APPLICATION NOTE

Liquid Chromatography

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Analysis of Common Sweeteners and Additives in Beverages with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Sweeteners are low or zero-calorie sugar substitutes that are added in drinks, processed foods and pharmaceutical products to provide the sweet taste of table sugar, which is also called sucrose. Sweeteners, especially artificial sweeteners, contain practically no calories because their metabolism follows a different pathway than that of sucrose. On the other hand, the intake of sucrose and the calories that derive from its metabolism is one of the leading causes of obesity and its related health problems including heart disease and diabetes. People with diabetes are unable to properly metabolize sucrose causing an abnormally high concentration of it in the blood stream with damaging effects on blood vessels and other vital body organs. In 2007 there were 23.6 million people in the U.S. living with diabetes with an alarming 1.6 million new cases each year at an annual cost of $174 billion. Worldwide, about 247 million people live with diabetes, with another seven million more cases each year making it a global epidemic. Substituting sucrose with artificial sweeteners, in addition to getting regular physical exercise and having healthy eating habits, is effective in fighting obesity and preventing or managing diabetes.

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The use of artificial sweeteners is regulated in most countries. In the U.S., artificial sweeteners are part of the Generally Recognized As Safe (GRAS) ingredients. However, the FDA has established an Acceptable Daily Intake (ADI) for all sweeteners. Therefore manufacturers are required to list the type and amount of sweeteners on a food label. This application note presents a fast and robust liquid chromatography method to test widely used artificial sweeteners such as acesulfame potassium, saccharine and aspartame. A common stimulant and a preservative, namely caffeine and potassium benzoate were tested as well. The method was developed using a 3 µm LC column to achieve very high throughput at a low flow rate to reduce the testing time and solvent usage. The throughput was compared to that of a conventional HPLC analysis with a 5 µm particle column. In addition to throughput comparisons, method conditions and performance data including precision and linearity are presented. The results of the method applied to two popular soft drinks and two popular coffee sweeteners are reported.

Experimental

A working standard solution containing 200 µg/mL of acesulfame potassium, potassium benzoate, aspartame, and 100 µg/mL of saccharine and caffeine was prepared by dissolving neat material in water.

Repeatability was studied with six injections of the working standard. Linearity was determined across the range of 2-200 µg/mL concentration. About 20 mg/mL of two cola drinks from two major competitive brands were prepared by dilution with water. About of 2 mg/mL of two popular sugar substitutes were prepared individually by dissolving the sample in water followed by two min. vortexing. The solutions were thoroughly mixed and filtered with a 0.2 µm nylon membrane prior to testing.

A PerkinElmer® Flexar® FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was used. The separation was achieved using a Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm column. The run time was 3.5 min with a back pressure of 6050 PSI (417 bar).

Table 1. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume, partial loop mode
Injection:	4 µL for UHPLC column, 10 µL for HPLC column
Detector:	Flexar FX PDA UHPLC Detector
Analytical Wavelength:	214 nm
Pump:	Flexar FX-15 UHPLC Pump
UHPLC Column:	Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm (Cat # 9414312)
HPLC Column:	PerkinElmer Brownlee™ Analytical C-18, 5 µm, 250 x 4.6 mm (Cat #N9303514)
Column Temperature:	Ambient, 30 °C
Mobile Phase:	A: 0.1% TFA in water B: 0.1% TFA in acetonitrile (HPLC grade solvent and ACS grade reagent)

Conventional C18 HPLC column

Time (min)	Flow rate (mL/min)	B %	Curve
10	1	10-35	1
2	1	75	1
2	1	75	1

UHPLC C18 column

Time (min)	Flow rate (mL/min)	B %	Curve
3.5	0.7	5-40	1

Sampling Rate:	5 pt/s
Software:	Chromera Version 3.0

Results And Discussion

Initially, the method was developed with a conventional C18, 250 x 4.6 mm, 5 µm particle size HPLC column. The optimal flow rate of this method was determined to be 1.0 mL/min. at ambient temperature. All the peaks eluted within 12 min. (see Figure 1). By using a shorter column with smaller particle size (C18 100 x 2.1 mm, 3 µm particle size) suitable for UHPLC, the run time was dramatically reduced from 12 min. to about 3.5 min. at 30 °C (see Figure 2).

In addition to the more than threefold reduction in chromatographic run time, the flow rate was reduced to 0.7 mL/min. from 1.0 mL/min. Thus, 70% reduction in testing time and 80% reduction in solvent usage was achieved by moving to the UHPLC method. This is important not only because of the relatively high cost of HPLC-grade solvents, but also because far less solvent must be disposed of as waste. This results in a much lower cost of ownership and a much greener lab operation.

Excellent method performance was achieved. The linearity of the analysis achieved a R-squared value of at least 0.999 for each additive tested and precisions values ranged from 0.9 - 1.5% RSD. Details of the method performance and results of the sample tested are presented in Table 2 and Table 3.

Table 2. **Precision and linearity.**

	Acesulfame K	Saccharine	Caffeine	Aspartame	Potassium benzoate
%RSD (n=6)	0.9	1.5	1.3	1.3	1.0
r^2	0.9997	0.9995	0.9993	0.9993	0.9999
Range (µg/mL)	4-200	2-100	2-100	4-200	4-200

Table 3. **Amount of additives in samples.**

Compound	Cola Drink 1 (mg/12 oz)	Cola Drink 2 (mg/12 oz)	Sweetener 1 (mg/g)	Sweetener 2 (mg/g)
Acesulfame K	ND	ND	ND	ND
Saccharine	ND	ND	ND	30
Caffeine	47	35	ND	ND
Aspartame	181	157	40	ND
Potassium benzoate	71	74	ND	ND

ND = None detected

A spectrum of each component was obtained from the analysis of the standard solution over a range of 190 nm to 700 nm, and the wavelength maximum was determined, enabling the selection of a suitable wavelength setting for the analysis.

PerkinElmer's Chromera® software helps in assessing the purity of each peak by comparing the spectra on the upslope and the down slope of the peak. Because a pure peak has matching spectra throughout the peak, a ratio of upslope/down slope absorbance greater than 1.5 could be an indication of a co-elution. The spectrum and the purity of one of the sweeteners tested are presented in Figure 3.

Although in liquid chromatography peak identification is usually based on the retention time, Chromera's ability to collect and store spectra offers another way of identification by matching any peak spectrum to spectra stored in its library. This feature of Chromera adds another level of confidence in the analysis as the same relative retention time does not necessarily mean the components are the same. Confirmation of the presence of caffeine, aspartame and potassium benzoate in the Cola Drink 1 sample is shown in Figure 4. In that figure, the spectra at the peak apex of each peak is compared with the spectra of the standard stored in the library. When a match is made, the name of the matching spectrum appears on each peak in question, confirming its identity.

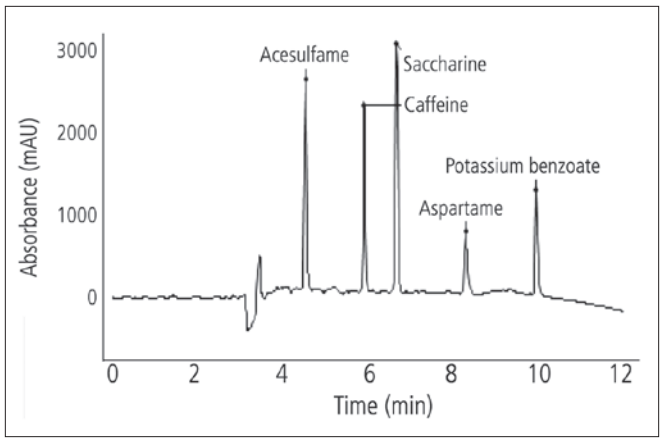

Figure 1. Chromatogram from the analysis of a standard with conventional HPLC C18 250 x 4.6 mm, 5 µm column.

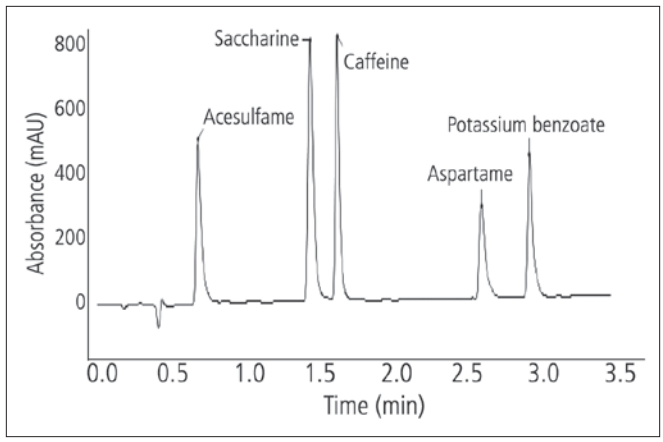

Figure 2. Chromatogram from the analysis of a standard UHPLC C18 100 x 2.1 mm, 3 µm column.

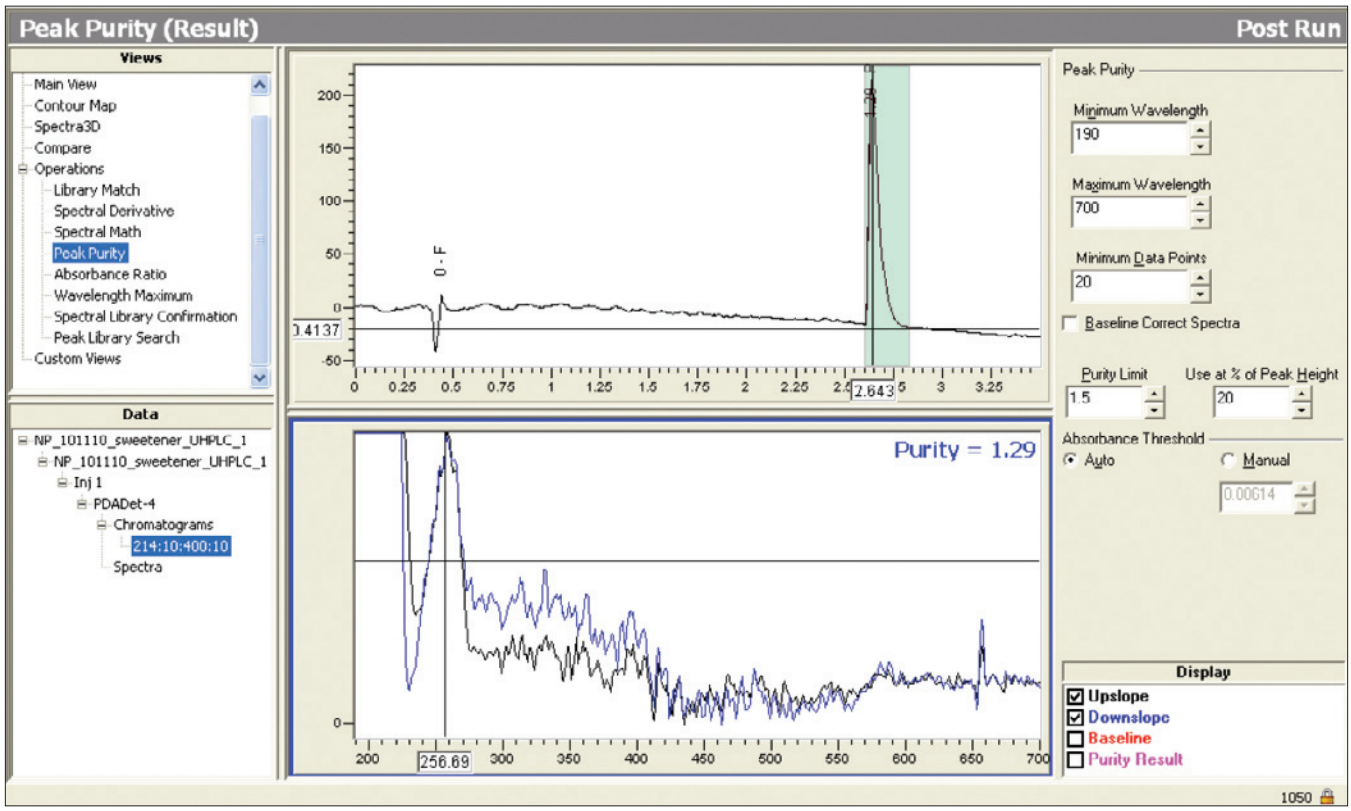

Figure 3. Chromatogram of the analysis of Sweetener 1 and the assessment of the peak purity.

Conclusion

The application of UHPLC to the analysis of artificial sweeteners and soft drink additives resulted in 70% reduction in run time, as well as 80% reduction in solvent usage. The PerkinElmer Flexar FX-15 UHPLC system and Restek® Pinnacle® DB C18, 3 µm, 100 x 2.1 mm resolved all five additives studied in about three and half minutes.

The method was shown to be linear and the peaks were well resolved. Both of the soft drinks tested were sweetened with aspartame: 181 mg/12 oz for Cola Drink 1 and 157 mg/12 oz for Cola Drink 2. The level of caffeine in drinks was similar to the label claim of 45 mg/12 oz for Cola Drink 1 and 35 mg/12 oz for Cola Drink 2. Both of the drinks have similar amounts of potassium benzoate: 71 mg/12 oz for Cola Drink 1 and 74 mg/12 oz for Cola Drink 2. The PerkinElmer

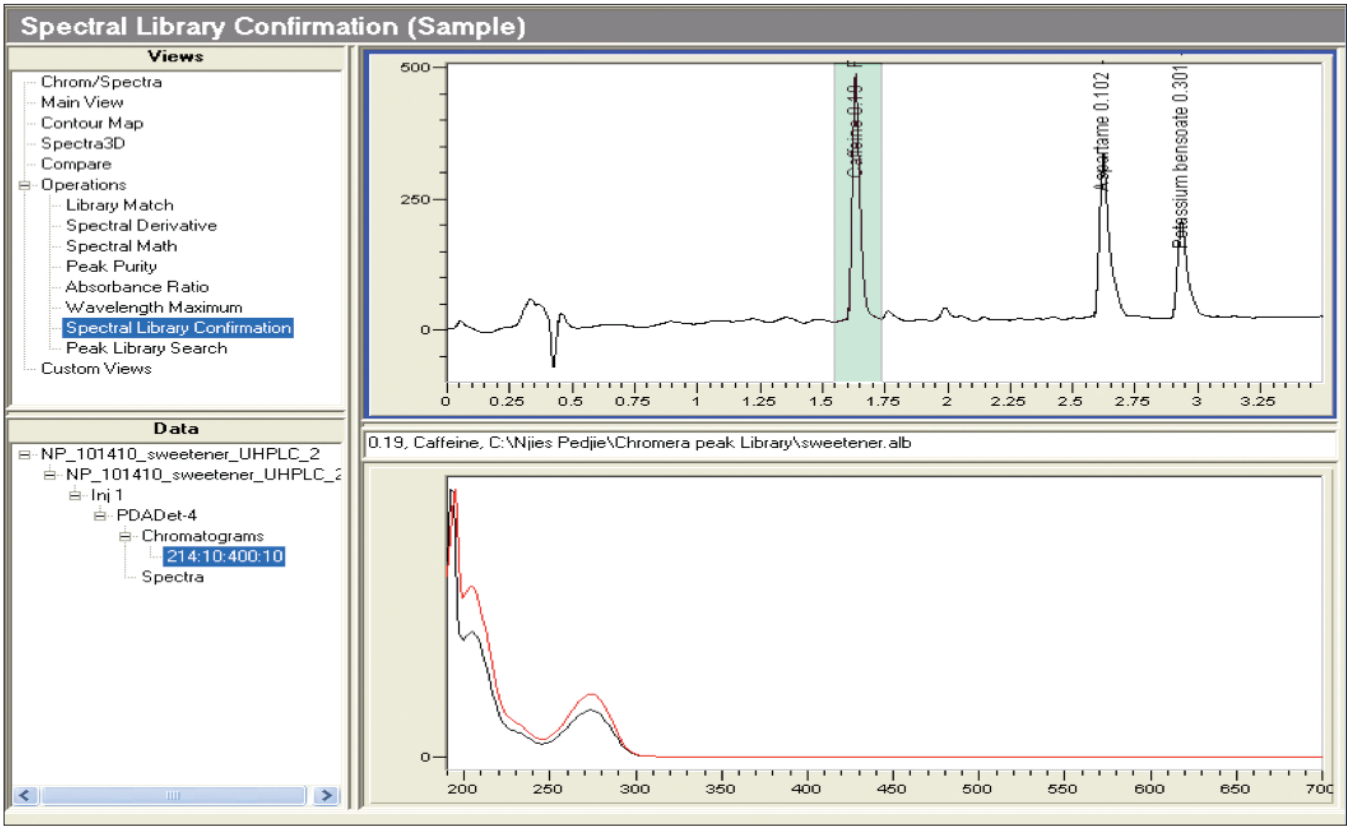

Figure 4. Chromatogram of the Cola Drink 1 and spectra library confirmation.

FX PDA detector provides rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectra library search function allowed the storage of standard peaks spectra that were later used for peak identification confirmation in the sample.

References

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