APPLICATION NOTE

Liquid Chromatography

AUTHORS

Jason Weisenseel, PhD
PerkinElmer, Inc., Johns Creek, GA, USA

Jamie Foss
PerkinElmer, Inc., Shelton, CT, USA

A Fast, Simple and Green Chemistry Method for the Analysis of Additives in Diet Soft Drinks by HPLC

Introduction

Soft drink products serve as a major revenue stream for food and beverage manufacturers, with a global market size of USD 237 billion in 2021.[1] Due to the popularity and wide consumption of these products, product consistency, which drives customer satisfaction, is of the utmost importance. For quality control purposes and to ensure product consistency, it is critical to analyze six major additives during production to ensure they fall within specified target concentration ranges. These six major additives (Figure 1) include three artificial sweeteners (acesulfame K, aspartame, saccharin), two preservatives (benzoate, sorbate), and a stimulant (caffeine).

This application note demonstrates a simple, fast, and robust green chemistry method for the accurate quantitation of these six additives in diet soft drinks using high performance liquid chromatography (HPLC) with photodiode array (PDA) detection. The method is ideal for users bottling soft drinks who are seeking an intuitive method that can be performed by users of varying skill levels, while also reducing hazardous solvent waste in support of social responsibility initiatives.

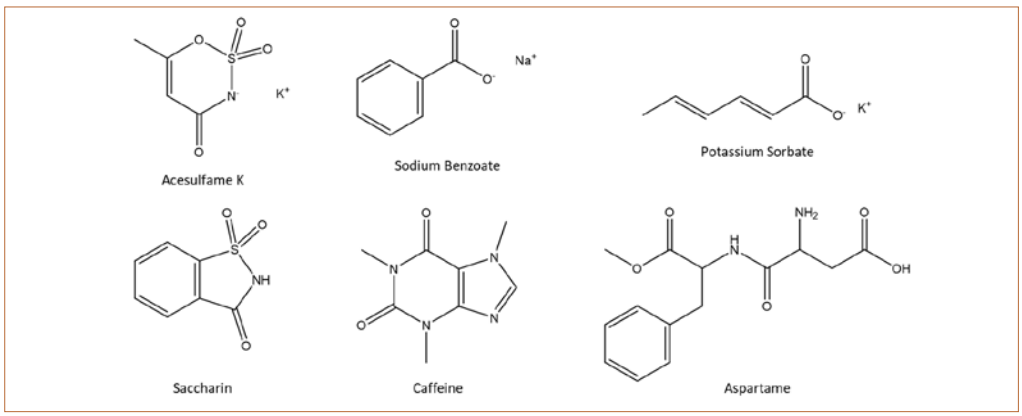

Figure 1. Chemical structures of the six beverage additives analyzed in this study.

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Experimental

Hardware/Software

Chromatographic separation was achieved using a PerkinElmer LC 300 HPLC system, consisting of an LC 300 HPLC Pump and an LC 300 HPLC Autosampler equipped with an integrated column oven. The autosampler was configured with a 100-µL syringe and 20-µL sample loop. Detection was achieved using an LC 300 Photodiode Array (PDA) detector. Instrument control, analysis and data processing were performed using the SimplicityChrom™ software platform.

Method Parameters

The LC parameters are shown in Table 1.

Table 1. LC Parameters.

Column	PerkinElmer Brownlee SPP C18, 2.7 µm, 3.0 x 75 mm (Part Number N9308409)
Guard Column	PerkinElmer Brownlee SPP C18, 2.7 µm, 3.0 x 5 mm (Part Number N9308514)
Mobile Phase	96% 10 mM Sodium Acetate pH 4.7 (±0.05), 4% Absolute Ethanol (Isocratic)
Flow Rate	1.2 mL/min
Analysis Time	6 minutes
Oven Temperature	40°C
Sample Temperature	Ambient
Injection Volume	3 µL (Partial loop)
PDA Wavelength	Analytical: 220 nm Bandwidth: 10 nm Reference: 400 nm Bandwidth: 20 nm
Data Collection Rate	5 pts/sec (Hz)
PDA Flow Cell	10 mm (standard)

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade. Unless otherwise specified, standard and sample dilutions were prepared using mobile phase.

Six standards were obtained from Sigma-Aldrich®, Inc (St. Louis, MO). These included: acesulfame K, saccharin, sodium benzoate, caffeine, potassium sorbate and aspartame.

A mixed stock standard solution was prepared by combining 0.0150 g of acesulfame K, 0.0100 g of saccharin, 0.0200 g of sodium benzoate, 0.0200 g of caffeine, 0.0200 g of potassium sorbate, and 0.0500 g of aspartame in a 100-mL volumetric flask containing ~50 mL of mobile phase. Approximately 40 mL of mobile phase was added, and the solution was sonicated until fully dissolved. The solution was then diluted to volume with mobile phase.

The mixed stock standard solution was diluted serially using mobile phase to create a 5-level calibration set covering the concentration ranges shown in Figure 3.

Three diet soft drink samples were purchased from a local grocery store and included two different diet colas and one diet lemon-lime soda.

For sample preparation, approximately 10 mL of the soft drink was placed into a 100-mL beaker and sonicated for 5 minutes to degas. The sample was then diluted 1:4 by transferring 300 µL to a 1.5-mL microcentrifuge tube, adding 900 µL of mobile phase diluent and vortexing. The sample was filtered using a 0.22-µm nylon syringe filter (Part Number 02542881) and 3 µL was injected for analysis.

Results and Discussion

The chromatogram of the Level 4 standard is shown in Figure 2, with all six analytes eluting in under six minutes, making the method suitable for high throughput production environments. This method provides baseline chromatographic resolution between all peaks.

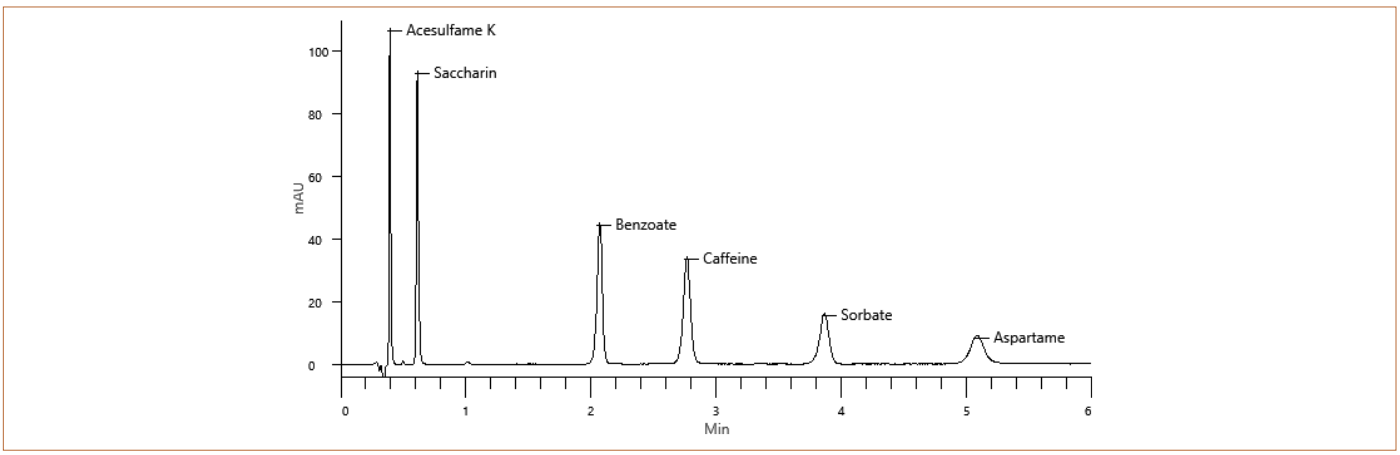

Figure 2. Chromatogram of the the Level 4 standard.

Figure 3 shows the 5-pt calibration curves for the six beverage additives. All analytes followed a linear (1st order) fit and had r^2 coefficients above 0.999 (n=3 at each level).

Using the same chromatographic conditions, three different diet soft drinks were analyzed. Chromatographic results for the three samples are shown in Figures 4, 5, and 6.

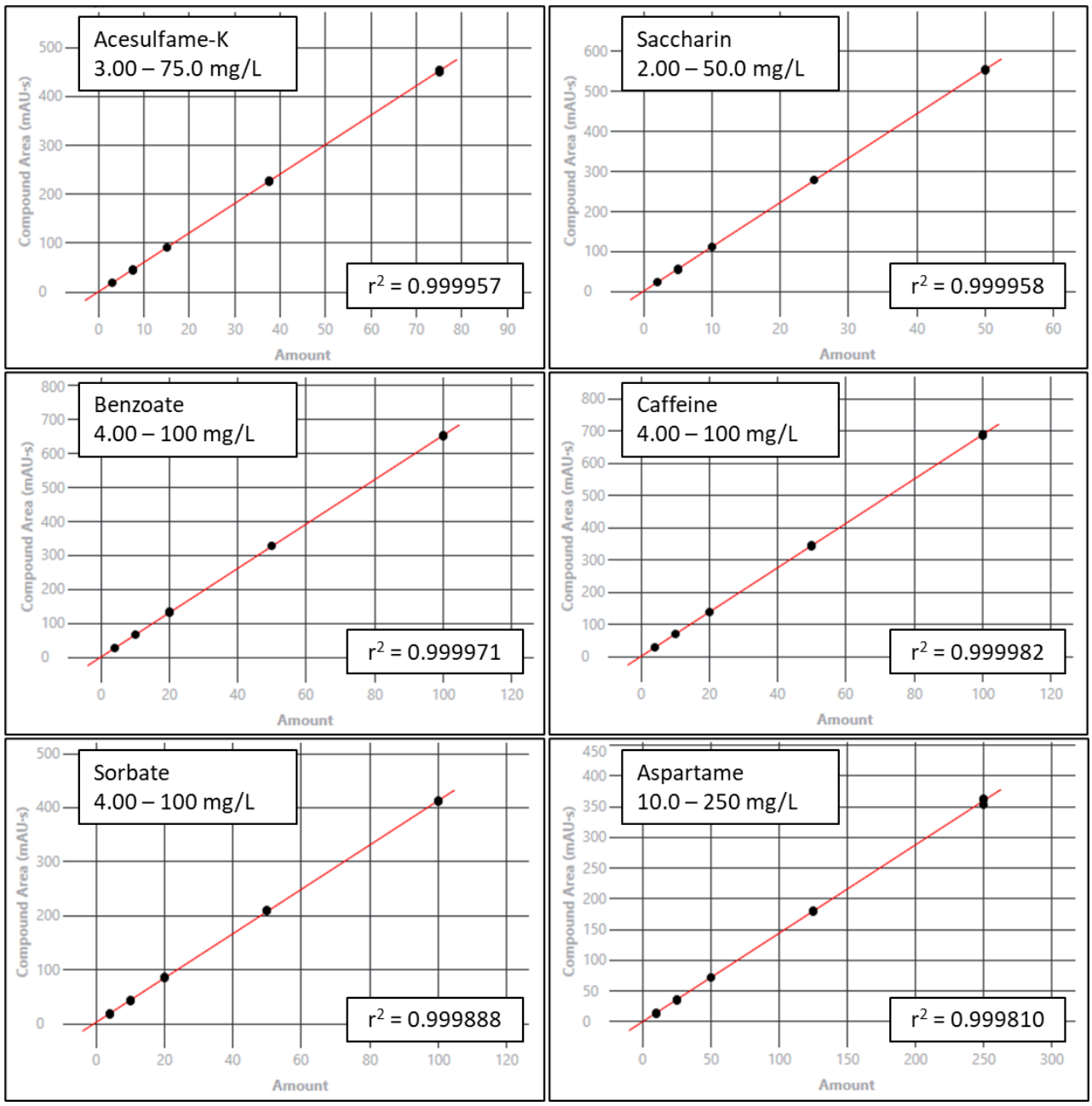

Figure 3. Calibration ranges and results of the 5-level calibration sets for the six additives.

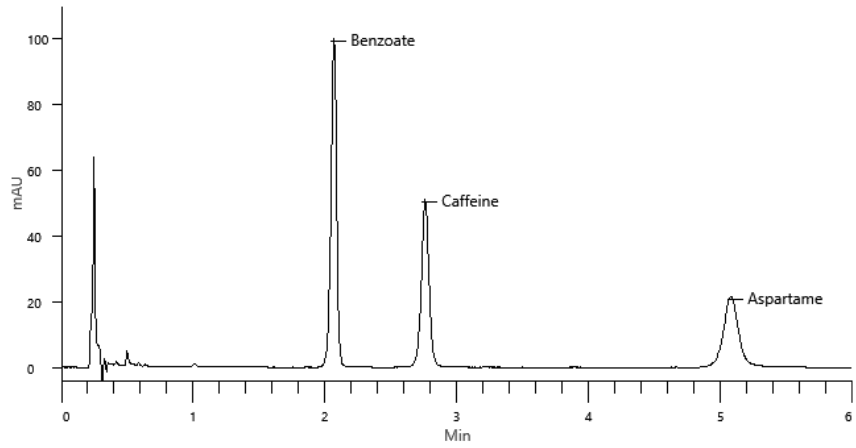

Figure 4. Chromatogram of diet cola A sample.

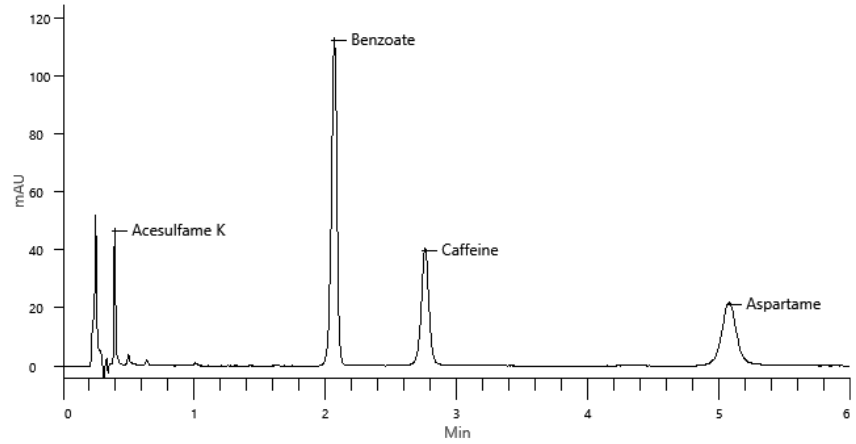

Figure 5. Chromatogram of diet cola B sample.

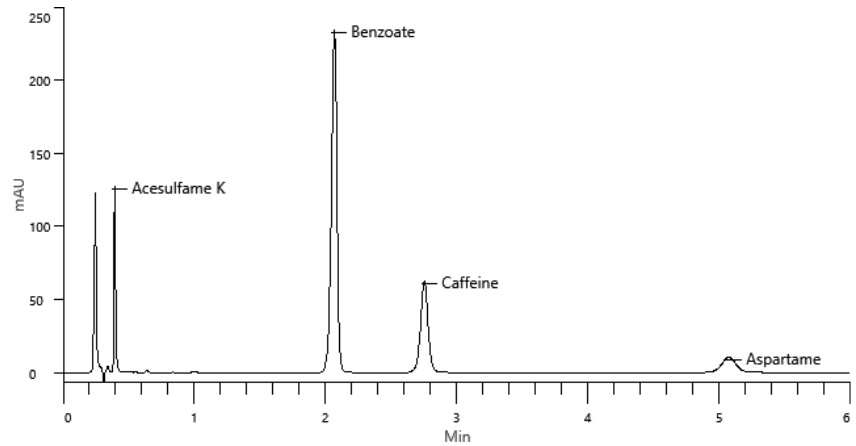

Figure 6. Chromatogram of diet lemon-lime soda sample.

Based on standard calibration, the quantitative results for each sample are shown in Table 2. While the specific amounts of ingredients are not listed on the ingredients label for the diet beverages, accurate labeling of ingredients is required. All analytes detected matched the list of ingredients for each sample.

Table 2. Amount (mg/L) and %RSD for the additives found in each diet soft drink. Sample results are based on the average of three analyses.

DIET COLA A		
Analyte	**Average Amount (mg/L)**	**%RSD**
Benzoate	180.64	0.25
Caffeine	119.75	0.59
Aspartame	481.86	0.45
DIET COLA B		
Analyte	**Average Amount (mg/L)**	**%RSD**
Acesulfame K	28.92	0.10
Benzoate	202.82	0.68
Caffeine	95.61	0.43
Aspartame	486.69	0.35
DIET LEMON-LIME SODA		
Analyte	**Average Amount (mg/L)**	**%RSD**
Acesulfame K	72.18	0.28
Benzoate	423.40	0.18
Caffeine	146.97	0.49
Aspartame	234.74	0.85

Although a UV/Vis detector can be used in place of the PDA detector, the PDA spectral processing capabilities increase the confidence in chromatographic peak identification. The two primary spectral processing tools for peak identification are peak purity index and spectral library confirmation. Peak purity index compares the upslope and downslope spectra of a peak at a defined % peak height.[2] While a peak purity index value of 1.0 indicates the spectra are exactly the same and thus the chromatographic peak pure, as a measure of practicality, a peak purity index value of 1.5 or less is taken to indicate a pure peak.[2,3] A peak purity index value greater than 1.5 indicates that two or more components with different spectra are present within the peak and the thus the peak is not pure. Spectral library confirmation compares the apex spectrum of a chromatographic peak to an identically named spectrum contained within a spectral library and returns a match value.[3] Library spectra are obtained from pure standards run under the same chromatographic conditions. A match value of 1000 is considered a perfect match, with a desirable match value being 950 or higher.

As part of the analysis of the soft drink samples, chromatographic peak identities were confirmed using the peak purity index and spectral library confirmation spectral processing tools. As can be seen in Table 3, peak purity index values were below 1.5 and spectral library confirmation match values were above 980 for all analytes.

Table 3. Example purity index and spectral library confirmation values obtained for the three samples analyzed.

DIET COLA A		
Analyte	**Purity Index (10% Peak Height)**	**Spectral Library Confirmation**
Benzoate	1.04	998
Caffeine	1.06	998
Aspartame	1.09	984
DIET COLA B		
Analyte	**Purity Index (10% Peak Height)**	**Spectral Library Confirmation**
Acesulfame K	1.36	990
Benzoate	1.02	998
Caffeine	1.09	998
Aspartame	1.11	983
DIET LEMON-LIME SODA		
Analyte	**Purity Index (10% Peak Height)**	**Spectral Library Confirmation**
Acesulfame K	1.06	999
Benzoate	1.01	998
Caffeine	1.04	998
Aspartame	1.13	985

Conclusion

This work has demonstrated the fast and robust chromatographic separation and quantitation of six additives commonly found in diet beverages, as a measure of product quality, using a PerkinElmer LC 300 HPLC system with PDA detection. The calibration curves demonstrate excellent linearity ($r^2 \geq 0.999$) across the full concentration ranges for each analyte. The described method was used to analyze three different diet soft drinks, two diet colas and one diet lemon-lime drink. Analytical results were consistent with the ingredients label for each sample. Minimal sample preparation and a fast 6-minute isocratic method, eliminating the need for column equilibration between runs, make this method suitable for high throughput production environments. The use of absolute ethanol as the organic modifier in the mobile phase, as opposed to hazardous solvents such as methanol or acetonitrile, provides a much greener analytical method that reduces the cost of solvent waste disposal. Spectral processing tools such as peak purity index and spectral library confirmation, available with PDA detection, can give extra confidence in chromatographic peak identification.

Although this work focuses on the analysis of finished soft drinks, the method can be easily adapted to the analysis of syrups, or concentrates, by further dilution of the syrup with mobile phase. This allows the method to be applied earlier in the production process at beverage bottling plants as a quality measure, ensuring target specifications are met prior to the bottling phase. Identifying a batch that does not meet specification prior to bottling can save the bottling plant thousands of dollars.

References

1. Grand View Research, Carbonated Soft Drink Market Size, Share & Trends Analysis Report (2021).
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3. *IRIS – HPLC Spectral Processing Software User Manual;* PerkinElmer: Shelton, CT, 2006.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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