

## ACQUITY UPLC for the Rapid Analysis of Soft Drinks

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### Abstract

This application note shows how an advanced chromatographic technique, UltraPerformance LC (UPLC) can reduce run times to under 75 seconds per sample resulting in a considerable time savings while providing the resolution necessary to deal with a range of analytes in a variety of samples.

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### Introduction

Soft drinks are widely consumed beverages throughout the world. Maintaining and confirming the quality and consistency of a given product from many individual bottling plants is a considerable challenge for central quality control laboratories. The complexity and variety of these samples compound the challenge further. Numbers of samples analyzed yearly can reach into the tens of thousands making analysis time important. For example, 10,000 samples analyzed by high performance liquid chromatography (HPLC) at a run time of 16 minutes would require approximately 333, 8-hour working days to complete. This application note shows how an advanced chromatographic technique, UltraPerformance LC (UPLC) can reduce run times to under 75 seconds per sample

(Figure 1) resulting in a considerable time savings while providing the resolution necessary to deal with a range of analytes in a variety of samples.

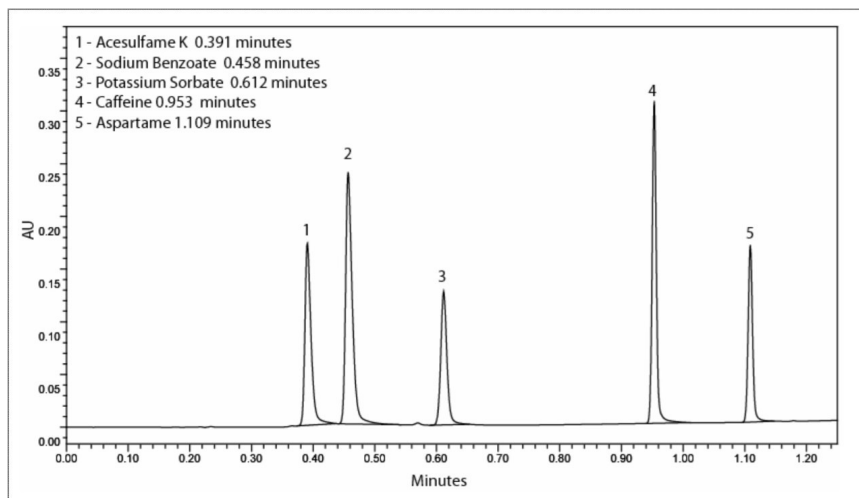


Figure 1. UPLC separation of a five component soft drink standard.

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## Experimental

### UPLC Conditions

The Waters ACQUITY UPLC System consisted of the ACQUITY UPLC Binary Solvent Manager, the ACQUITY UPLC Sample Manager (5  $\mu$ L loop), and the ACQUITY UPLC TUV Detector. All instruments were controlled and data collected and analyzed using Waters Empower Software. Separations were performed on a 2.1 X 50 mm ACQUITY UPLC BEH  $C_{18}$  Column, 1.7  $\mu$ m particle size at a flow rate of 0.90 mL/minute. Column temperature was set to 40  $^{\circ}$ C and injection volumes for all samples and standards were set to 1  $\mu$ L. Water was used as the weak wash solvent (700  $\mu$ L), and a mixture of water and acetonitrile (1:1) was used as the strong wash solvent (200  $\mu$ L). Mobile phase components and gradient conditions are outlined in Table 1. Soft drink sample components were detected at a wavelength of 214 nm using a sampling rate of 20 points per second and a filtering constant of 0.1 seconds.

Time (min)	%A	%B	Curve
Initial	98	2	–
1.20	70	30	7
1.21	98	2	6

*Table 1. Gradient separation conditions, where:  
Solvent A = 10 mmol Sodium Phosphate Buffer,  
pH 6 Solvent B = Acetonitrile.*

## Calibration

A stock standard solution of acesulfame K (380 mg/L), sodium benzoate (517 mg/L), potassium sorbate (273 mg/L), caffeine (253 mg/L), and aspartame (500 mg/L) was prepared in 30% acetonitrile. Six dilutions (in water) of the stock solution were prepared with concentrations outlined in Table 2. Calibration curves for all five components were generated from these analyses and fit to linear equations and are shown in Figure 2.

Compound	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Acesulfame K	76	114	152	190	228	266
Sodium benzoate	103	155	207	258	310	362
Potassium Sorbate	54	812	109	136	163	190
Caffeine	51	76	101	126	152	177
Aspartame	100	150	200	250	300	350

*Table 2. Standard concentrations (mg/L)*

## Sample Preparation

Samples were prepared by subjecting an aliquot (~20 mL) of the given soft drink to sonication for 5 minutes to remove dissolved carbon dioxide. Degassed sample was placed directly into 2 mL glass vial fitted with pre-slit septa. Small injection volumes (1 µL) minimized sample preparation by allowing the sample to be injected without a dilution step.

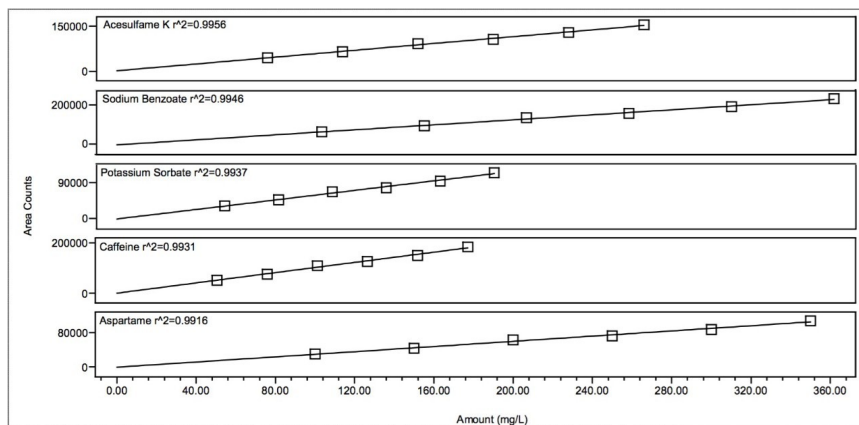
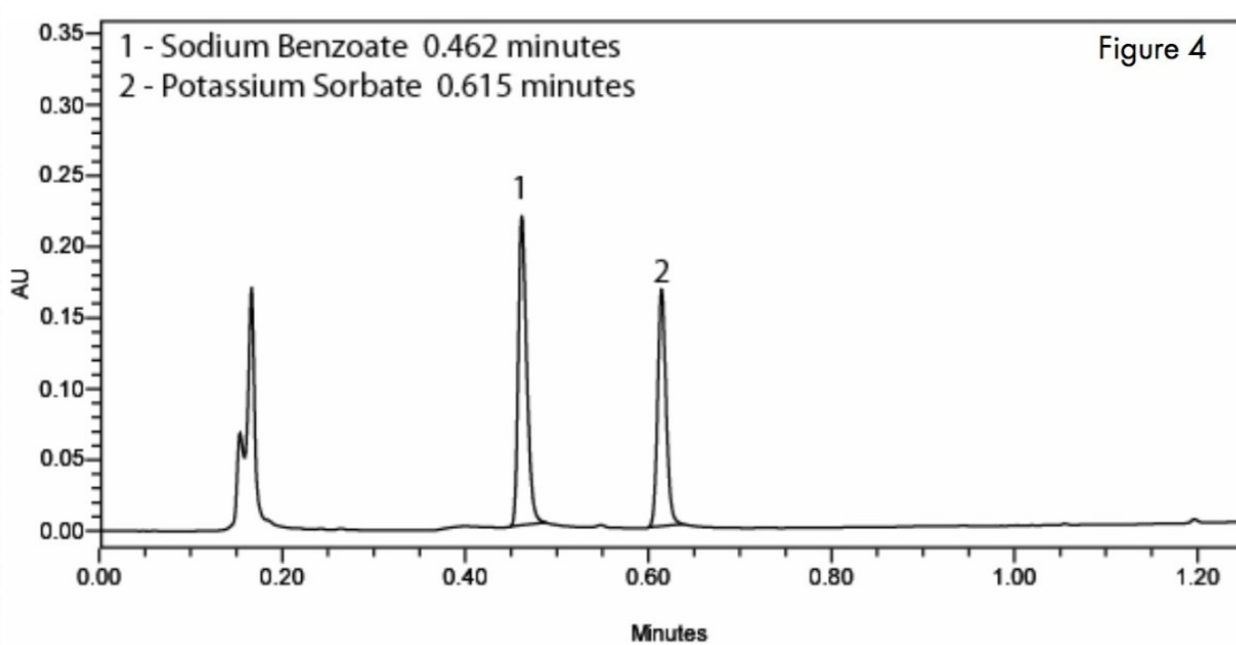
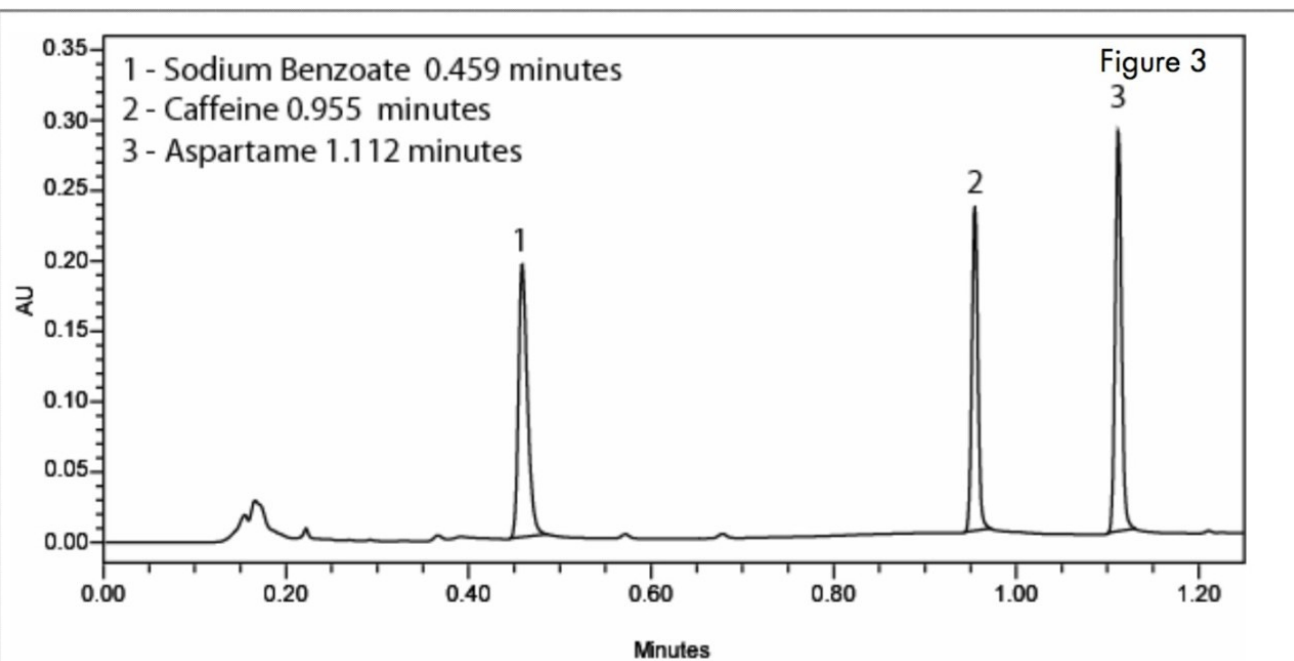
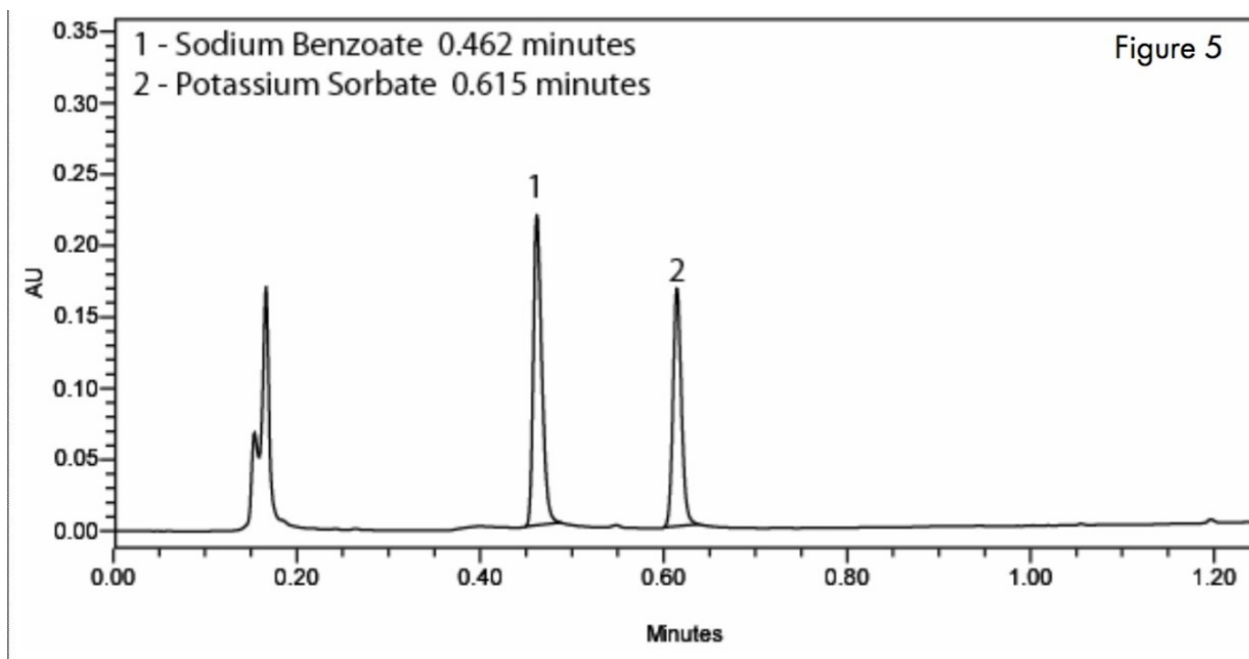


Figure 2. Calibration curves for acesulfame K, sodium benzoate, potassium sorbate, caffeine, and aspartame.

## Results and Discussion

Area counts from injections of a diet cola, lemonade, and lemon flavored diet cola, Figures 3–5 respectively, were compared to the calibration curves (Figure 2) and the amounts (mg/L) were calculated for each component (Table 3). Retention times of all peaks were consistent with standard deviations of less than 0.10 seconds for all five compounds. The total cycle time for each analysis was 2 minutes and 29 seconds. Cycle time consists of the run time (1.25 minutes), system and column re-equilibration (45 seconds), injection time (29 seconds consisting of vial selection, sample draw, loop fill, and injection). Using the same calculation as presented in the introduction, 10,000 samples can be analyzed in just over 51 working days compared to the 333 working days...6.5 times faster!





Figures 3–5. Analysis of Diet Cola (Figure 3, top), Lemonade (Figure 4, center), and Lemon-flavored Diet Cola (Figure 5, bottom).

This represents a substantial increase in sample throughput and subsequent laboratory productivity without sacrificing analysis quality. Further, a significant reduction in the total amount of mobile phase used (and corresponding waste generated) can be realized; 160 liters total for the traditional method as compared to just 22.5 liters for the ACQUITY UPLC method.

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## Conclusion

The ACQUITY UPLC System combined with ACQUITY UPLC Column technology provides a rapid analysis of soft drinks, with the necessary resolution to separate multiple components, that is significantly faster than traditional HPLC techniques. The ability to reduce QC test costs while maintaining the quality of shipped manufactured product is desired in many food, beverage, and pharmaceutical organizations. This enhanced capability and cost savings can be easily adapted to the analysis of a wide variety of food, beverage, pharmaceutical and industrial compounds.

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