

ROUTINE ANALYSIS OF VANILLA-FLAVORED SOFT DRINKS USING A BEVERAGE ANALYSIS KIT

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INTRODUCTION

- Soft drinks are non-alcoholic beverages containing water, sweeteners, preservatives, flavorings, and functional ingredients
- Common soft drink additives include: Ace-K, aspartame, saccharin, sodium benzoate, potassium sorbate, and caffeine
- Waters™ Beverage Analysis Kit enables a simple analysis with ready-to-use ethanol-based solvents and pre-mixed standards, suitable for QC testing in bottling plants
- Challenge: In vanilla-flavored soft drinks, vanillin co-elutes with caffeine/other components, impacting accuracy

OBJECTIVES

This study aimed to develop a solution using the existing Waters Beverage Analysis Kit for the vanilla-flavored soft drinks.

EXPERIMENTAL

Soft drinks from major brands were purchased from a local store. Details of these samples are provided in Table 1.

Table 1. Soft drink sample codes and description.

Sample ID	Sample description
SD 1	Diet cola, carbonated.
SD 2	Diet cola with a cherry flavor, carbonated.
SD 3	Cola with a cherry and a vanilla (cream) flavor, carbonated.

Sample Prep.:

Samples were degassed and filtered through a 0.2 µm, 13 mm PVDF membrane filter disc (Waters, p/n WAT200806).

Mobile phase preparation:

Use a 1000 mL Class A graduated cylinder to measure 900 mL of Mobile Phase Reagent (p/n 186006006) from the Waters Beverage Analysis kit (Waters, p/n 176002534) and transfer it quantitatively to a 1-liter glass bottle. Then, use a 100 mL Class A graduated cylinder to measure 100 mL of Waters Beverage Analysis Kit Wash Solvent (p/n 186006007) and transfer it quantitatively to the same 1-liter glass bottle. Mix well.

LC conditions:

LC system: Waters Arc™ HPLC System with a 2998 PDA Detector
 Runtime: 35.0 min
 Column: XBridge™ BEH™ Phenyl 3.5 µm, 4.6 x 150 mm (PN 186003335) with an In-line Filter (p/n 205000343).
 Col. temp.: 35 °C
 Mobile phase: Mixture of Waters Beverage Mobile Phase Reagent and Wash Solvent at a 9:1 volume ratio.
 Flow rate: 1.5 mL/min
 Injection vol.: 5 µL
 Detection: UV at 214 nm
 Software: Empower™ 3
 Sample Manager Purge: Same as the Mobile phase.
 Sample Manager Wash: Beverage Analysis Wash Solvent.
 Seal wash: Beverage Analysis Wash Solvent.

RESULTS AND DISCUSSION

Chromatographic separation optimization

- Column temperature — minimal impact on resolution
- Mobile phase composition — effective in improving resolution

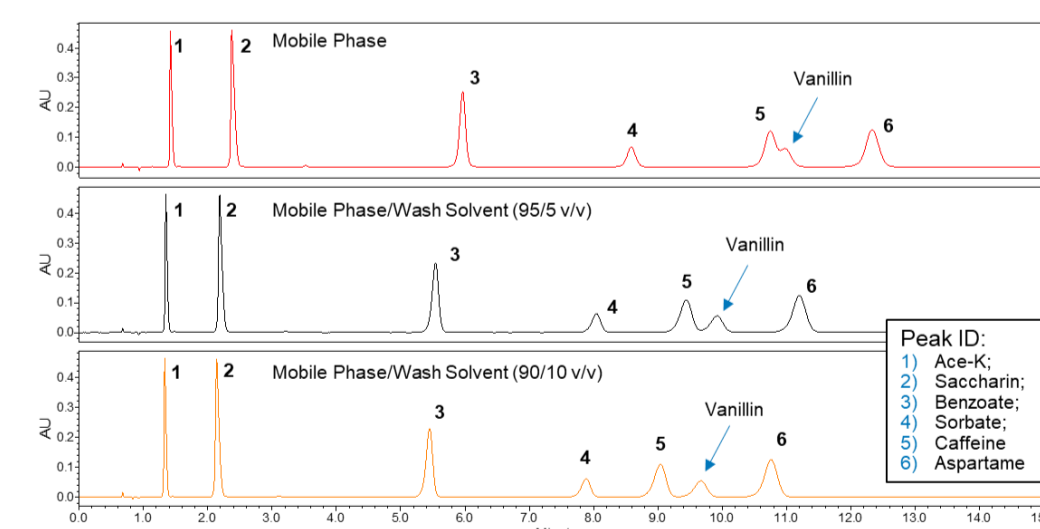


Figure 1. Chromatograms of a standard mixture using different mobile phase compositions. Conditions: XBridge BEH Phenyl Column 2.5 µm, 4.6 x 100 mm. Isocratic elution. Flow rate: 1.6 ml/min.

Linearity

- Excellent R² and low relative deviation for multiple concentration levels (see Table 2)
- Excellent linearity (through zero) supports a single-point calibration in routine analysis

Table 2. Calibration results for six beverage ingredients

Compound	Conc. range (mg/L)	Calibration Equation*	R ²	Max % Deviation*
Ace-K	1.5 - 148	Y = 7500 X	0.999990	1.20%
Saccharin	0.99 - 98.9	Y = 19500 X	0.999986	0.97%
Benzoate	5.9 - 197	Y = 9450 X	0.999956	1.99%
Sorbate	0.98 - 98.1	Y = 7150 X	0.999970	3.80%
Caffeine	0.98 - 97.9	Y = 16000 X	0.999959	4.46%
Aspartame	14.7 - 490	Y = 225 X	0.999914	1.54%

Note: * Calibration equation for peak area (Y) vs. concentration (X), except for aspartame, where the peak height vs concentration is calibrated. * The maximum relative deviation between the fitted and the determined values for all concentration levels.

Ethylvanillin in vanilla flavor

- Ethylvanillin is a synthetic flavoring agent sometimes used to enhance vanilla flavor
- Ethylvanillin elutes at 30 min.
- Due to the long retention time of ethylvanillin, a run time of 35 min is required for vanilla flavored soft drinks

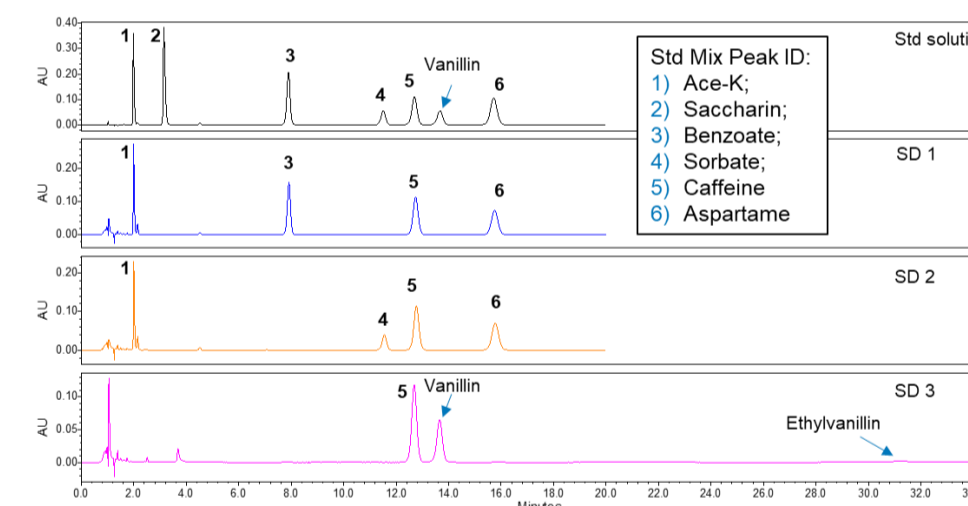


Figure 2. Chromatograms of a standard solution and soft drinks.

Intermediate precision

- Intermediate precision evaluated across 3 soft drinks, multiple days, and 3 column batches (see Table 3)
- Excellent intermediate precision
 - Aspartame: RSD < 3%
 - Other analytes: RSD < 1.8%
- Measured caffeine levels within ±5% of label claims
- Baseline separation achieved for all analytes (Rs ≥ 2)

Table 3. Separation resolution and intermediate precision in sample analysis

Sample	Content*	Ace-K	Saccharin	Benzoate	Sorbate	Caffeine	Vanillin	Aspartame
SD 1	Average (mg/L)	105	ND	150	ND	102	ND	347
	RSD (n=8) % of Label value	1.83%		0.44%		1.06%		2.59%
SD 2	Average (mg/L)	86	ND	ND	70	102	ND	328
	RSD (n=8) % of Label value	1.79%			0.59%	1.12%		2.99%
SD 3	Average (mg/L)	ND	ND	ND	ND	105	92	ND
	RSD (n=8) % of Label value					1.01%	1.16%	
Resolution*								
Std-Mix	Average*	-	10.1	21.4	11.8	2.8	2.4	3.9
	SD (n=11)	-	0.1	4.0	0.6	0.3	0.3	0.3

Note: * Determined from multiple measurements using 3 different columns (different batches). * The average resolution between the previous and the current peaks.

CONCLUSION

- The co-elution of vanillin with other ingredients in the analysis of vanilla-flavored soft drinks has been successfully resolved using the Waters Beverage Analysis Kit.
- Results:
 - Baseline resolution (Rs ≥ 2) of vanillin and other ingredients
 - Excellent ruggedness demonstrated using three XBridge BEH Phenyl Columns (3.5 µm, 4.6 x 150 mm) from different production batches
 - Excellent detection linearity and intermediate precision
- This optimized method offers a reliable and simple solution for the QC of vanilla-flavored soft drinks.

Reference

- 1) Yang, J., Rainville, P., and Harden, S., Using Waters Beverage Analysis Kit to Analyze Vanilla-Flavored Soft Drinks, Application Note 720008924, Waters Corporation, 2025.



Please scan the QR Code on the right to download the application note for additional details.