

## Liquid Chromatography

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## Rapid UHPLC Determination of Common Antioxidants in Edible Oils

### Introduction

Phenolic antioxidants and ascorbyl palmitate (Figure 1 – Page 2) are commonly used in food to prevent the oxidation of oils. Oxidized oils will 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  $\mu\text{m}$  column to achieve very high throughput at a low flow rate, reducing solvent consumption. The throughput of an HPLC method with a 5  $\mu\text{m}$  particle-size column will be compared with that of a UHPLC method and 1.9  $\mu\text{m}$  particle-size column. In addition to throughput comparisons, method conditions and performance data, including precision, linearity, and recovery from spiked samples, will be presented.

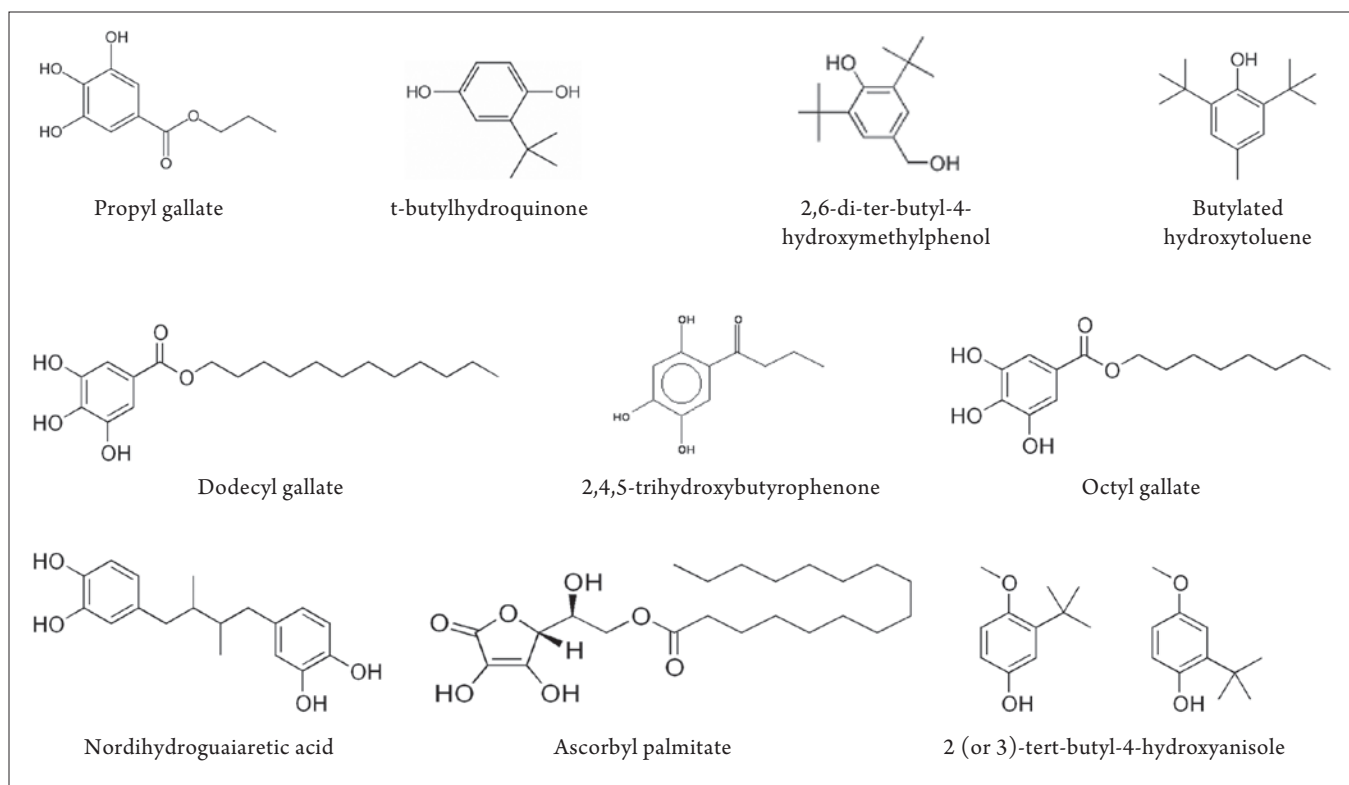


Figure 1. Names and codes of antioxidants.

## Experimental

The separation was characterized and system calibrated with a mixture of antioxidants diluted from neat material. One stock solution contained 0.5 mg/mL of propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), nordihydroguaiaretic acid (NDGA), 2 (or 3)-tert-butyl-4-hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,6-di-tert-butyl-4-hydroxymethylphenol (Iox 100) in methanol; a second stock contained about 0.5 mg/mL of 2,4,5-trihydroxybutyrophenone (THBP) in methanol; a third stock contained about 0.5 mg/mL of t-butylhydroquinone (THBQ) in methanol; and a fourth stock contained about 0.5 mg/mL of ascorbyl palmitate (AP) 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 respectively were added to the methanol to prevent the degradation of ascorbyl palmitate. Working standards with 10 µg/mL of each antioxidant were prepared from the stock standards.

Repeatability was studied with six injections of the working standard. Linearity was determined across the range of 0.2 – 10 µg/mL, with injections at: 0.2, 0.5, 1, and 10 µg/mL. Recovery from sample analysis was tested with oils samples spiked with 50 mg/kg of each antioxidant. A canola-oil and a corn-oil sample were tested. The samples were diluted

with methanol containing 1 mg/mL of citric acid and 1 mg/mL of isoascorbic acid, vortexed for 5 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant were filtered with a 0.2 µm nylon syringe filter prior to dispensing into UHPLC vials.

A PerkinElmer® Flexar™ FX-15 with a Flexar UV/Vis detector provided the UHPLC platform for this application. The separation was completed on a PerkinElmer Brownlee™ Analytical C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 2 min with a back pressure of 8000 psi (552 bar).

## Results and Discussion

Initially, the method was developed with phosphoric acid as the modifier in mobile phase A and samples were run using a C18 100 x 4.6 mm, 5 µm particle-size column. The optimal flow rate of this method was determined to be 1.8 mL/min at ambient temperature. All the antioxidants eluted in 7 min. By using a UHPLC shorter column with smaller particle size (C18 50 x 2.1 mm, 1.9 µm particle size), the run time was dramatically reduced from 7 min to about 2 min. The resolution of analyte peaks and sensitivity of the determination were improved by changing the phosphoric acid modifier to formic acid. The optimal flow rate with formic acid was 0.7 mL/min at a temperature of 44 °C. An improved separation with sharper peaks and better signal-to-noise characteristics was obtained.

<b>Table 1. Detailed UHPLC system and chromatographic conditions.</b>		
<b>Autosampler:</b>	Flexar FX UHPLC, Part No. N2930664	
	Setting: 50 $\mu$ L loop and 15 $\mu$ L needle volume, partial loop injection	
	Injection: 10 $\mu$ L C18 Conventional HPLC column 2 $\mu$ L C18 HPLC column	
<b>Detector:</b>	Flexar UV/Vis Detector, Part No. N2920013	
	280 nm for phenolics antioxidants and 255 nm for ascorbyl palmitate	
<b>Pump:</b>	Flexar FX-15, Part No. N2910531	
<b>Columns:</b>	PerkinElmer Brownlee Analytical C18, 1.9 $\mu$ m, 50 x 2.1 mm, Part No. N9303853	
	PerkinElmer C18, 5 $\mu$ m, 100 x 4.6 mm	
<b>Column temperature:</b>	Ambient, 44 °C	
<b>Mobile phase:</b>	B: 70/30 (v/v) acetonitrile/methanol, A: 1% phosphoric acid in water	
	B: 70/30 (v/v) acetonitrile/methanol, A: 0.02% formic acid in water	
	HPLC and ACS <sup>®</sup> reagent-grade solvents	
<b>Flow rate:</b>	1.8 mL/min C18 Conventional HPLC column	
	0.7 mL/min C18 UHPLC column	
<b>Gradient:</b>	A with phosphoric acid modifier	A with formic acid modifier
	(C18 Conventional HPLC column)	(C18 UHPLC column)
	0.5 min 35% B	0.3 min 38% B
	2 min 35% - 45% B	0.5 min 38% - 70% B
	2 min 45% - 100% B	0.7 min 70% - 100% B
	2.5 min 100% B	0.7 min 100% B
<b>Software:</b>	Chromera <sup>®</sup> Version 2.1.0.1631	
<b>Sampling rate:</b>	50 pts/s	

The final analysis was completed in 2.2 minutes with a total solvent usage of 1.5 mL for each injection, an impressive improvement from 7 min run time and 12.6 mL solvent usage when the conventional HPLC column was used. Representative chromatograms of standard solution analysis under conventional HPLC and UHPLC conditions are presented in Figures 2 and 3 (Page 4), representative chromatograms of spiked canola oil and corn oil under UHPLC conditions are presented in Figures 4 and 5 (Page 4).

The method performance was outstanding. The linearity of the analysis achieved an average  $r^2$  value of 0.998. The average precision was less than 1% relative standard deviation (n=6). The sample preparation resulted in recovery results between 97% and 114% for both corn and canola oils, with an average recovery of approximately 103%. Details of the method performance are presented in Table 2.

## Conclusion

The application of UHPLC to the analysis of common antioxidants in edible oils has resulted in a 4.8 min or about 70% reduction in run time as well as a reduction of solvent usage of 11.1 mL or about 90%. The PerkinElmer Flexar FX-15 UHPLC system and Brownlee Analytical C18, 1.9  $\mu$ m 50 x 2.1 mm column resolved all antioxidants in about 2 minutes. The method was shown to be linear, the antioxidant peaks were well resolved and the recovery was good.

## Reference

- Perrin C., and Meyer L., *J. Am. Oil Chem*, vol 80, no.2 (2003) 115-118.

<b>Table 2. Precision, linearity and recovery.</b>										
	PG	THBP	TBHQ	NDGA	BHA	Ionox-100	OG	BHT	DG	AP
<b>Precision (% RSD)</b>	0.9	0.7	0.9	0.8	1.1	1.0	0.8	1.3	1.0	1.3
<b>Linearity (R<sup>2</sup>)</b>	0.9996	0.9999	0.9991	0.9985	0.9990	0.9931	0.9999	0.9944	0.9959	0.9999
<b>Corn oil recovery (%)</b>	107	102	103	102	102	101	101	100	100	105
<b>Canola oil recovery (%)</b>	97	105	105	105	105	104	106	108	106	114

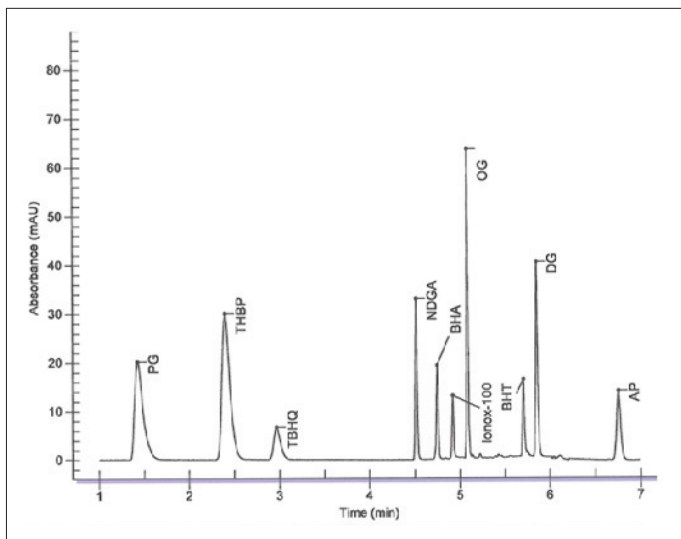


Figure 2. Chromatogram from the analysis of a standard solution with 10  $\mu\text{g/mL}$  of 10 antioxidants using a conventional HPLC C18 100 x 4.6 mm, 5  $\mu\text{m}$  particle-size column.<sup>1</sup>

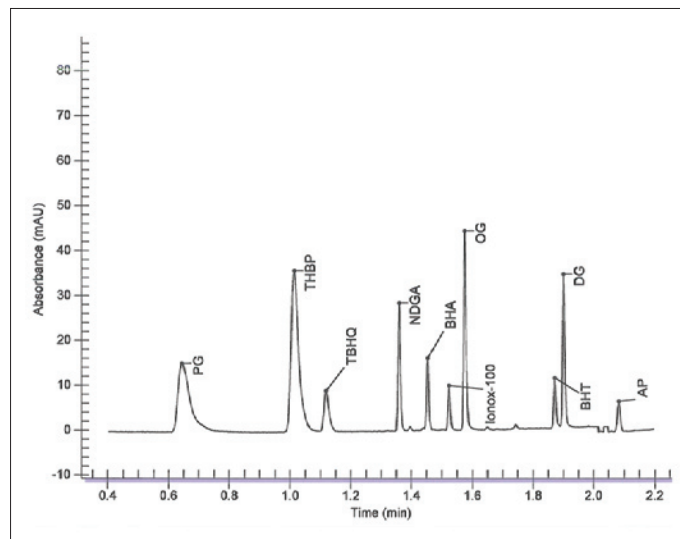


Figure 4. Chromatogram from the analysis of canola oil spiked with common antioxidants.

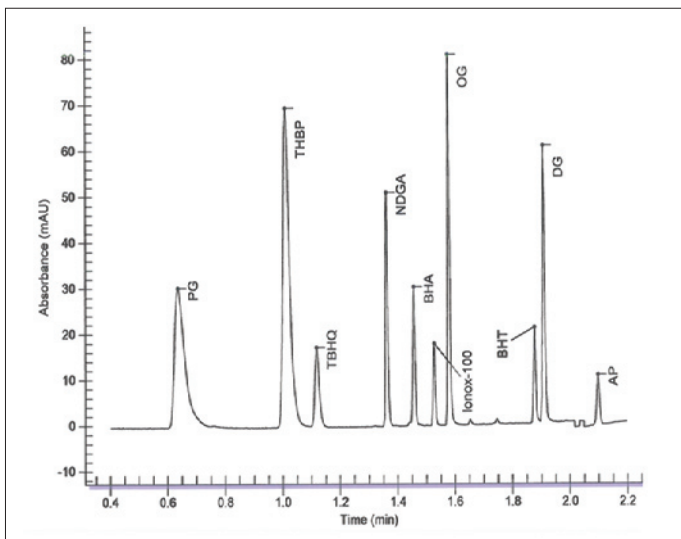


Figure 3. Chromatogram from the analysis of a standard solution with 10  $\mu\text{g/mL}$  of 10 antioxidants using a UHPLC C18 50 x 2.1 mm, 1.9  $\mu\text{m}$  particle-size column.

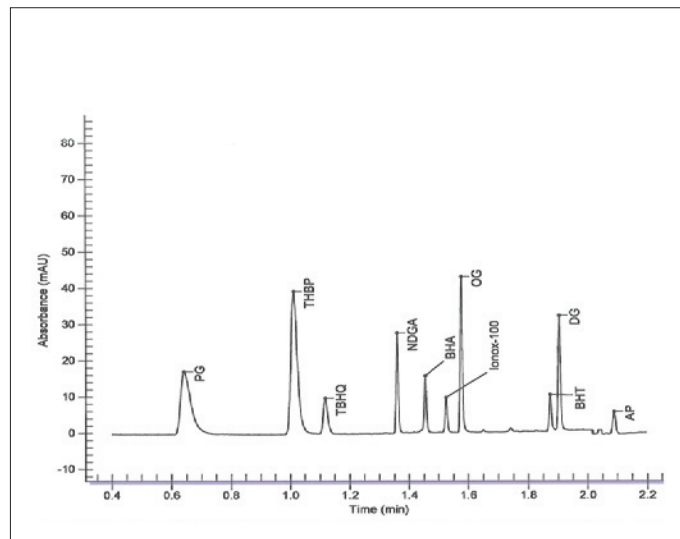


Figure 5. Chromatogram from the analysis of corn oil spiked with common antioxidants.