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應用手冊

Using Waters Beverage Analysis Kit to Analyze Vanilla-Flavored Soft Drinks

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Abstract

The Waters™ Beverage Analysis Kit was developed to help beverage manufacturers analyze common ingredients in soft drinks. However, use of this kit for the analysis of vanilla-flavored soft drinks, a relatively new formulation in the market, has shown that vanillin co-elutes with caffeine or other ingredients. This co-elution leads to inaccurate results in the quality control (QC) of these products. To address this issue related to vanilla-flavored soft drinks, the approach used in the beverage analysis kit has been optimized using a new mobile phase, which is prepared by mixing the Mobile-Phase Reagent and the Wash Reagent from the Waters Beverage Analysis Kit in a 9:1 volume ratio (Mobile-Phase Reagent:Wash Reagent = 9:1 v/v). By using the new mobile phase while keeping the other analytical conditions the same as the kit, a baseline separation of vanillin from caffeine and other ingredients has been successfully achieved. The robustness of the separation of vanillin from other common ingredients has been demonstrated using XBridge™ BEH™ Phenyl Columns (3.5 µm, 4.6 x 150 mm) from three different production batches. Excellent detection linearity and intermediate precision have also been demonstrated in sample analyses. This optimized beverage analysis method offers a reliable and simple solution for the QC analysis of vanilla-flavored soft drink products.

Benefits

- Accurate quantitation of common ingredients in vanilla-flavored soft drinks
- Ready to use, environmentally-friendly reagents
- Minimal sample preparation

Introduction

Soft drinks are non-alcoholic beverages that generally include water, sweeteners, caffeine, preservatives, flavorings, or colorings. Common additives in soft drinks include acesulfame potassium (Ace-K, sweetener), aspartame (sweetener), saccharin (sweetener), sodium benzoate (preservative), potassium sorbate (preservative), and caffeine. The Waters Beverage Analysis Kit offers a simple and easy solution for the routine analysis of these ingredients.[1,2] It includes an ethanol-based mobile phase and a wash solvent that are ready to use, along with pre-mixed standards, making it ideal for QC checks at bottling plants or other manufacturing plants. The mobile phase can be reused for up to a week, depending on the number of analyses performed each day, which helps keep the cost low.

However, analysis of vanilla-flavored soft drinks, a relatively new soft drink formulation, using the Waters Beverage Analysis Kit reveals that vanillin co-elutes with caffeine or other ingredients, causing inaccuracies in the analysis of soft drink ingredients. This study addressed this issue by optimizing the analysis conditions provided with the kit to improve the separation resolution (Rs) of vanillin from other ingredients. A simple solution for analyzing vanilla-flavored soft drinks was developed using the existing Beverage Analysis Kit. The method development, analytical performance, and robustness of the separation are included in this application note.

Experimental

Standard Preparation

Follow the Beverage Analysis Kit Care and Use Manual to prepare the standards solution.[3] Specifically, pour the entire contents (100 mL) of the 5 Beverage Analysis Standards (p/n: 186006008 < https://www.waters.com/nextgen/global/shop/standards--reagents/186006008-beverage-analysis-5-standards-

solution.html>) into the bottle containing aspartame powder (p/n: 186006010 < https://www.waters.com/nextgen/global/shop/standards--reagents/186006010-beverage-analysis-standard-solid.html>). Cap the bottle and shake it vigorously until the aspartame is completely dissolved. If necessary, sonicate the bottle to break up any solid clumps. Transfer the solution to vials for direct use and label them as "Beverage Analysis Standard". Store any remaining unused vials in the refrigerator. Refrigerated Beverage Analysis Standard vials expire after 1 week.

Sample Preparation

Three soft drinks from major brands were purchased from a local store. These samples include carbonated diet or regular cola, with or without cherry and vanilla flavors. Details of these samples are provided in Table 1. These samples were degassed (using vacuum and sonication) and filtered through a 0.2 µm, 13 mm polyvinylidene fluoride (PVDF) membrane filter disc (p/n: WAT200806 < https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/wat200806-acrodisc-syringe-filter-pvdf-13-mm-02--m-aqueous-100-pk.html>).

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.

Table 1. Soft drink sample codes and description.

Mobile Phase Preparation

The following mobile phase preparation procedure differs from the Beverage Analysis Kit Care and Use Manual. This procedure has been optimized for analyzing vanilla-flavored soft drinks. Briefly, use a 1000 mL Class A graduated cylinder to measure 900 mL of Mobile-Phase Reagent (p/n: 186006006 < https://www.waters.com/nextgen/global/shop/standards--reagents/186006006-beverage-analysis-mobile-phase-reagent.html>) from the Waters Beverage Analysis kit (p/n: 176002534 < https://www.waters.com/nextgen/global/shop/application-kits/176002534-beverage-analysis-kit.html>) 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 Reagent (p/n: 186006007 <

https://www.waters.com/nextgen/global/shop/standards--reagents/186006007-beverage-analysis-wash-reagent.html>) 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 (20 minutes for soft drinks without a vanilla flavor)
Column:	XBridge BEH Phenyl Column 3.5 µm, 4.6 x 150 mm (p/n: 186003335) with an In-line Filter (p/n: 205000343)
Col. temperature:	35 °C
Mobile phase:	Mixture of Waters Beverage Mobile-Phase Reagent and Wash Reagent at a 9:1 volume ratio, isocratic elution.
Flow rate:	1.5 mL/min
Injection volume:	5 µL
Detection:	UV at 214 nm
Chromatography data system:	Empower™ Chromatography Data System (CDS)
Sample manager purge:	Same as the Mobile phase
Sample manager wash:	Beverage Analysis Wash Reagent

Seal wash:	Beverage Analysis Wash Reagent

Results and Discussion

Method Optimization for Vanilla-Flavored Soft Drinks

The effects of column temperature and mobile phase composition on the separation of vanillin from six common ingredients were investigated. Testing column temperatures between 30 °C and 45 °C showed minimal improvement in separation resolution. Adjusting the mobile phase composition, however, was found to be effective in improving the resolution. Figure 1 shows the effects of various mobile phase compositions on the separation of vanillin and common ingredients. Baseline separation was achieved using a 9:1 volume ratio mixture of the Mobile-Phase and Wash Reagent from the Waters Beverage Analysis Kit.

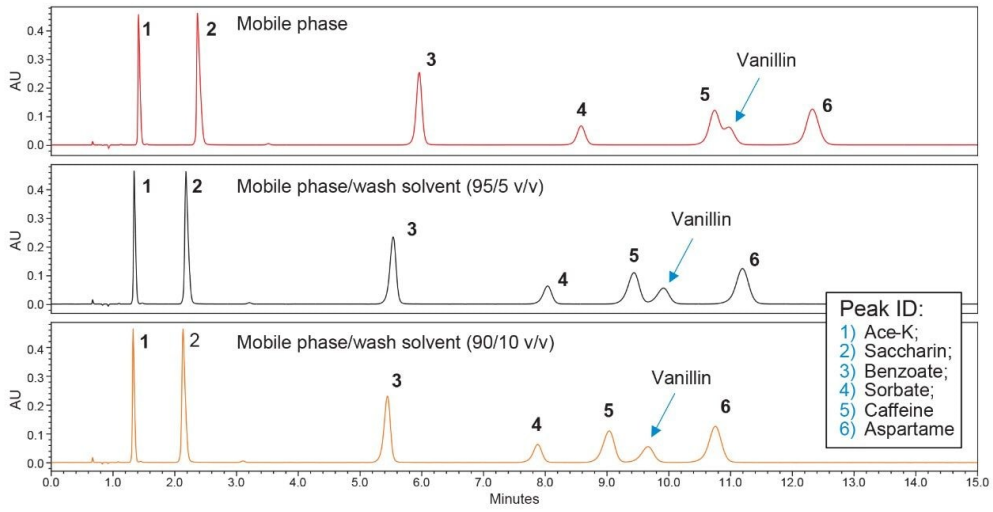

Figure 1. Chromatograms of a standard mixture using different mobile phase compositions (mobile phase compositions and peak IDs are shown in the chromatograms). Conditions: XBridge BEH Phenyl Column 2.5 µm, 4.6 x 100 mm. Mobile-Phase and Wash Reagent are from Waters Beverage Analysis Kit. Isocratic elution. Flow rate: 1.6 ml/min. Column temperature 35 °C. UV Detection wavelength: 214 nm.

Linearity

Standard solutions at various concentration levels were analyzed, and their peak areas (or peak heights) were plotted against their concentrations for each standard. These data points were then fitted to a line passing through zero using least square regression. Table 2 presents typical calibration results for the six standards. The high coefficients of determination (R^2>0.999) in the fitted calibration curves and the low relative deviation (<4.5%) between the fitted and the determined values indicate excellent linearity in the relationship between detector response and concentration. These findings validate the single-point calibration approach in the routine beverage analysis.

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.

Table 2. Calibration results for six beverage ingredients.

Ethylvanillin in Vanilla Flavor

Figure 2 displays HPLC-UV chromatograms of a standard solution and three soft drinks. For the vanilla-flavored soft drink SD 3, a late eluting peak (around 30 minutes) was observed. This peak was identified as ethylvanillin by comparing its UV/Vis spectrum and retention time with an ethylvanillin standard (data not shown). Ethylvanillin is a synthetic flavoring agent that is commonly used to enhance vanilla flavor. Due to the longer retention of ethylvanillin, a runtime of 35 minutes was required for vanilla-flavored soft drinks. For samples containing no vanilla flavor, a runtime of 20 minutes was sufficient.

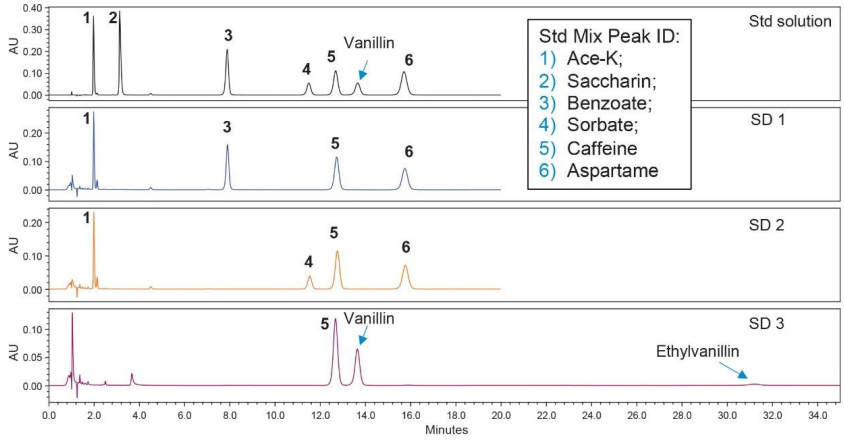

Figure 2. Chromatograms of a standard solution and three soft drinks. Conditions: XBridge BEH Phenyl Column 3.5 µm, 4.6 x 150 mm. Mobile phase: Beverage Analysis Kit Mobile-Phase/Wash Reagent 9:1 v/v. Flow rate: 1.5 ml/min. Column temperature 35 °C. UV Detection wavelength: 214 nm.

Intermediate Precision

Table 3 presents the intermediate precision of sample analysis results for the three soft drinks. These analyses were conducted on different days using XBridge BEH Phenyl Columns (3.5 µm, 4.6 x 150 mm) from three different production batches. The relative standard deviations (RSD) of the replicated determined values (n=8) were below 3% for aspartame and less than 1.8% for other ingredients. The slightly higher RSD for aspartame is likely due to its lower stability (easier to degrade than others) in solution. The low RSD values for the other ingredients indicate excellent intermediate precision in this analysis. The determined caffeine contents in these soft drinks differ by less than 5% from the labeled values, which is within the expected range for soft drink products. Table 3 also shows the separation resolutions for vanillin and other ingredients. Baseline separation (Rs ≥ 2) for all analytes has been verified.

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)	1.83%		0.44%		1.06%		2.59%
	% of Label value					95.4%		
SD 2	Average (mg/L)	86	ND	ND	70	102	ND	328
	RSD (n=8)	1.79%			0.59%	1.12%		2.99%
	% of Label value					95.7%		
SD 3	Average (mg/L)	ND	ND	ND	ND	105	92	ND
	RSD (n=8)					1.01%	1.16%	
	% of Label value					97.7%		
	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.

Table 3. Intermediate precision in sample analysis and resolution.

System and Column Considerations

This method can be readily implemented on legacy HPLC systems, such as Waters Breeze™ QS HPLC System, where the system pressure limit is around 6000 psi. For labs equipped with HPLC systems that have a higher system pressure limit, such as the Waters Arc HPLC System, the XBridge BEH Phenyl Column with a smaller particle size (2.5 µm) and a shorter column length (100 mm) can be used. This XBridge BEH Phenyl Column (2.5 µm, 4.6 x 100 mm) has been tested (see Figure 1) and produced satisfactory results within a shorter run time (15 minutes). However, due to the relatively high system pressure (approximately 4700 psi) generated by this 2.5 µm XBridge BEH Phenyl Column, the 3.5 µm XBridge BEH Column (3.5 µm, 4.6 x 150 mm) is more suitable for the legacy HPLC systems, such as the Breeze System. Also, please be advised that although the new mobile phase mixture (prepared from mixing the Mobile Phase and the Wash Reagent at volume ratio of 9:1) provides excellent results across different columns, the mixing volume ration (in preparing the mobile phase mixture) could be further fine-tuned for the best available resolution for a specific LC system and column setup.

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 with a slightly modified mobile phase. By mixing the Mobile Phase Reagent with the Wash Reagent at a 9:1 volume ratio, while keeping the other conditions the same, the separation resolution of the vanillin from other ingredients has been improved to achieve a baseline

separation (Rs ≥ 2). This separation (Rs ≥ 2) has been shown to be robust across XBridge BEH Phenyl Columns (3.5 µm, 4.6 x 150 mm) from three different production batches. Excellent detection linearity and intermediate precision have also been demonstrated in the sample analysis. This optimized method offers a reliable and simple solution for the QC of vanilla-flavored soft drinks.

References

1. Benvenuti, M.E., Giska, R., and Burgess, J.A. Rapid analysis of soft drinks using the Acquity UPLC H-Class system with the Waters Beverage Analysis Kit. Waters Application Note 720004016, 2011.
2. Yang, J., and Rainville, P.D. Analysis of soft drink additives with no interference from aspartame degradants using Arc HPLC system with PDA detection. Waters Application Note 720007219, 2021.
3. Beverage Analysis Kit Care and Use Manual. Waters literature code: 715003129 < https://help.waters.com/help/global/support/library-details.html?documentid=715003129> Rev C, 2015.

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