

Liquid Chromatography

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Analysis of Phenolic Antioxidants in Edible Oil/Shortening Using the PerkinElmer Altus UPLC System with PDA Detection

Introduction

Phenolic antioxidants are commonly used in food to prevent the oxidation of oils. Oxidized oil and fats cause foul odor and rancidity in food products, which is a major cause for concern to the food industry. Globally, regulations vary, but current maximum allowable levels are as low as 100 µg/g (100 ppm).

This application note presents a UHPLC method for the analysis of the ten most common phenolic

antioxidants that may be found in such products. The application was carried out with minor modifications to the AOAC Official Method 983.15⁽¹⁾. This method applies to the analysis of finished food products. A 2.7-µm SPP (superficially porous particle) C18 column was used, allowing one to achieve very high throughput at a back-pressure considerably lower than that for UHPLC columns.

This method was then applied to a commercial vegetable shortening product, which per label claim, was reported to contain at least one of the antioxidants being analyzed.

Method conditions and performance data, including linearity and repeatability, are presented.

Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer® Altus™ UPLC® System was used, including the Altus A-30 Solvent delivery Module, Sampling Module, A-30h Column Module and PDA (photodiode array) Detector with a 10-mm path-length flow cell. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method parameters

The HPLC method parameters are shown in Table 1.

Table 1. UHPLC Method Parameters

HPLC Conditions																																																	
Column:	PerkinElmer Brownlee™ 2.7 µm 2.1 x 100 mm C18 (Part# N9308404)																																																
Mobile Phase:	Solvent A: Water; Solvent B: Acetonitrile Solvent program:																																																
	<table border="1"><thead><tr><th></th><th>Time (min)</th><th>Flow Rate (mL/min)</th><th>0%A</th><th>%B</th><th>%C</th><th>%D</th><th>Curve</th></tr></thead><tbody><tr><td>1</td><td>Initial</td><td>0.600</td><td>60.0</td><td>40.0</td><td>0.0</td><td>0.0</td><td>Initial</td></tr><tr><td>2</td><td>4.50</td><td>0.600</td><td>45.0</td><td>55.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>3</td><td>7.00</td><td>0.600</td><td>18.0</td><td>82.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>4</td><td>10.00</td><td>0.600</td><td>18.0</td><td>82.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>5</td><td>10.10</td><td>0.600</td><td>60.0</td><td>40.0</td><td>0.0</td><td>0.0</td><td>11</td></tr></tbody></table>		Time (min)	Flow Rate (mL/min)	0%A	%B	%C	%D	Curve	1	Initial	0.600	60.0	40.0	0.0	0.0	Initial	2	4.50	0.600	45.0	55.0	0.0	0.0	6	3	7.00	0.600	18.0	82.0	0.0	0.0	6	4	10.00	0.600	18.0	82.0	0.0	0.0	6	5	10.10	0.600	60.0	40.0	0.0	0.0	11
	Time (min)	Flow Rate (mL/min)	0%A	%B	%C	%D	Curve																																										
1	Initial	0.600	60.0	40.0	0.0	0.0	Initial																																										
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5	10.10	0.600	60.0	40.0	0.0	0.0	11																																										
	Equil. Time ("Next inj. Delay Time"): 3 minutes																																																
Analysis Time:	10 min.																																																
Flow Rate:	0.6 mL/min. (maximum pressure during run: 6600 psi)																																																
Oven Temp.:	35 °C																																																
Detection:	Altus A-30 PDA; wavelength channels: 280 and 220 nm																																																
Injection Volume:	1 µL																																																

Solvents, Standards and Samples All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The phenolic antioxidant standard kit #2 (catalog# 40048-U) was obtained from Supelco® (Irvine, CA). This included nordihydroguaiaretic acid (NDGA), propyl gallate (PG), octyl gallate (OG), lauryl gallate (dodecyl gallate (DG)), 2-tert-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-hydroxymethylphenol (Ionox 100), tert-butylhydroquinone (TBHQ), 3,5-di-*t*-butyl-4-hydroxytoluene (BHT) and ethoxyquin. In addition, a 2,4,5-trihydroxybutyrophenone standard (THBP; catalog# 2620-1-X9) was obtained from SynQuest® (Alachua, FL).

Using a 100-mL volumetric flask, a 100-ppm stock standard was made up by dissolving 10 mg of each of the ten antioxidant standards in methanol and then bringing the flask up to the mark with methanol. Individual calibrant standards were prepared using the 100-ppm stock solution.

The sample ("Sample X") was a commercially available vegetable shortening purchased at a local food market. The sample was prepared by dissolving 3 grams of Sample X in 15 mL of hexane in a 50-mL centrifuge tube and vortexing for 5 minutes. The resulting solution was then extracted with three 30-mL portions of acetonitrile, combining the three extracts into a 250-mL evaporation dish. The combined extract was evaporated down to 1-2 mL and reconstituted to 6 mL with methanol.

Prior to injection, all calibrants and samples were filtered through 0.22-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 10 phenolic antioxidants in under nine minutes. Figure 2 shows the overlay of 10 replicate 50-ppm standard injections,

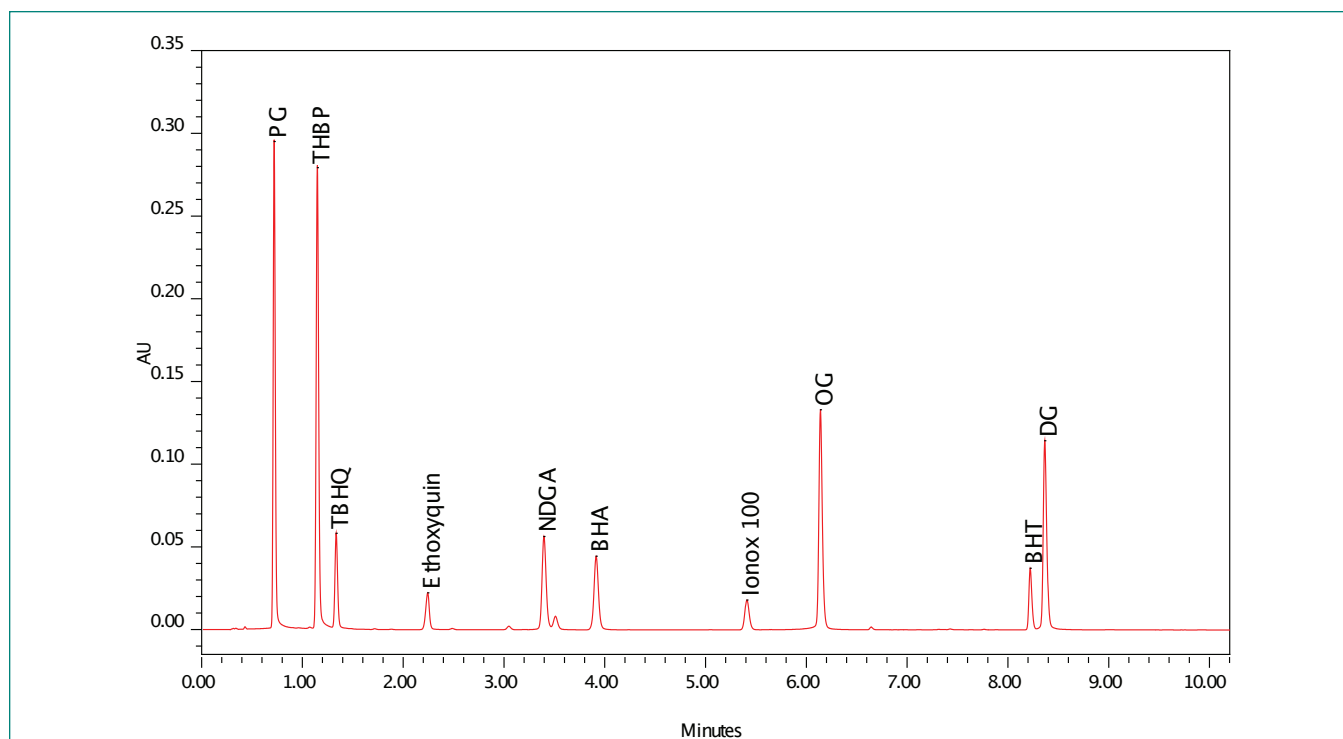


Figure 1. Chromatogram of 50-ppm phenolic antioxidant standard; wavelength = 280 nm.

demonstrating exceptional reproducibility. Retention time % RSDs ranged from 0.10 (early eluters) to 0.03 (later eluters).

In a previous application note⁽²⁾, it has been noted that ethoxyquin may not be well detected at 280 nm. However, we did not observe this, and we could easily detect the analyte at 5-ppm levels. The same injection was also captured on a

separate channel, set to 220 nm, as shown in Figure 3. At this wavelength, it is evident that the ethoxyquin has approximately two times the signal intensity. However, this additional signal intensity was not really required here, as current maximum allowable concentrations for phenolic antioxidants only go down to 100 ppm, which was easily handled at 280 nm.

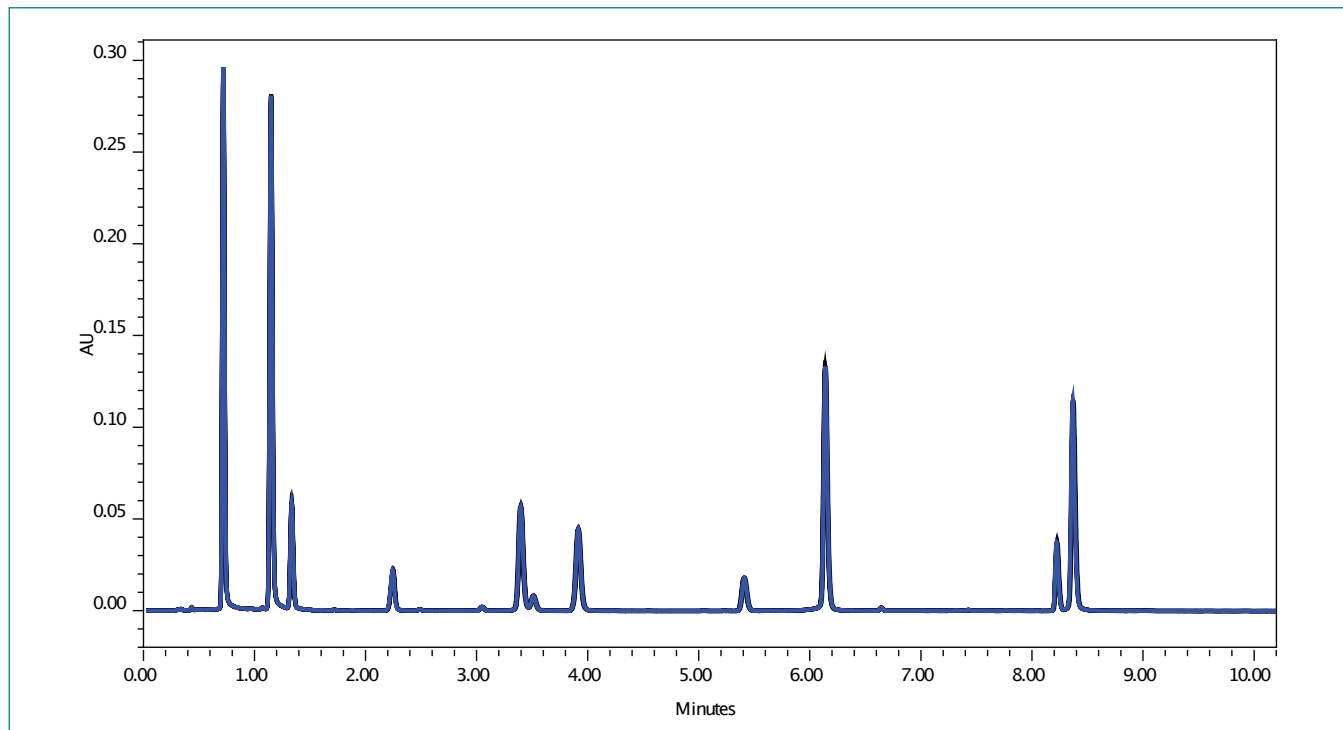


Figure 2. Overlay of 10 replicates of 50-ppm check standard; wavelength = 280 nm.

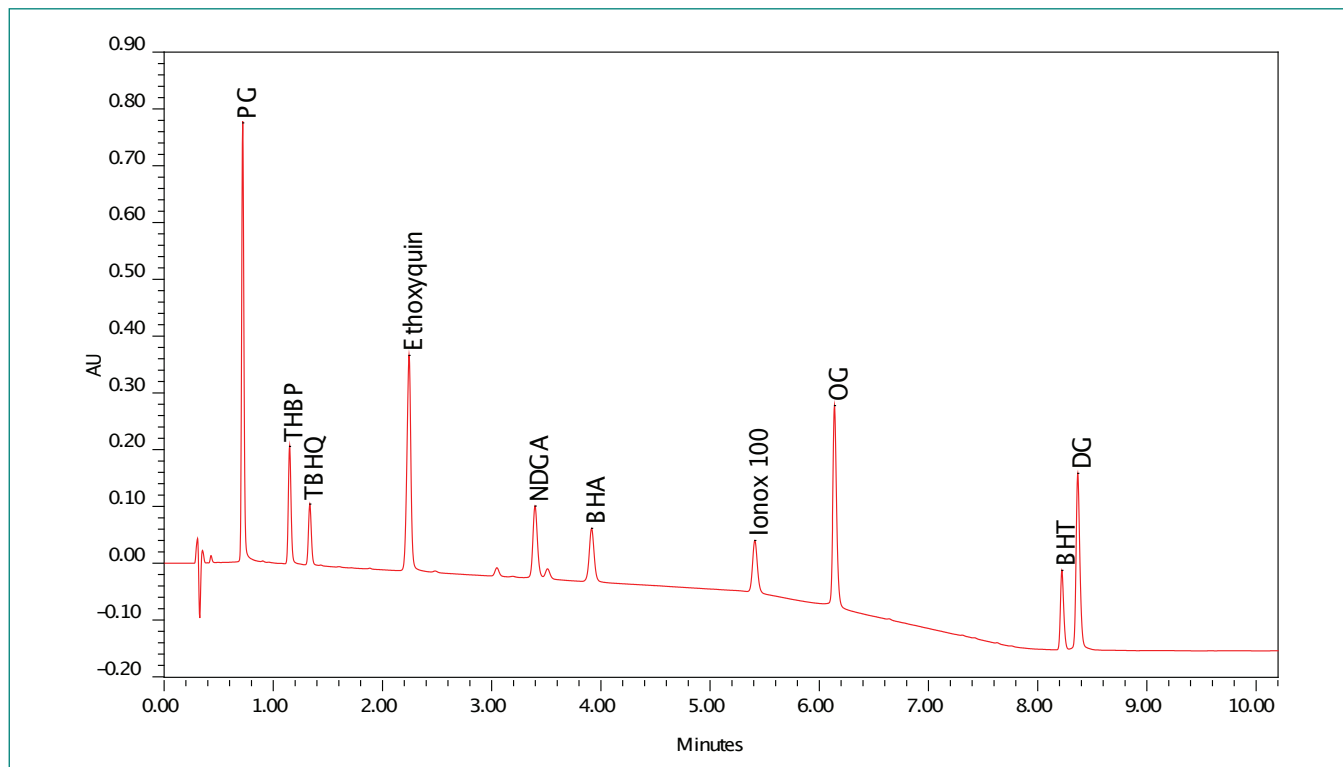


Figure 3. Chromatogram of 50-ppm phenolic antioxidant standard; wavelength = 220 nm.

Figure 4 shows three representative calibration results over a concentration range of 5 to 100 ppm. All ten components had linearity coefficients > 0.999 (n = 3 at each level).

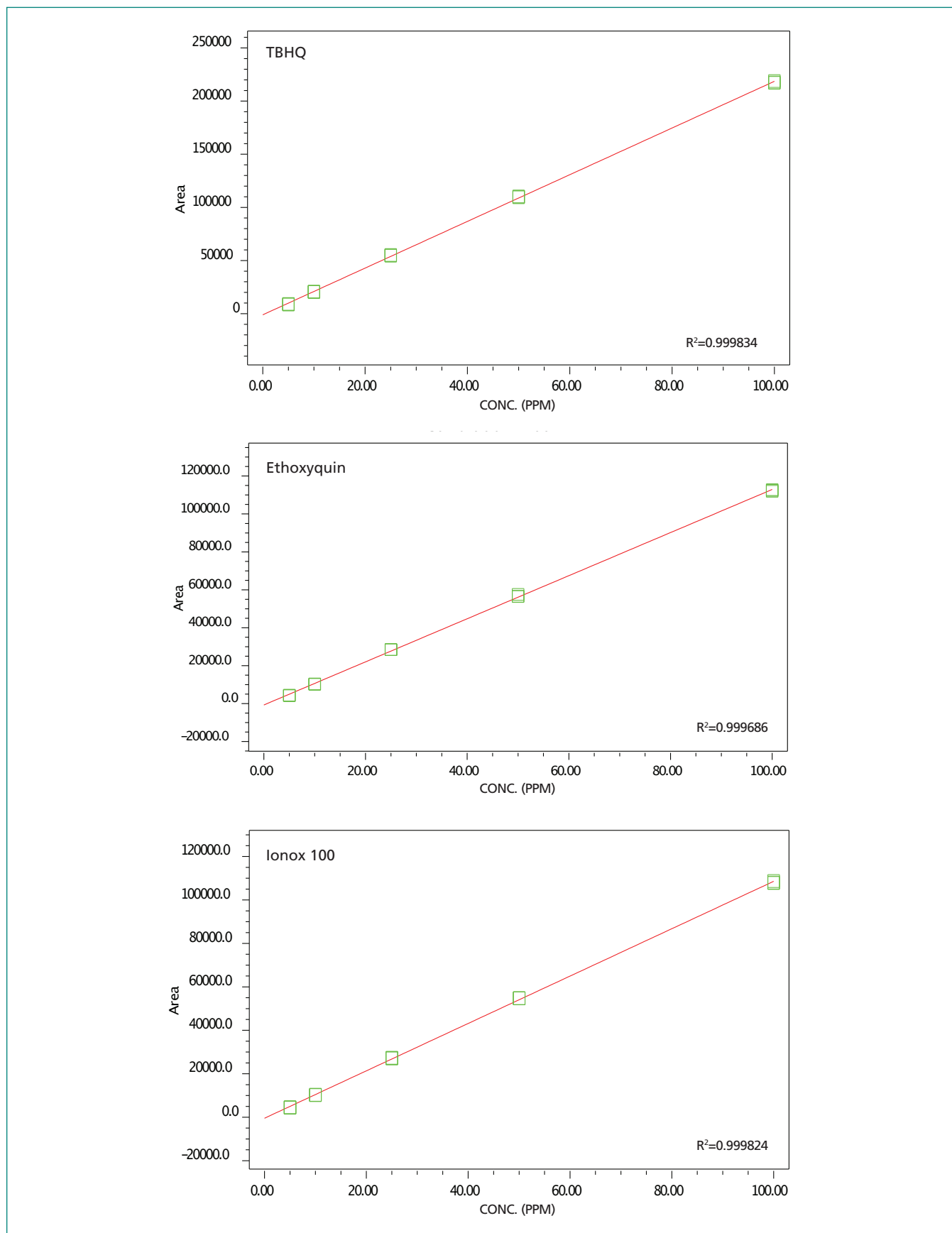


Figure 4. Three representative results of 5-level calibration sets for the phenolic antioxidants; wavelength = 280 nm.

Figure 5 shows the chromatographic results of Sample X overlaid with the 50-ppm standard. A peak eluting at exactly the time of TBHQ (tert-butylhydroquinone) was observed. This was consistent with the product label claim. By back-calculating the concentration in the original sample, it was determined that Sample X contained approximately 12-ppm of TBHQ. The actual concentration could not be verified as it was not provided in the product's label claim.

Per Figure 6, upon closer examination of the chromatogram of Sample X, a small peak at about 8.23 minutes was also observed. This matched the elution time for DG (dodecyl gallate) in the standard mix. If this was indeed DG, its concentration was below the calibration curve, estimated to be <0.5 ppm. Further verification of the identity of this peak was not pursued.

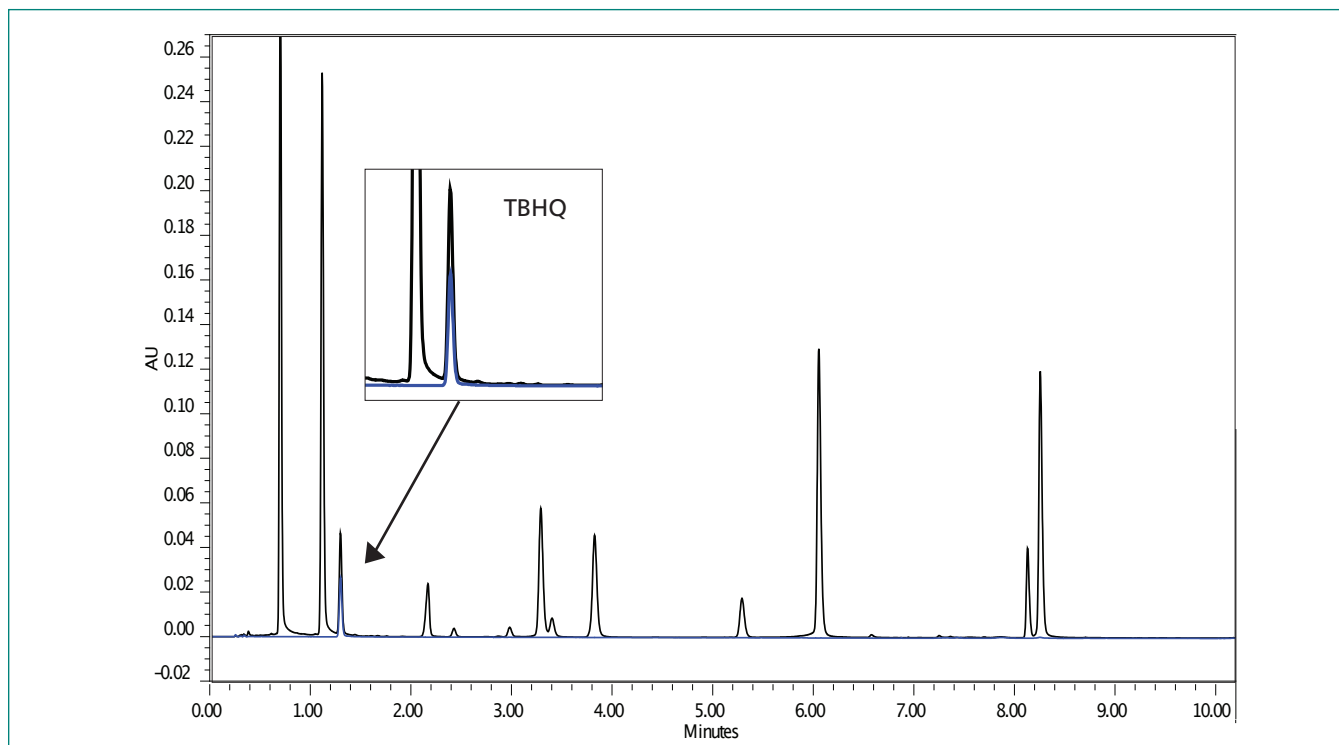


Figure 5. Chromatogram of Sample X (blue) overlaid with 50-ppm standard (black); wavelength = 280 nm.

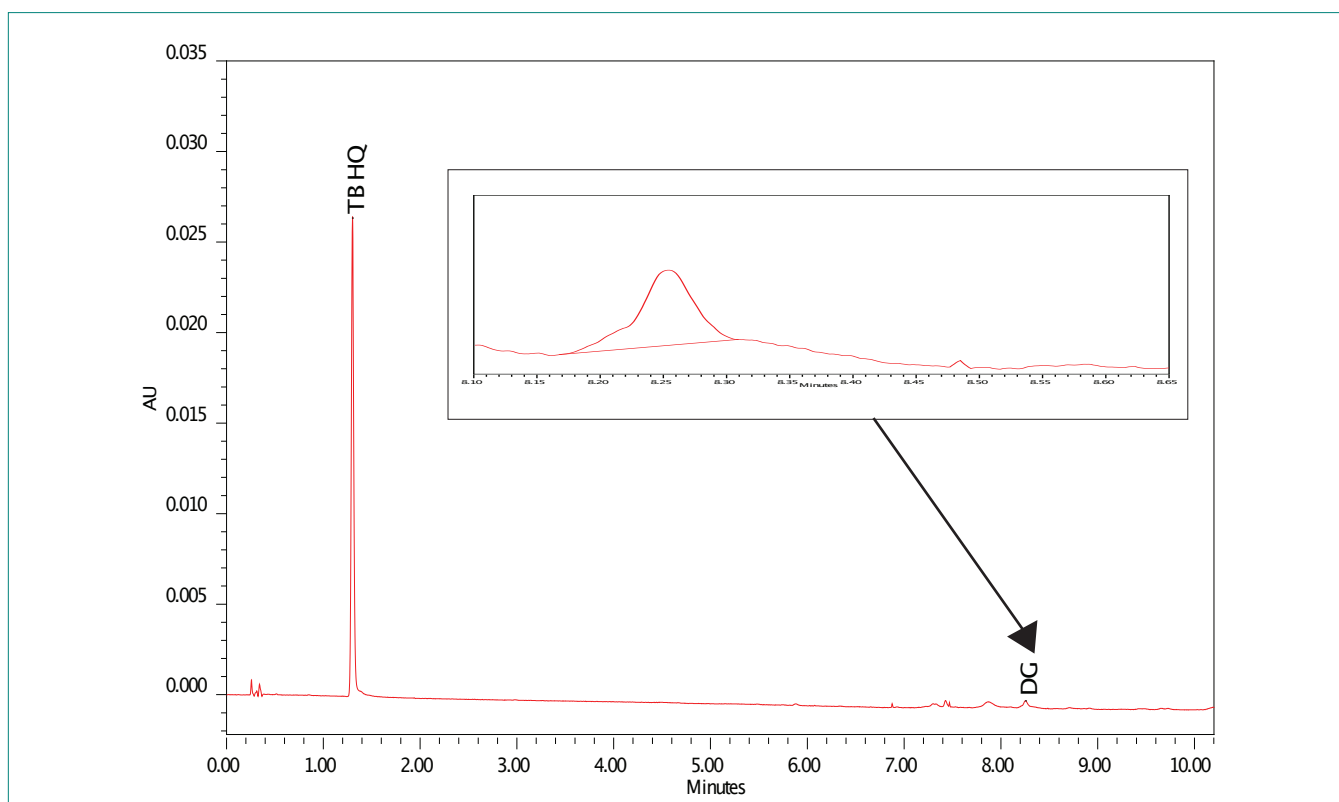


Figure 6. Chromatogram of Sample X with zoomed in area just after 8 minutes; wavelength = 280 nm.

Conclusion

This work has demonstrated the effective chromatographic separation of ten phenolic antioxidants using a PerkinElmer Altus UPLC® with a PDA detector and the Empower® 3 CDS system. The results exhibited excellent retention time repeatability as well as exceptional linearity over the tested concentration ranges. At an analytical wavelength of 280 nm, the sensitivity for all 10 phenolic antioxidants was found to be more than adequate to accommodate the current maximum allowable concentration limit of 100 ppm.

We were able to identify and quantitate the phenolic antioxidant content in a commercial vegetable shortening product and the results matched the label claim of the manufacturer.

From a food quality perspective, considering the ever growing emphasis on food monitoring, this application is intended to serve as a valuable guide for the monitoring of edible oils/shortening. It should be noted that in the U.S., per label claims, only some of the vegetable shortenings reported any amount of phenolic antioxidant. None of the edible oils that were found in stores reported any phenolic antioxidants. However, although only edible vegetable shortening was tested for this study, the provided sample preparation procedure and chromatographic application easily lend themselves to the analysis of edible oils as well.

References

1. Official Methods of Analysis, Method 983.15, Association of Official Analytical Chemists (AOAC), Arlington, VA USA
2. Monitor Antioxidant Additives in Foods, Using HPLC (AOAC Method 983.15) or Capillary GC and a Supelco Reference Standards Kit, Application Note 78, Sigma-Aldrich/Supelco, 2004
3. Analysis of Common Antioxidants in Edible Oil with the PerkinElmer Flexar™ FX-15 System Equipped with a PDA, Application Note, PerkinElmer, Inc.