APPLICATION NOTE

UHPLC

Author

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Analysis of Common Antioxidants in Edible Oils with a Superficially Porous Particle C18 Column and a Conventional C18 Column

Introduction

Phenolic antioxidants are commonly used in food to prevent the oxidation of oils. Oxidized oils cause foul odor and rancidity in food products. This application note presents three chromatographic conditions to analyze nine antioxidant compounds used in edible oils (Figure 1). A conventional C18 column and a superficially porous particle column (SPP) are used for the separations.

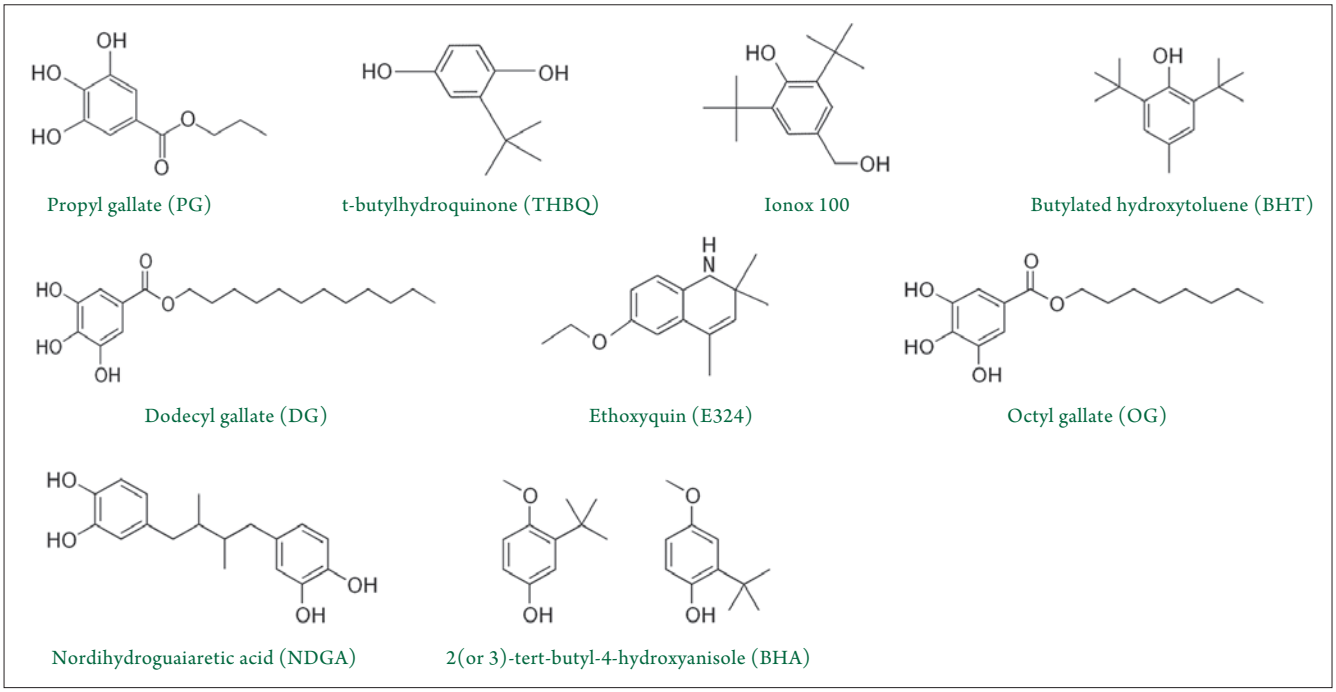

Figure 1. Names and structures of Antioxidants analyzed.

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Experimental

A stock solution with 0.7 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-ter-butyl-4-hydroxymethylphenol (Ionox 100) was prepared with methanol as diluent; similarly, a second stock with 0.7 mg/mL of t-butylhydroquinone (THBQ) was prepared. A third stock with 0.7 mg/mL of ethoxyquin was prepared in the same way as the other stock solutions. From the stock solutions a 45 µg/mL working standard was prepared; unlike the stock solutions, the working standard preparation was diluted with a solution of 1 mg/mL of citric and isoascorbic acid in methanol (oxygen quencher, and chelating agent) was used as diluents.

A PerkinElmer® Flexar® FX-15 with a UV Detector provided the UHPLC platform for this application.

General chromatographic conditions:

Autosampler:	Flexar FX UHPLC
	Setting: 50 µL loop and 15 µL needle volume Partial loop mode Flush solvent: 1:1 methanol/water
Injection:	4 µL
Detector:	UV detector at 280 nm or 220 nm
Column:	Brownlee™ SPP C18, 100 x 3.5 mm, 2.7 µm Part No. N9308410
	Brownlee Validated C18 100 x 2.1 mm, 3 µm Part No. N9303551
Column Temperature:	40 °C
Mobile Phase:	B: 50:50 (v/v) methanol/Acetonitrile, (HPLC grade solvent and ACS grade reagent)
Software:	Chromera™ v3.0
Sampling Rate:	5 pts/s

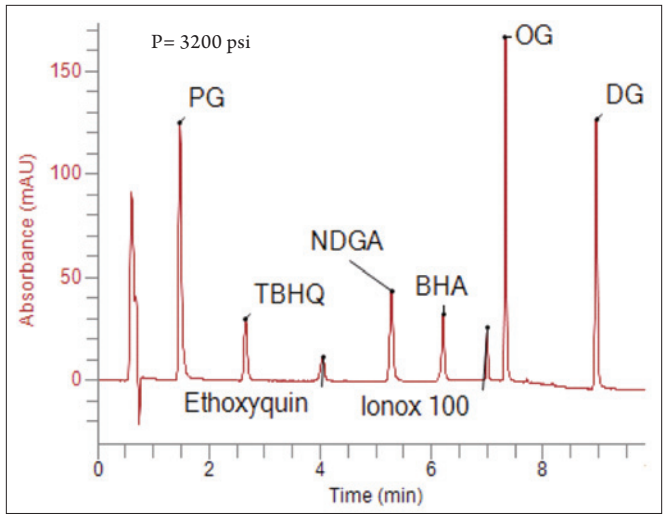

Figure 2. Antioxidant solution analyzed with a 0.05% formic acid and as mobile phase A and SPP C18 100 x 3.5 mm 2.7 µm column at 280 nm.

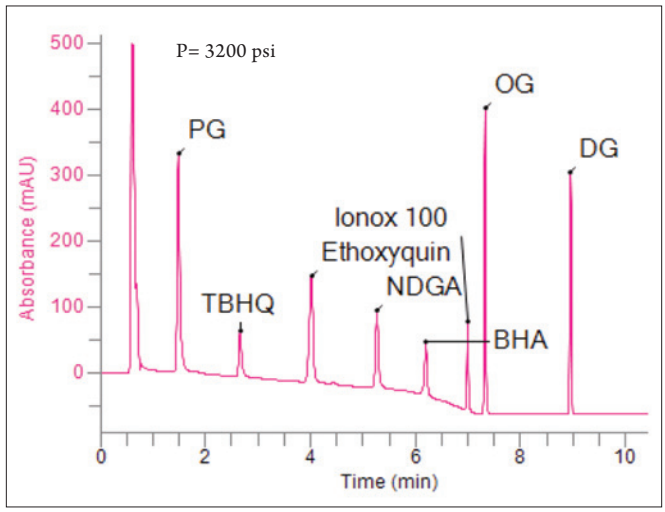

Figure 3. Same solution as in Figure 2 with optimized wavelength at 220 nm.

Note: DG and BHT elute at the same time, DG is larger and masks BHT.

Specific chromatographic conditions:

Brownlee SPP C18 100 x 3.5 mm SPP 2.7 µm, Part No. N9308404

Mobile phase A: 0.05% formic acid in water

Mobile phase B: 50:50 methanol/Acetonitrile

Time (min)	Flow (mL/min)	B%	Curve
4.5	0.6	40 - 45	1
2.5	0.6	55 - 90	1
3	0.6	90	1
3 min. equilibration			

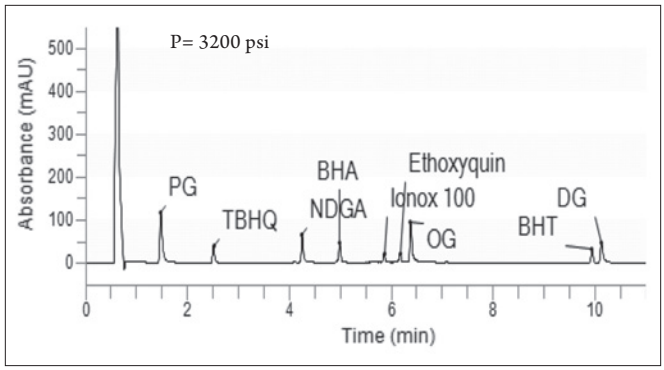

Figure 4. Antioxidant solution analyzed with an ammonium phosphate buffer as mobile phase A and a SPP C18 100 x 3.5 mm 2.7 µm column at 280 nm.

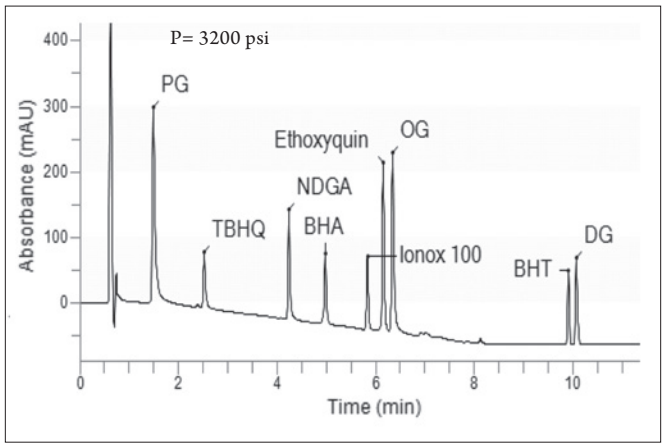

Figure 5. Same solution as in Figure 4 with wavelength optimized at 220 nm.

Specific chromatographic conditions:

Brownlee SPP C18 100 x 3.5 mm 2.7 µm, Part No. N9308404

Mobile phase B: 50:50 methanol/acetonitrile

Mobile phase A: 4 mM ammonium acetate water (0.1% Acetic acid)

Time (min)	Flow (mL/min)	B%	Curve
2.5	0.6	40 - 45	1
2.5	0.6	55 - 70	1
4	0.6	70 - 85	1
2	0.6	85	1
3 min. equilibration			

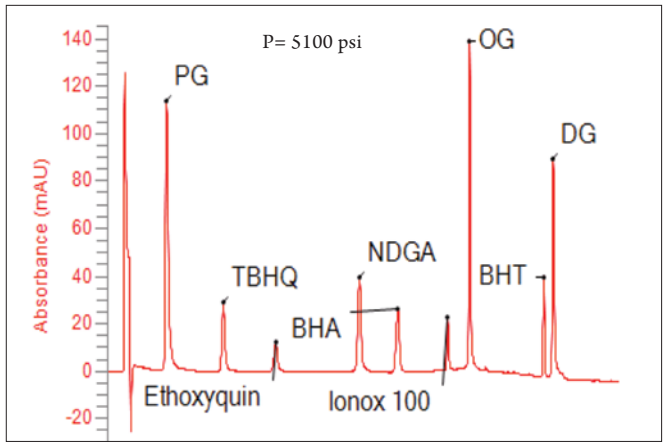

Figure 6. Antioxidant solution analyzed with a 0.05% formic acid and as mobile phase A and a Brownlee C18 100 x 2.1 mm 3 µm column at 280 nm.

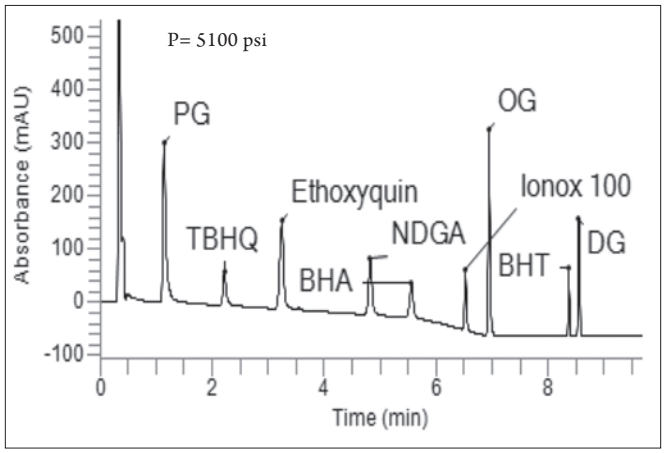

Figure 7. Same solution as in Figure 6 with wavelength optimized at 220 nm.

Specific chromatographic conditions:

Brownlee Validated C18, 100 x 2.1 mm, 3 µm
Part No. N9303551

Mobile phase B: 50:50 methanol/acetonitrile

Mobile phase A: 0.05% formic acid in water

Time (min)	Flow (mL/min)	B%	Curve
4.5	0.6	40 - 45	1
2.5	0.6	55 - 90	1
3	0.6	90	1
3 min. equilibration			

Conclusion

All nine antioxidant peaks were well resolved with straightforward chromatographic conditions. Results obtained using a Brownlee Validated C18 column were similar to those obtained using a Brownlee SPP C18 column. However, the back pressure was lower with the SPP column (3200 psi) when compared to the Brownlee Validated C18 conventional column (5100 psi).

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