

## Soft Drink Analysis on an ACQUITY Arc System

Mark Benvenuti and Jennifer Burgess  
Waters Corporation, Milford, MA, USA

### APPLICATION BENEFITS

Soft Drink analysis using the ACQUITY® Arc™ System, along with Waters® Beverage Mobile Phase, XBridge® Phenyl Column Chemistry, and a choice of Waters Beverage Standards, or Waters Big-4 Standard concentrate provides multiple benefits such as:

- Ethanol-based, ecologically friendly mobile phase.
- Choice of a single-point calibration suitable for lower levels of analytes common to most soft drinks, or a multi-point calibration especially suited to the higher levels of caffeine found in energy drinks when saccharin and aspartame are not required.
- Dual wavelength monitoring to avoid known co-eluting compounds.
- Use of 2.5 micron particle size columns at system pressures up to 9500 psi.
- Dual flow paths to emulate HPLC or UHPLC separations.

### WATERS SOLUTIONS

[ACQUITY Arc System](#)

[2489 UV/Visible \(UV/Vis\) Detector](#)

[ACQUITY QDa® Mass Detector](#)

[XBridge Phenyl XP Column](#)

[Beverage Mobile Phase](#)

[Beverage Analysis Standard](#)

[Big-4 Calibration Stock Standard](#)

### KEYWORDS

Soft drink, LC, non-nutritive sweetener, acesulfame K, saccharin, caffeine, aspartame, benzoate, sorbate, beverage

### INTRODUCTION

The soft drink market is an important revenue source for many major food and beverage producers. Such beverages include traditional carbonated soft drinks, high energy drinks, and recently popular healthy formulations such as vitamin waters and teas.

These products often contain caffeine as a stimulant, benzoate, and sorbate as preservatives; and for diet preparations, non-nutritive sweeteners such as acesulfame K, aspartame, and saccharin.

For quality control purposes the conformance of target concentrations of analytes to specified ranges is critical. In this application note, we show that the Waters ACQUITY Arc System, XBridge Phenyl XP Columns, along with Waters Beverage Mobile Phase, can separate these compounds in under 10 minutes.



Figure 1. ACQUITY Arc System.

## EXPERIMENTAL

### LC conditions

LC system:	ACQUITY Arc
Runtime:	10.0 min
Column:	XBridge Phenyl <b>XP</b> 2.5 µm, 4.6 x 50 mm
Column temp.:	35 °C
Mobile phase:	Waters Beverage Mobile Phase
Flow rate:	1.0 mL/min
Flow path:	1 (HPLC emulation)
Injection volume:	5 µL
Detector:	2489 UV/Visible (UV/Vis)
Detection:	214, 247 nm

### Standard preparation

#### A: Beverage analysis standard (single point)

One bottle of Waters Beverage Analysis 5 Standards Solution, p/n [186006008](#), was poured into one bottle of Waters Beverage Analysis Standards Solid (aspartame), p/n [186006010](#), and mixed until all of the aspartame was dissolved. This resulted in a standard with a concentration of 150 mg/L acesulfame K, 100 mg/L saccharin, 200 mg/L benzoate, 100 mg/L sorbate, 100 mg/L caffeine, and 500 mg/L aspartame.

#### B: Standard concentrate (multi-point)

Waters Big-4 Calibration Stock Standard, p/n [186007980](#), (1000 mg/L each of acesulfame K, benzoate, sorbate, and caffeine) was diluted in water to produce eight separate levels with concentrations listed in Table 1.

Std	Big 4 analytes (mg/L)
1	500
2	400
3	200
4	100
5	50
6	40
7	20
8	10

Table 1. Standard amounts for the Big-4 analytes: acesulfame K, benzoate, sorbate, and caffeine.

### Sample preparation

Carbonated beverages were sonicated to remove carbon dioxide. All beverages were filtered through a 0.2-µm PVDF filter, then injected.

## RESULTS AND DISCUSSION

Chromatograms resulting from separation of beverage standards using the ACQUITY Arc System with an XBridge Phenyl **XP** Column are shown in Figure 2. Depending on the requirements of the analysis, there are two different options for the standards. The first standard, shown in Figure 2A, is the Waters Beverage Standard. This standard contains (in order of elution): acesulfame K, saccharin, benzoate, sorbate, caffeine, and aspartame. Each component has a different UV absorbance spectrum so that the peak height ratios are different as a function of the wavelength. As can be seen from the chromatogram in Figure 2A, two different wavelengths can be programmed and monitored in the same run. In Figure 2A and 2B chromatograms for both 214 nm and 247 nm are shown. Figure 2B shows the chromatograms for the Big-4 Standard, which has the same analytes as the Waters Beverage Standard, with the exception of saccharin and aspartame. These two sweeteners are not required for the analysis of beverages that do not use these ingredients.

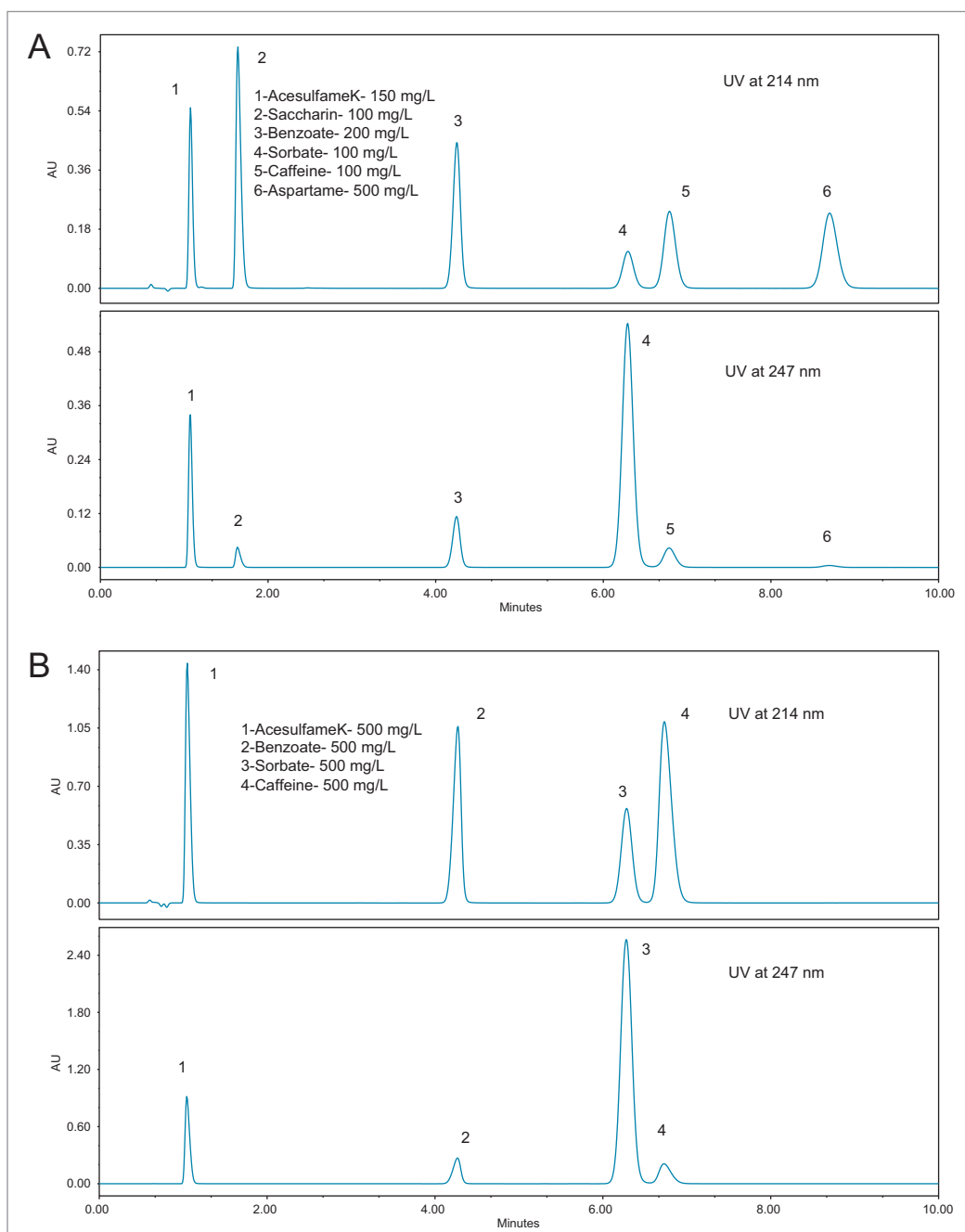


Figure 2A. Beverage Standard.  
Figure 2B. Waters Big-4  
Beverage Standard.

In order to assess the method with the sample types that are typically encountered, five different beverages were purchased from a local store for analysis. The resulting chromatograms from the analysis of these beverages are shown in Figures 3 to 7. The more traditional-style carbonated beverages are represented by two examples, a diet cola (Figure 3), and a lemon-lime soft drink (Figure 4). For each sample, the amounts of each analyte were calculated using both wavelengths for comparison. In Figures 3 and 4, the calculated amounts using the two different wavelengths agree within 4% for all of the analytes. A single point calibration was used to calculate the amounts shown in Figures 3 to 7.

Newer formulations of soft drinks often include ingredients or additives that have known or perceived health benefits. Examples include essential nutrients, antioxidants, and plant extracts. Figures 5, 6, and 7 show three different examples. Figure 5 displays the chromatograms for a diet vitamin water, and Figure 6 for a diet energy drink. For both of these samples, the quantification at both wavelengths is in agreement (within 2.1%) with the exception of acesulfame K. The diet lemon tea showed excellent agreement at both wavelengths (within 3.4%), even for acesulfame K.

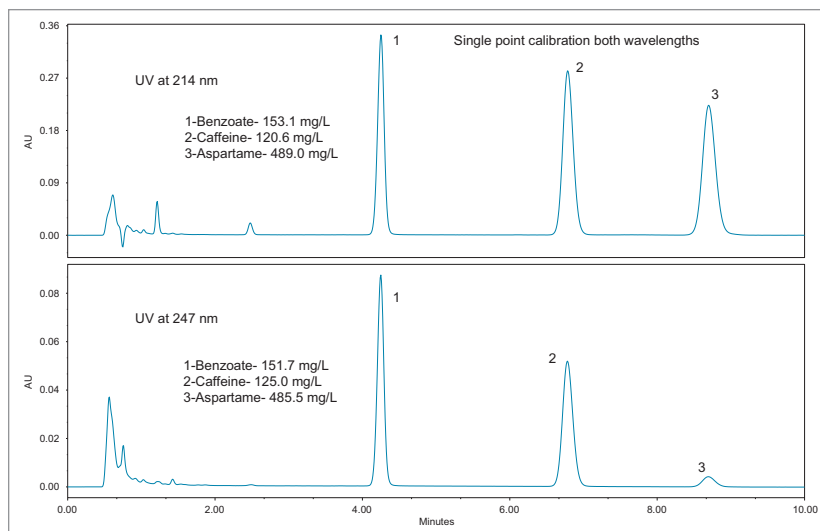


Figure 3. Diet cola chromatograms.

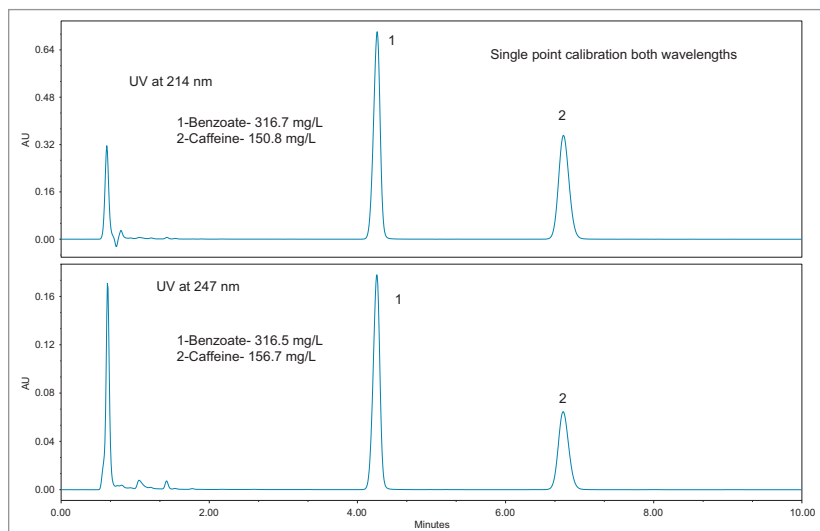


Figure 4. Lemon-lime soft drink chromatograms.

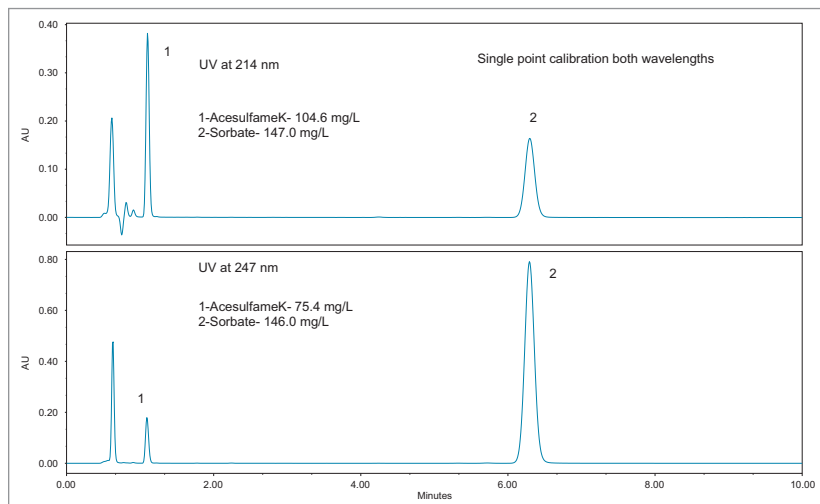


Figure 5. Diet vitamin water chromatograms.

Further investigation of the difference in quantification for acesulfame K in the diet vitamin water and diet energy drink revealed that both of these beverages contain B vitamins, where the other soft drinks did not. Using the ACQUITY QDa Detector, which enables mass analysis of the analytes, it was apparent that the B vitamins closely elute with acesulfame K. Figure 8 shows an overlay of the vitamin water chromatograms of acesulfame K at 214 nm and the individual mass-to-charge ratios of the B vitamins acquired using the ACQUITY QDa Detector. Note that vitamin B6 coelutes with acesulfame K.

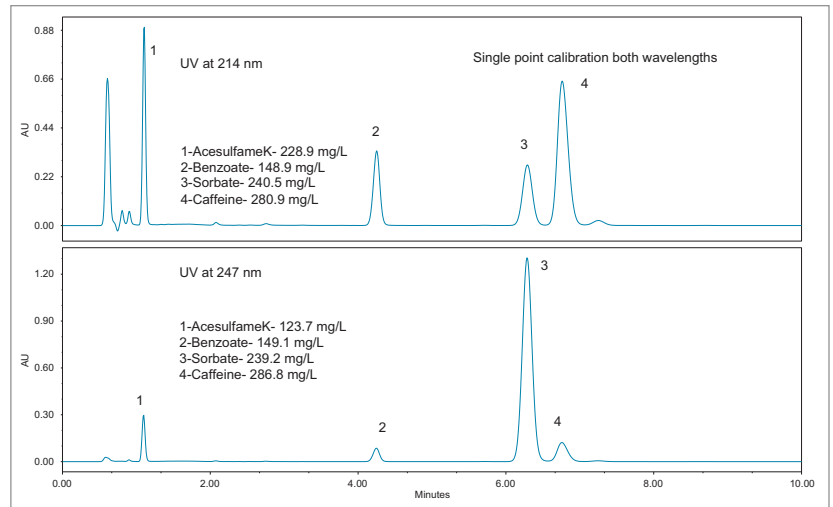


Figure 6. Diet energy drink chromatograms.

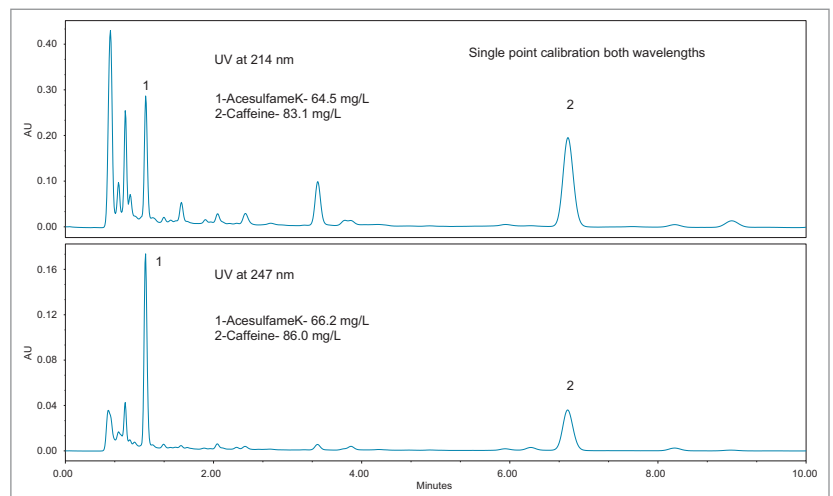


Figure 7. Diet lemon tea chromatograms.

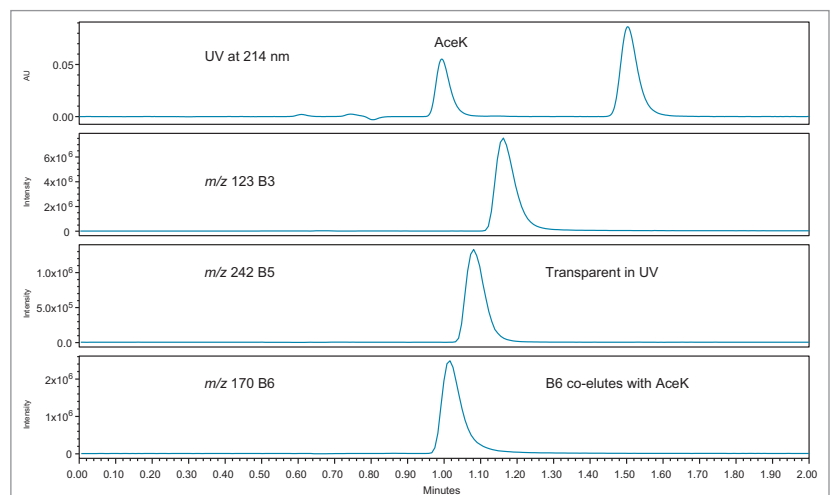


Figure 8. Overlay of UV chromatogram of Acesulfame K with SIR channels of B vitamins.

The UV spectra of vitamin B6 and acesulfame K are shown in Figures 9A and 9B, respectively. At 214 nm, which is non-specific, vitamin B6 has high absorbance and contributes to the overall response at 214 nm, along with acesulfame K. This results in over-estimation of the amount when using 214 nm. As can be seen, vitamin B6 is almost transparent at 247 nm, making this the wavelength of choice for beverages containing acesulfame K and vitamin B6.

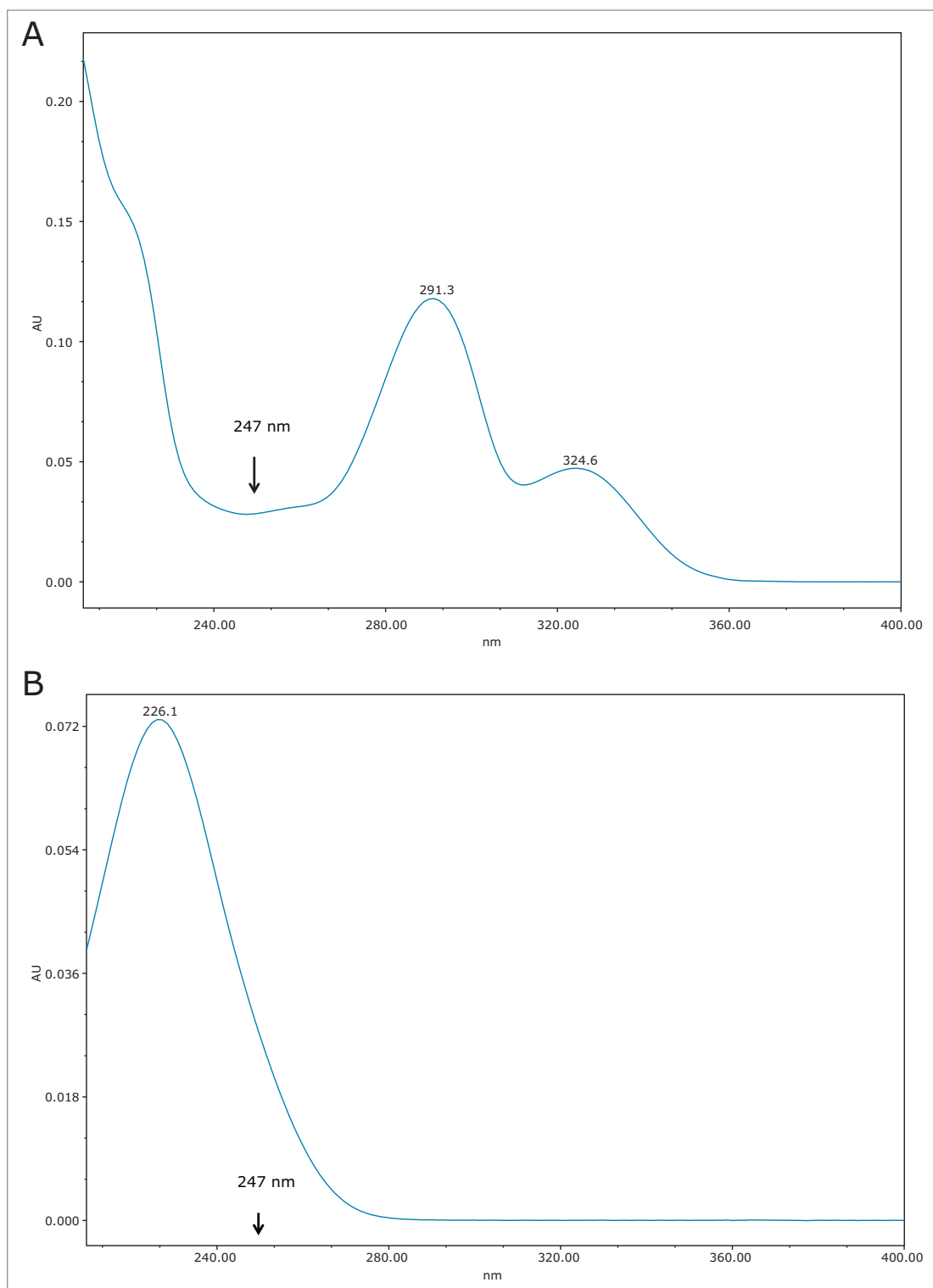


Figure 9A. UV spectrum of vitamin B6. Figure 9B. UV spectrum of acesulfame K.

The calibration curves created using the multi-point calibration from the Waters Big-4 Standard are shown in Figure 10.  $R^2$  values were  $>0.999$  for all compounds. Table 2 lists the retention time reproducibility for six injections of the Waters Beverage Analysis Standard. The %RSD was  $<0.2\%$  for all analytes. Table 3 lists the reproducibility data for retention time and amounts for seven injections of the lemon-lime soft drink, which contained benzoate and caffeine. Quantification was made using the single-point calibration. Retention time reproducibility was  $0.05\%$  or less and the reproducibility for amount was  $<0.25\%$ . Table 4 compares the quantification of the energy drink using both the single- and multi-point calibration at 247 nm to eliminate B vitamin interference.

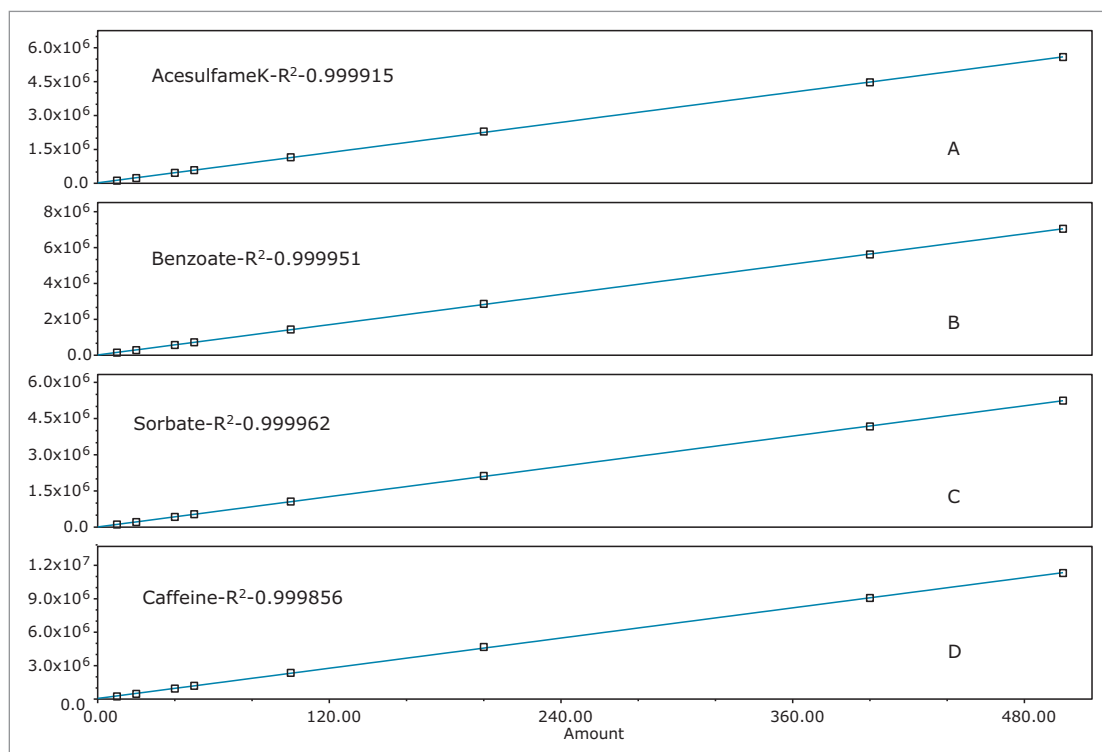


Figure 10. A-D: calibration curves of Big-4 analytes.

Analyte	RT (min)	%RSD
Acesulfame K	1.08	0.16
Saccharin	1.64	0.10
Benzoate	4.25	0.04
Sorbate	6.30	0.03
Caffeine	6.79	0.03
Aspartame	8.70	0.02

Table 2. Retention time reproducibility for six injections of Waters Beverage Standard.

Analyte	RT	%RSD	Amount	%RSD
Benzoate	4.27	0.05	316.64	0.17
Caffeine	6.78	0.04	150.80	0.21

Table 3. Retention time and reproducibility amount for seven injections of the lemon-lime soft drink.

Analyte	Single-point at 247nm	Multi-point at 247 nm	%RSD
AcesulfameK	123.7	123.9	0.24
Benzoate	149.1	148.9	0.13
Sorbate	239.2	245.1	1.24
Caffeine	286.8	288.6	0.33

Table 4. Comparison of the quantitation of the diet energy drink (mg/L) using single- and multi-point calibration at 247 nm.

## CONCLUSIONS

Soft drink analysis using the ACQUITY Arc System provides a simple method for the analysis of soft drink additives. Implementation of such a procedure in a manufacturing environment has the capacity to improve overall workplace efficiency as well as:

- The choice of a single- or multi-point calibration, depending on the analytes and analyte levels required.
- Ethanol based mobile phase allows for simple, cost-effective disposal.
- Simple sample preparation requiring only sonication and filtering.
- Choice of UV wavelengths to remove vitamin B6 interference from acesulfame K quantification.

# Waters

**THE SCIENCE OF WHAT'S POSSIBLE.®**

Waters, ACQUITY, QDa, XBridge, and The Science of What's Possible are registered trademarks of Waters Corporation. Arc is a trademark of Waters Corporation. All other trademarks are the property of their respective owners.

©2017 Waters Corporation. Produced in the U.S.A. March 2017 720005474EN AG-PDF

Waters Corporation  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)