APPLICATION NOTE

UHPLC

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Simultaneous Analysis of Nine Food Additives with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Food additives are natural or synthetic substances that are added in food, beverage and pharmaceutical products for their microbicidal, preservative and flavoring properties. Among the commonly used additives, benzoic acid and its salts are widely used in beverage and food for preservation. Artificial sweeteners are widely used as sugar substitute in calorie-conscious societies, where their intake provides practically no calories and also helps fight obesity and its related ailments.

In most countries, the use of additives is regulated. In the U.S., most additives are part of the Generally Recognized As Safe (GRAS) ingredients although the FDA has established Acceptable Daily Intake (ADI) for each of them. There is a need for analytical techniques to identify and quantify additives because the food industry is required to list the type and amount of each ingredient on product labels to help consumers make dietary choices and manage food allergies.

This application note presents a fast and robust liquid chromatography method to simultaneously test nine widely used additives. Among the additives tested are: preservatives (benzoic acid, sorbic acid, dehydroacetic acid and methylparaben); artificial sweeteners (acesulfame potassium, saccharin and aspartame); flavoring agent (quinine); and a stimulant (caffeine). Method conditions and performance data including precision, accuracy and linearity are presented. The method is applied to a mouthwash and a tonic soda and the type and amount of additives are confirmed.

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Experimental

Nine stock standard solutions of each additive at 1 mg/mL concentration were prepared by dilution with water, followed by one minute vortex and five minutes sonication. A working standard of 0.1 mg/mL was prepared by transferring one mL of each of the stock solution into 10 mL volumetric flask. The solution was brought to volume with water and mixed well.

Precision was evaluated with five injections of the working standard. Linearity was determined across a range of 2.5 – 100 µg/mL. To assess accuracy, purified water was spiked with the working standard to obtain a 0.005 mg/mL solution. About 0.25 g/mL of a popular mouthwash and 0.5 g/mL of a tonic soda was prepared by dilution with water. 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) served as a platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particle) column.

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

Autosampler:	Flexar FX UHPLC
	Setting: 50 µL loop and 15 µL needle volume, partial loop mode, 350 µL mixer
	Injection: 2 µL; injector wash and carrier: water
PDA Detector:	Scanned from 190 – 400 nm, recording setting 214 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm 2.7 µm (superficially porous particles) at 45 °C, Part No. N9308402
Mobile Phase:	A: 20 mM sodium acetate in water adjusted to pH 4.57 with acetic acid
	B: acetonitrile

Time (min)	Flow rate (mL/min)	B %	Curve
6	0.4	5-30	1
1	0.4	40	1

	3 minutes equilibration after each run (HPLC grade solvent and ACS grade reagent)
Sampling Rate:	5 pts/s
Software:	Chromera® Version 3.0

Results And Discussion

The optimal flow rate of this method was determined to be 0.4 mL/min. at 45 °C and the pressure stabilized around 5500 PSI. All the peaks eluted within seven minutes. Prior to running the samples, from one injection of the working standard solution, the maximum wavelength of each peak was determined and the wavelength setting was optimized (see Figure 1, 2). The chromatogram of a popular mouth-wash tested is presented in Figure 3. Excellent method performance was achieved: the linearity of the analysis shows a R-squared of not less than 0.997 for each additive, and a precision relative standard deviation (%RSD) average of 0.84% with values ranging from 0.47% to 1.37%. The spiked purified water tested has an average recovery of 97.1% with value ranging from 91.3% to 108.7%. Details of the method performance and results of the samples tested are presented in Table 2.

Although in liquid chromatography peak identification is usually based on the retention time, Chromera's ability to collect and store spectra (Figure 4) 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 aspartame and quinine in the tonic soda sample is shown in Figure 5. In that figure, spectra at the peak apexes are compared to the spectra stored in the library. When a match is made, the name of the matching spectrum appears on each peak in question, confirming its identity.

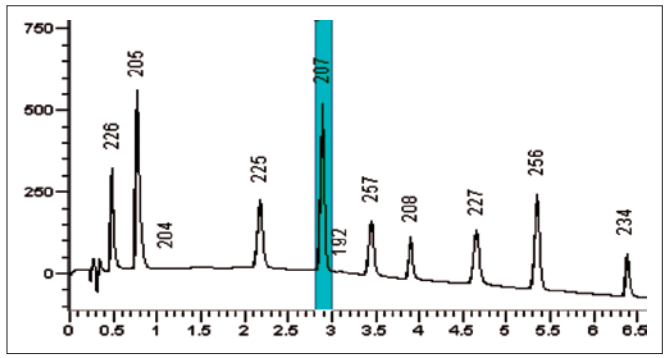

Figure 1. Chromatogram from the analyses of the standard solution with the maximum absorbance for each peak.

Table 1. **Precision, linearity, accuracy and samples.**

Compound	%RSD	r^2	Range (µg/mL)	Mouthwash (mg/12 oz)	Tonic Soda (mg/12 oz)	Spiked Recovery %
Acesulfame K	1.33	0.9997	2.5 – 100	ND	ND	108.7
Saccharine	0.88	0.9999	2.5 – 100	151	ND	94.1
Benzoic Acid	1.12	1	5.0 – 100	177	ND	93.1
Caffeine	0.57	0.9994	2.5 – 100	ND	ND	97.0
Sorbic Acid	0.80	0.9991	2.5 – 100	ND	ND	94.6
Aspartame	1.14	0.9965	5.0 – 100	ND	117	94.9
Dehydroacetic Acid	0.76	0.9994	5.0 – 100	ND	ND	99.5
Methylparaben	0.54	0.9999	2.5 – 100	ND	ND	100.3
Quinine	0.47	0.9967	5.0 – 100	ND	69	91.3
Average	**0.85**	**0.9990**	**NA**	**NA**	**NA**	**97.1**

ND = None detected *NA = Not applicable*

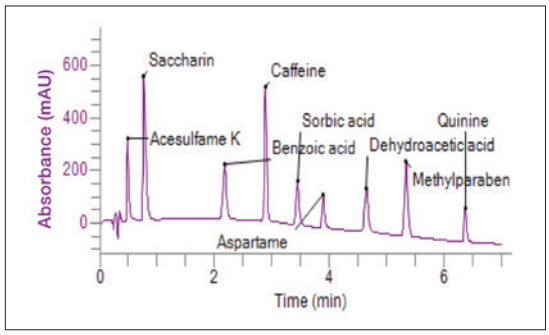

Figure 2. Chromatogram from the analysis of the standard solution.

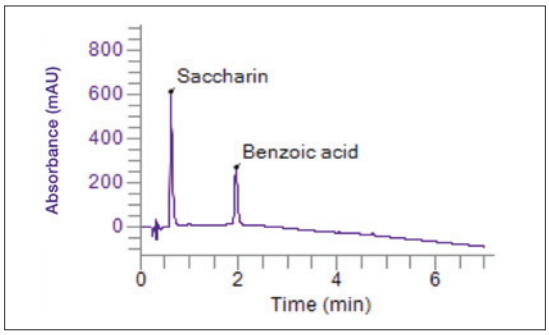

Figure 3. Chromatogram from the analyses of a popular mouthwash.

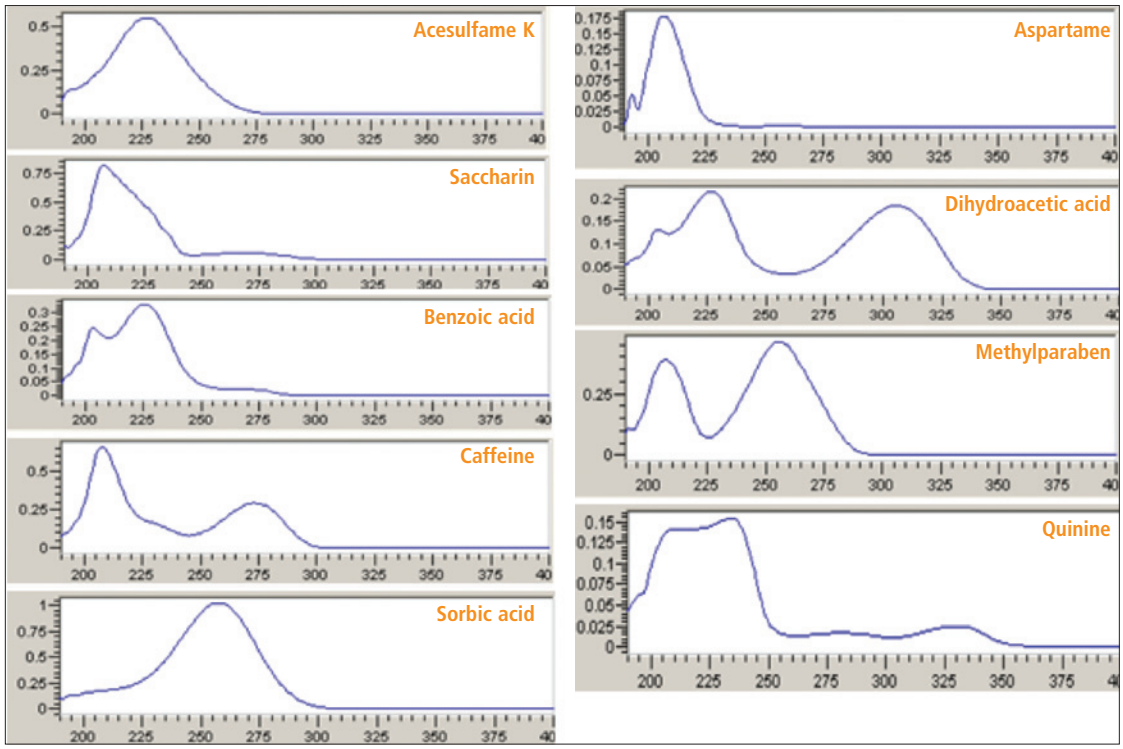

Figure 4. Spectra of the additives from the standard solution analysis.

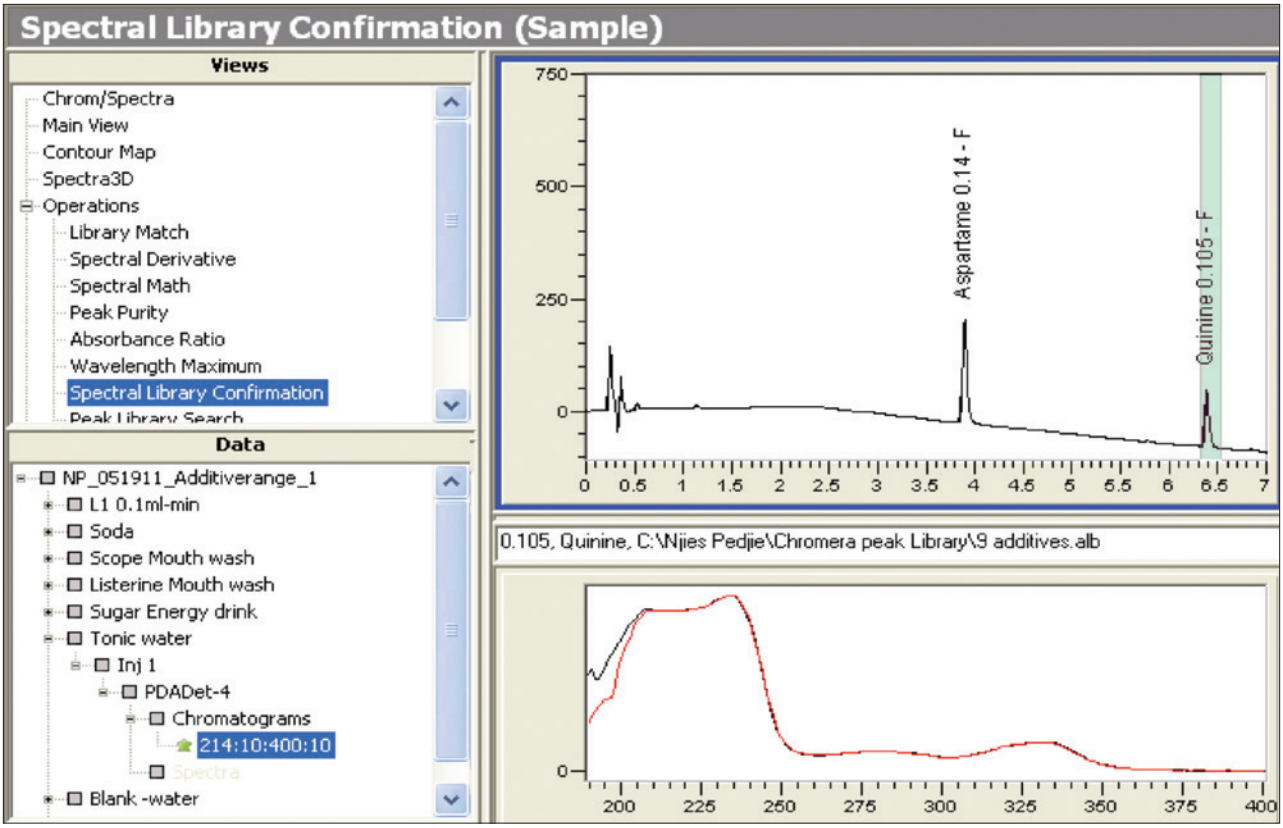

Figure 5. Peak identification in the tonic soda sample using Chromera spectral library.

Conclusion

The application of UHPLC to the analysis of nine additives resolves the nine components' peak within seven minutes. The method was shown to be linear with $r^2 \geq 0.997$, precise with %RSD ≤1.33; and accurate with an average recovery of 97.1%. The mouthwash tested is sweetened with 151 mg/12 oz saccharine and has 178 mg/12 oz of benzoic acid. The tonic soda is sweetened with 117 mg/12 oz of aspartame and has 69 mg/12 oz of benzoic acid. PerkinElemer's Flexar 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 3-D and contour maps, which are powerful tools that give insight to the information content of a 3-D 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 samples.

References

1. American Diabetes Association, 2007 National Diabetes Facts; Standards of Medical Care in Diabetes, 2008; Diabetes Care. 2009; 32:S13-S61, 2009.
2. FDA, Generally Recognized As Safe (GRAS) 21 CFR 170.30(b), 170.30(c) and 170.3(f).
3. Food Ingredient and Colors IFIC and FDA, November 2004, revised 2010.
4. Leo M.L. Nollet. Food Analysis by HPLC. Marcel Dekker, NY, 2000, pp. 99, 546.

Note: This application is subject to change without prior notice.

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