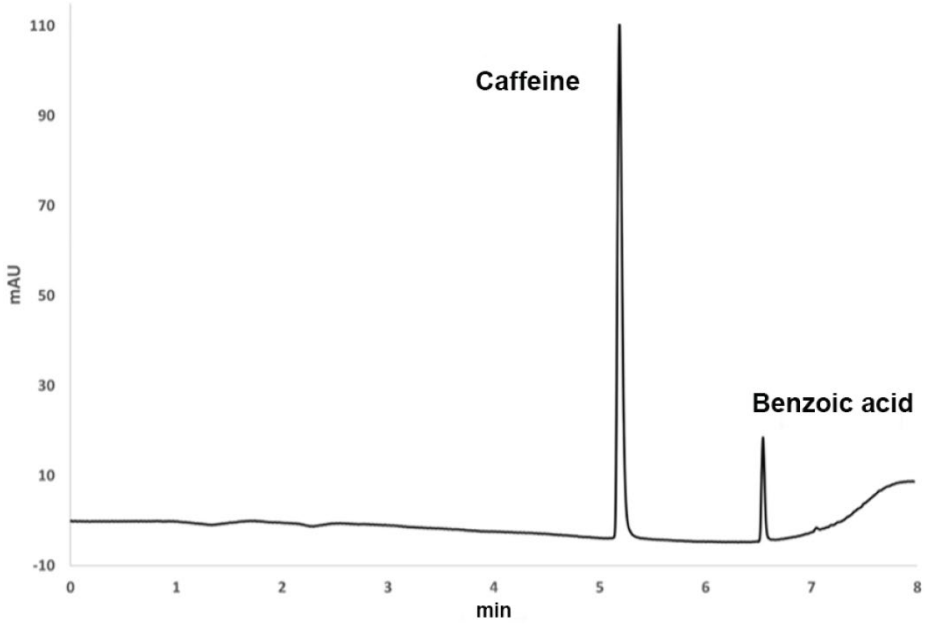

Figure 1. Chromatogram of calibration standard solution containing 50 ppm each caffeine and benzoic acid.

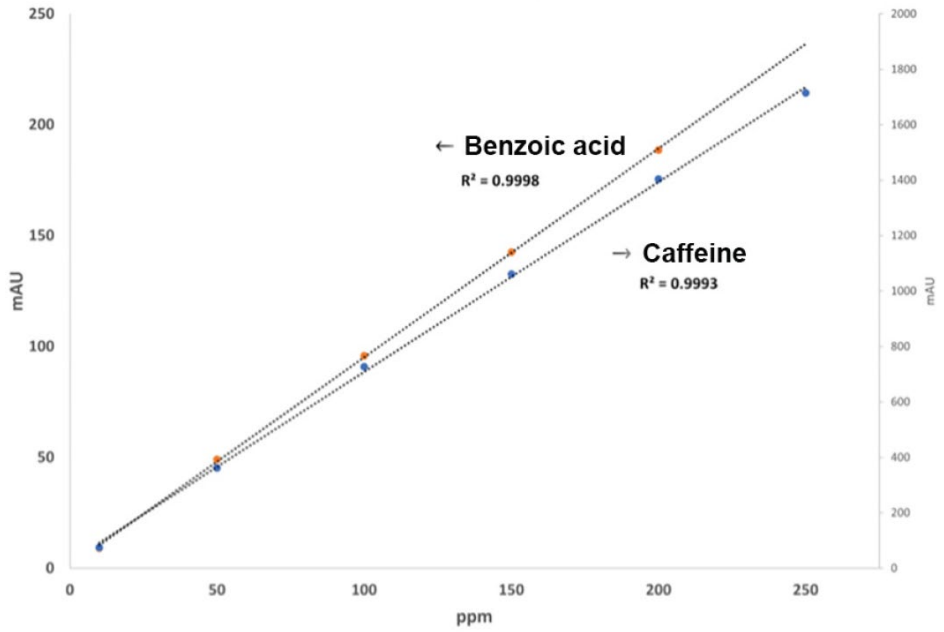

Figure 2. Calibration plots using chromatographic data obtained from analysis of caffeine and benzoic acid calibration solutions.

Analysis of Commercial Soft Drink

Table 2 lists the experimental data obtained from 6 repeat analyses. Figure 3 shows a chromatogram obtained from one of these runs of the commercial soft drink sample.

	Caffeine		Benzoic acid	
	Ret. time (min)	*Area counts*	*Ret. time (min)*	*Area counts*
Run 1	5.07	1574.5	6.61	171.8
Run 2	4.96	1596.5	6.64	170.3
Run 3	4.91	1607.0	6.65	172.6
Run 4	5.22	1562.6	6.58	178.5
Run 5	4.94	1604.4	6.65	174.3
Run 6	5.22	1579.0	6.61	177.0
Average	5.05	1587.3	6.63	174.1
Std dev	0.14	17.9	0.03	3.2
% RSD	2.8	1.1	0.4	1.8

Table 2. Results obtained from six repeat analyses of a commercial soft drink.

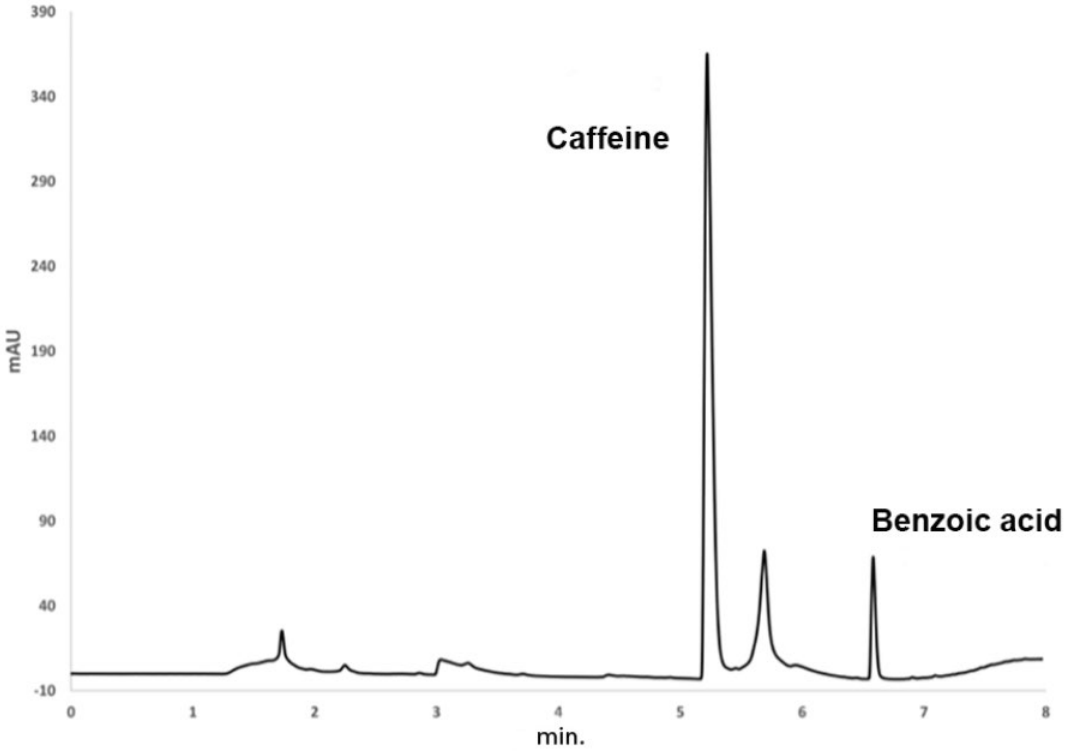

Figure 3. Chromatogram obtained from HPLC analyses of a commercial soft drink.

Conclusions

The portable, compact Axcend Focus LC can easily analyze commercial soft drinks for caffeine and benzoic acid with excellent accuracy, linearity, and repeatability. Analysis costs are significantly reduced because of the very small volumes of mobile phase solvents required for each analysis. The Axcend Focus LC can likewise be applied to the analysis of samples from a wide range of industries including chemical manufacturing, consumer products, cosmetics, foods, beverages, pharmaceuticals, environmental contaminants, petrochemicals, and many others, often with analysis facilitated by the small size of the instrument.