APPLICATION NOTE

Liquid Chromatography

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Analysis of Common Antioxidants in Edible Oil with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Phenolic antioxidants and ascorbyl palmitate (Figure 1, Page 2) are commonly used in food to prevent the oxidation of oils. Oxidized oils cause foul odor and rancidity in food products. This application note will present a UHPLC analysis of edible oils to determine the type and amount of ten different antioxidants.

The method was developed with a 1.9 µm particle size column to achieve very high throughput at a low flow rate, reducing solvent consumption. The throughput of an HPLC method with a 5 µm particle size column will be compared with that of a UHPLC method with a 1.9 µm particle size column. In addition to throughput comparisons, method conditions and performance data, including precision and linearity are presented. The results of the method applied to a spiked oil sample and a sample of vegetable shortening are reported.

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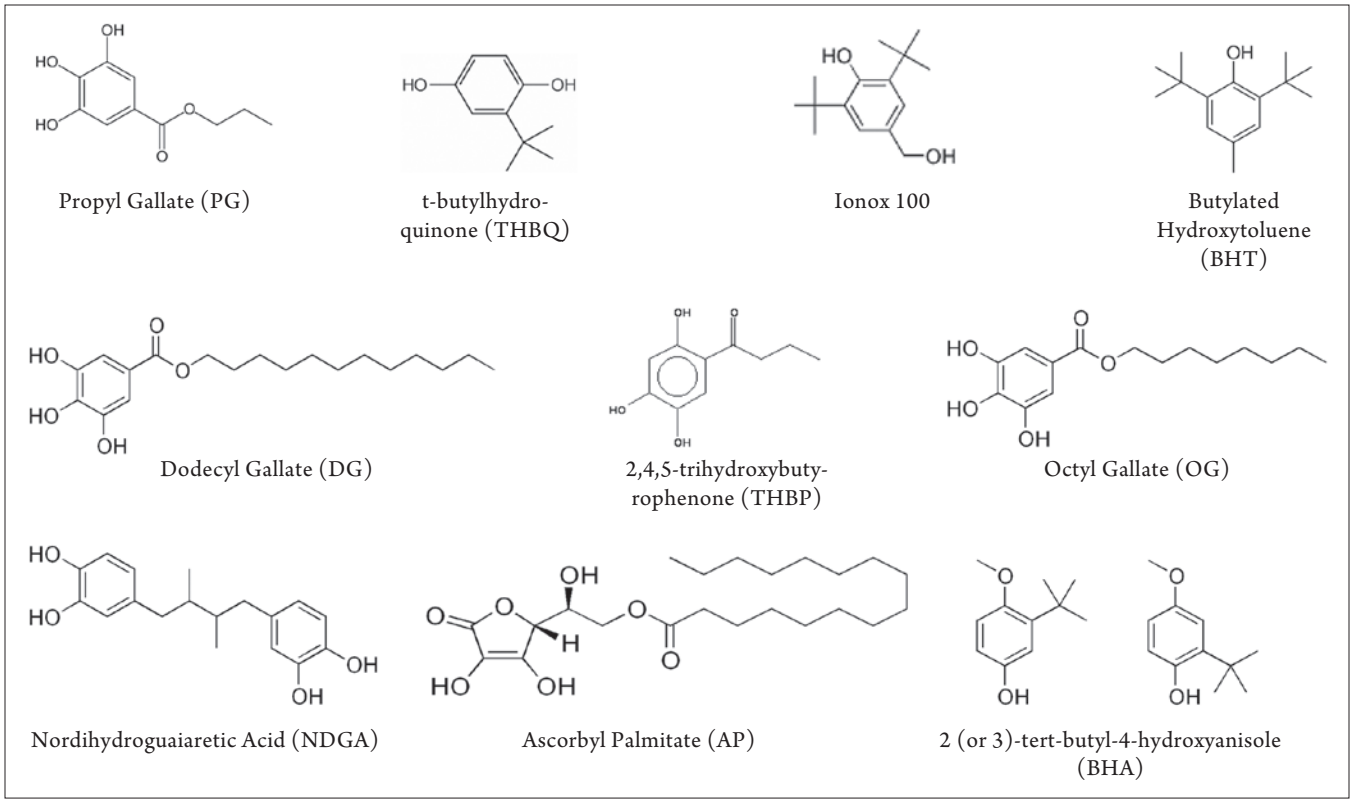

Figure 1. Names and codes of ten antioxidants.

Experimental

The separation was characterized and the system was calibrated with a mixture of antioxidants diluted from neat material. The stock solution contained 0.5 mg/mL of propyl gallate, octyl gallate, dodecyl gallate, nordihydroguaiaretic acid, 2 (or 3)-tert-butyl-4-hydroxyanisole, butylated hydroxy-toluene, 2, 6-di-ter-butyl-4-hydroxymethylphenol (Ionox 100) in methanol; a second stock contained about 0.5 mg/mL of 2,4,5-trihydroxybutyrophenone in methanol; a third stock contained about 0.5 mg/mL of t-butylhydroquinone in methanol; and a fourth stock contained about 0.5 mg/mL of ascorbyl palmitate in methanol with 1 mg/ml of citric acid and 1 mg/mL of isoascorbic acid. The isoascorbic acid and the citric acid as an oxygen quencher and chelating agent were added to methanol to prevent the degradation of ascorbyl palmitate. The working standard with 10 µg/mL of each antioxidant was prepared from the stock standards.

Repeatability was studied with six injections of each standard. Linearity was determined across the range of 0.2 – 10 µg/mL with injections at concentrations: 0.2, 0.5, 1, and 10 µg/mL. Recovery from the sample analysis was tested with canola/olive oil mixture samples spiked with 50 mg/kg of each anti-oxidant and a sample of 0.1 g/mL of vegetable shortening. The samples were diluted with methanol containing 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid, vortexed for five minutes and centrifuged at 5000 RPM for ten minutes. The supernatants were filtered with a 0.2 µm nylon filter prior to dispensing into UHPLC vials.

A PerkinElmer® Flexar™ FX-15 with Photodiode Array Detector provided the UHPLC platform for this application. The sepa-ration was completed on a Restek® Ultra II C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 3.3 min with a back pressure of about 7500 PSI (517 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:	10 µL conventional C18 HPLC column 2 µL C18 UHPLC column
Detector:	PDA detector 280 nm for phenolics antioxidants and 255 nm for ascorbyl palmitate
Pump:	FX-15
Column:	PerkinElmer C18, 5 µm, 100 x 4.6 mm Restek® Ultra II C18, 1.9 µm, 50 x 2.1 mm Cat # 9604252
Column Temperature:	Ambient, 45° C
Mobile Phase:	B: 70/30 (v/v) acetonitrile/methanol, A: 0.02% formic acid in water (HPLC grade solvent and ACS grade reagent)

Table 1. **Detailed UHPLC System and Chromatographic Conditions, *continued.***

Gradient:	**C18 Conventional HPLC column**			
	Time (min)	**Flow (mL/min)**	**B%**	**Curve**
	0.5	1.8	35	1
	2	1.8	35-45	1
	2	1.8	45-100	1
	2.5	1.8	100	1
	C18 UHPLC column			
	Time (min)	**Flow (mL/min)**	**B%**	**Curve**
	0.3	0.7	40	1
	1	0.7	40-75	1
	1	0.7	75-100	1
	1	0.7	100	1
Software:	Chromera® Version 3.0			
Sampling Rate:	5 pts/s			

Results And Discussion

Initially the method was developed with a C18 100 x 4.6 mm conventional HPLC column with 5 µm particle size. The optimal flow rate of this method was determined to be 1.8 mL/min. at ambient temperature. All the antioxidants eluted in seven min (Figure 2). By using a UHPLC shorter column with smaller particle-size (C18 50 x 2.1 mm, 1.9 µm particle size column) the run time was dramatically reduced from 7 min to 3.3 min; the optimal flow rate was 0.7 mL/min and the temperature 45 °C.

The total solvent usage for each injection was 2.3 mL, an impressive improvement from 12.6 mL solvent usage when the conventional HPLC column was used. Thus, more than 80% reduction in solvent usage and more than 50% reduction in testing time was achieved by moving to the UHPLC method. This is important because of the relative high cost of HPLC grade solvent as well as the significant cost of its disposal, resulting in a much lower cost of ownership and a much greener laboratory operation. Representative chromatograms of the standard solution analysis under UHPLC conditions showing the wavelength maximum are presented in Figure 3.

A spectrum of each antioxidant was obtained from the analysis of the standard solution over a range of 190 nm to 700 nm. The spectrum of two antioxidants and an annotated chromatogram of the standard solution under UHPLC conditions are presented in Figures 4 and 5. A representative chromatogram of the spiked canola/olive oil is presented in Figure 6.

Confirming the identity of compounds in the chromatogram of a known or an unknown sample is an important aspect of quality assurance. Confirmation of the presence of TBHQ in the vegetable shortening is demonstrated in Figure 7. In that figure, the spectrum of the peak apex of the highlighted peaks is compared to the spectra of the standard to confirm peak identity.

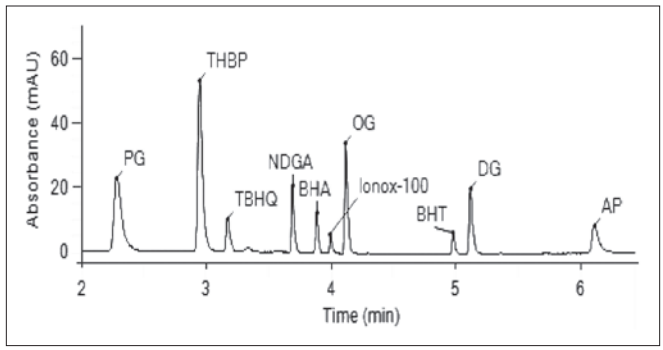

Figure 2. Chromatogram from the analysis standard solution with 10 antioxidants using a C18 HPLC column.

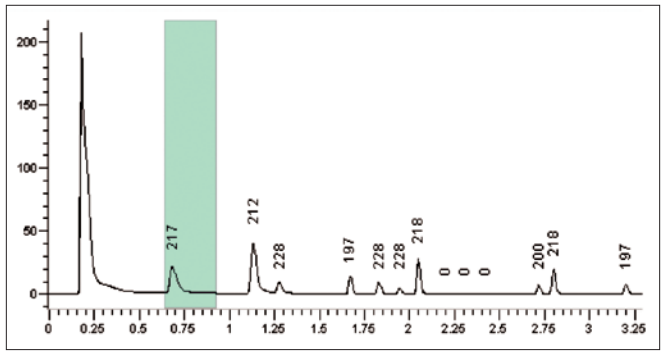

Figure 3. Chromatogram from the analysis of a standard with a C18 UHPLC column showing maximum wavelength absorbance for each peak.

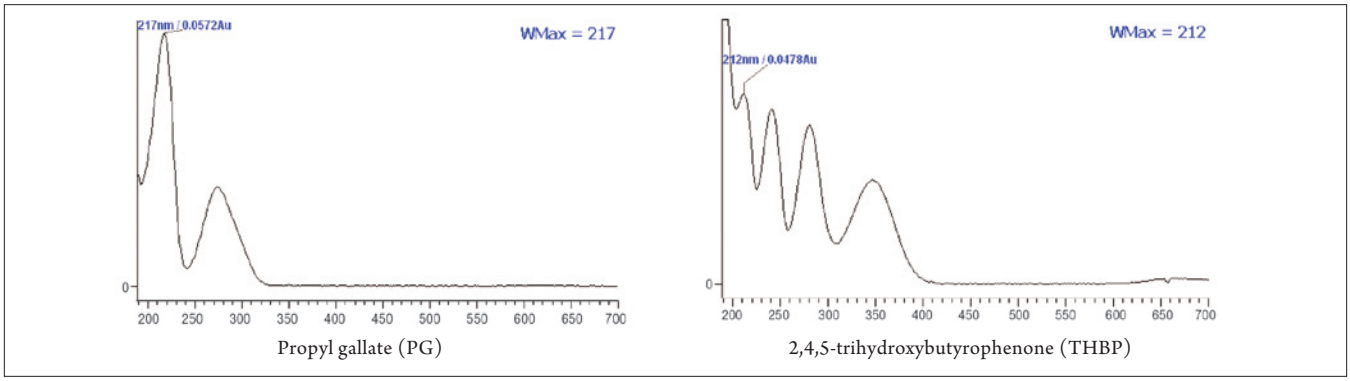

Figure 4. Stored spectra of two antioxidants from the analysis of a standard solution.

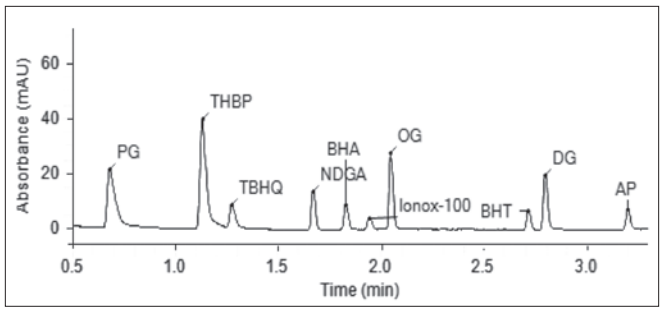

Figure 5. The chromatogram from the analysis standard solution of 10 antioxidants with a C18 UHPLC column.

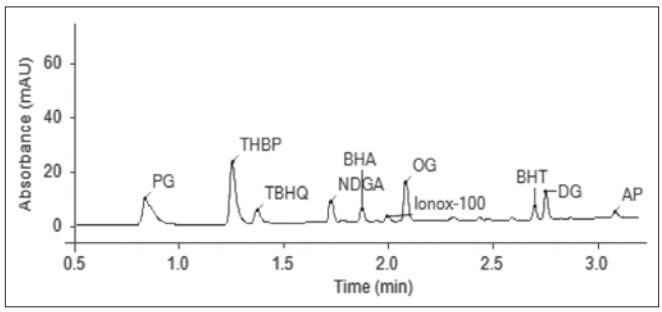

Figure 6. Chromatogram of the analysis of canola/olive oil spiked with 10 antioxidants with a C18 UHPLC column.

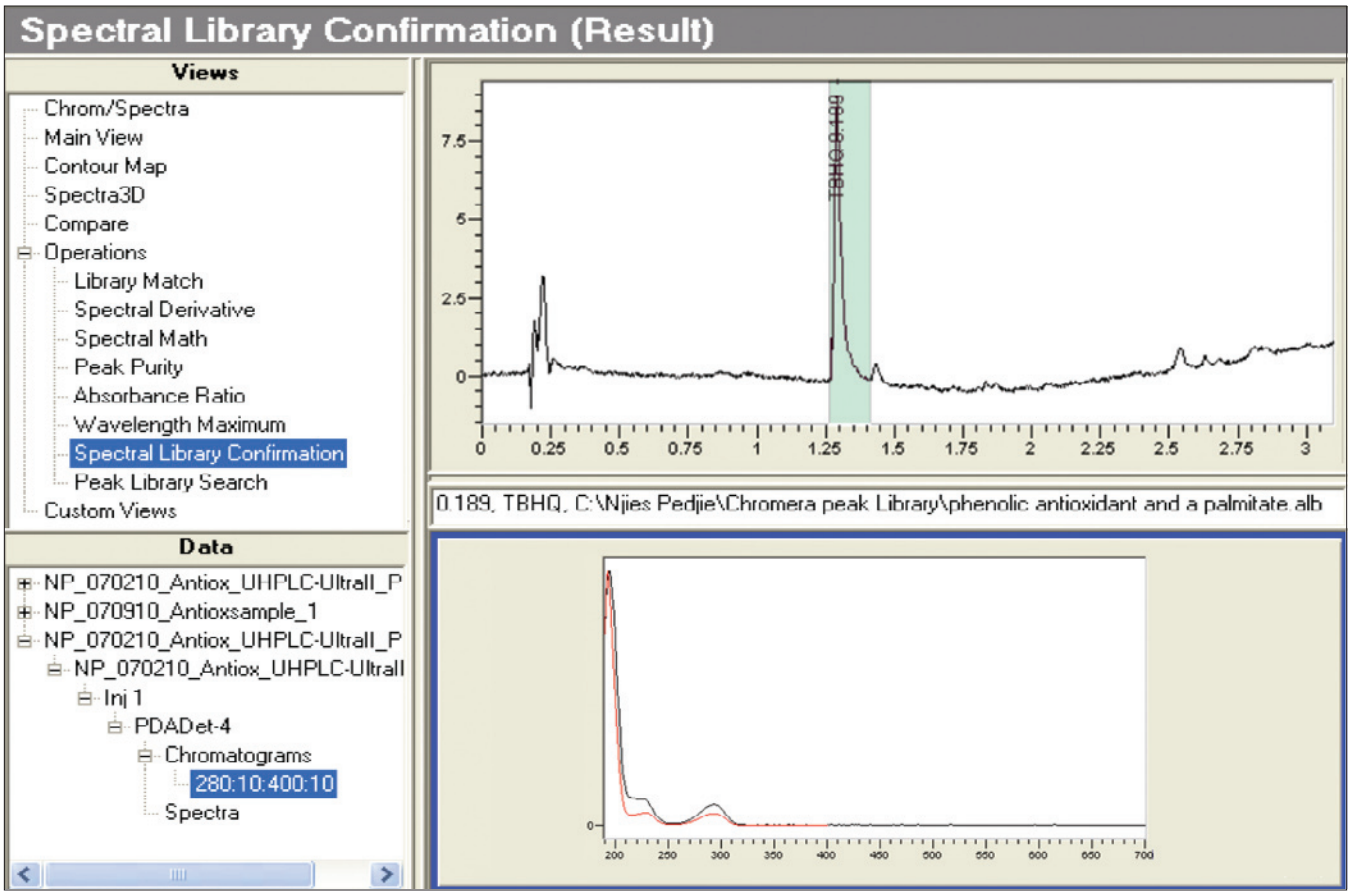

Figure 7. Chromatogram of the analysis of vegetable shortening and the spectra confirmation using a Chromera PDA spectral library.

The method performance was outstanding. The linearity of the analysis achieved an average r^2 value of 0.998. The precision was less than 2.0% relative standard deviation (n=6) for each antioxidant. The canola/olive oil sample resulted in recovery results from 70% and 113%, with an average recovery of approximately 97%. The vegetable shortening tested has 0.01% of TBHQ. Details of the method performance are presented in Table 2.

***Table 2.* Precision, Linearity and Recovery.**

Compound	% RSD (n=6)	Linearity r^2	Canola/olive oil recovery	Vegetable shortening
PG	0.8	0.9992	87%	ND
THBP	1.4	0.9991	99%	ND
TBHQ	1.7	0.9948	96%	0.01%
NDGA	1.1	0.9975	99%	ND
BHA	1.8	0.9991	100%	ND
Ionox-100	1.5	0.9992	96%	ND
OG	1.6	0.9992	103%	ND
BHT	1.3	0.9951	70%	ND
DG	1.5	0.9947	113%	ND
AP	1.1	0.9992	103%	ND

ND = None detected

Conclusion

The application of UHPLC to the analysis of common antioxidants in edible oils has resulted in a 53% reduction in run time as well as an 82% reduction in solvent usage per sample. The PerkinElmer Flexar FX-15 UHPLC and Restek® Ultra II C18, 1.9 µm 50 x 2.1 mm column resolved all antioxidants in about three minutes. All the peaks were well resolved and the method was shown to be linear. The spiked sample recovery was good and the vegetable shortening tested met the TBHQ requirement of not more than 0.02% based on fat content set by the U.S. Code of Federal Regulation. PerkinElmer's PDA provides a rugged and accurate detection over a range of 190 nm to 700 nm encompassing UV and Vis wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, 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 the antioxidant spectra that was later used for peak identification and confirmation in the vegetable shortening sample.

References

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