Application Note 20978

Improved Determination of Antioxidants in Edible Oils Using Solid Core LC Columns

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Key Words
Accucore C18, Accucore Polar Premium, AOAC 983.15, antioxidant, food testing

Abstract
Phenolic antioxidants are commonly used as preservatives in foods containing edible oils and fats to prevent development of rancidity. AOAC Method 983.15 is used to assay levels of these compounds in finished food products. The method uses a C18 column but can suffer from matrix interference effects. In this work, we demonstrate the suitability of both Thermo Scientific™ Accucore™ C18 and Thermo Scientific™ Accucore™ Polar Premium columns for this application.

Introduction

Phenolic antioxidants are commonly used as preservatives for edible oils and fats to prevent rancidity. Synthetic phenolic antioxidants are subject to maximum limits for reasons of safety [1] and to minimum limits for reasons of effectiveness [2]. AOAC Official Method 983.15 was developed to assay levels in finished food products [3]. The method specifies a general-purpose C18 column, which is satisfactory in many cases; however, food is a complex matrix and the method is prone to interferences. Choosing the right column to manage interferences is a necessary part of developing a rugged method. The AOAC method originally included only nine synthetic antioxidants, but this has been extended to include vitamin E, vitamin E acetate and ethoxyquin.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

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Experimental Details

Consumables	Part Number
Acetonitrile, LC/MS	
Methanol, LC/MS	
Water, purified with Millipore® Milli-Q® Advantage A10® system	
Acetic acid	
n-Heptane	
Phenolic antioxidants kit containing	
Nordihydroguairetic acid (NDGA)	
Lauryl gallate	
Octyl gallate	
2- & 3- tert-butyl-4-hydroxy-anisole (BHA)	
3,5-di-t-butyl-4-hydroxytoluene (BHT)	
tert-butylhydroquinone (TBHQ)	
2,6-di-t-butyl-4-hydroxymethylphenol (Ionox-100)	
2,4,5-trihydroxybutyrophenone (THBP)	
Ethoxyquin	
Propyl gallate	
Vitamin E and vitamin E acetate were purchased separately.	

Solutions

Stock solutions of standards were prepared at 1.0 mg/mL in acetonitrile.

Sample Preparation

Samples were prepared according to AOAC Official Method 983.15. The foodstuffs were warmed to separate the water from the fat. Approximately 5.5 g of oil or fat was dissolved in 20 mL of heptane and extracted three times with 50 mL of acetonitrile. The combined acetonitrile fraction was evaporated to about 4 mL, and reconstituted with isopropanol to make 10 mL.

Separation Conditions					Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 system consisting of LPG-3400RS quaternary pump, WPS-3000RS autosampler, DAD-3000RS diode array detector, and TCC-3000RS column oven				
Columns:	Accucore C18, 2.6 µm, 100 × 3 mm				7126-103030
	Accucore Polar Premium, 2.6 µm, 100 × 3 mm				28026-103030
Mobile phases:	A: acetonitrile / methanol / acetic acid (49.25:49.25:0.5 v/v)				
	B: water / acetic acid (99.5:0.5 v/v)				
Flow rate:	0.80 mL/min				
Gradient	Time	%A	%B	Curve	
	-4	25	75	5	
	0	25	75	5	
	5	100	0	4	
	10	100	0	5	
Column temperature:	30 °C				
Injection volume:	2 µL				
UV detection:	Diode Array 200–400 nm, 280 nm shown; data rate 5 Hz, filter 1.0 s				

Data Processing

Software:	Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System

Results

Only Accucore C18 and Accucore Polar Premium columns resolved all 12 peaks in the standards mix from a number of evaluated columns. There were minor differences in the elution order and the selectivity against matrix interferences was different. The margarine and butter contained an unidentified component that coeluted with propyl gallate from the C18 column (Figure 1). This is not a declared ingredient for either sample. The UV spectra of the peaks in the standard and the samples did not match.

Using the Accucore Polar Premium column, the interference was resolved from the propyl gallate and was shown not to be present, as expected (Figure 2). The margarine does declare TBHQ in its ingredients; for both columns it was resolved from the matrix and assays at 180 µg/g of oil.

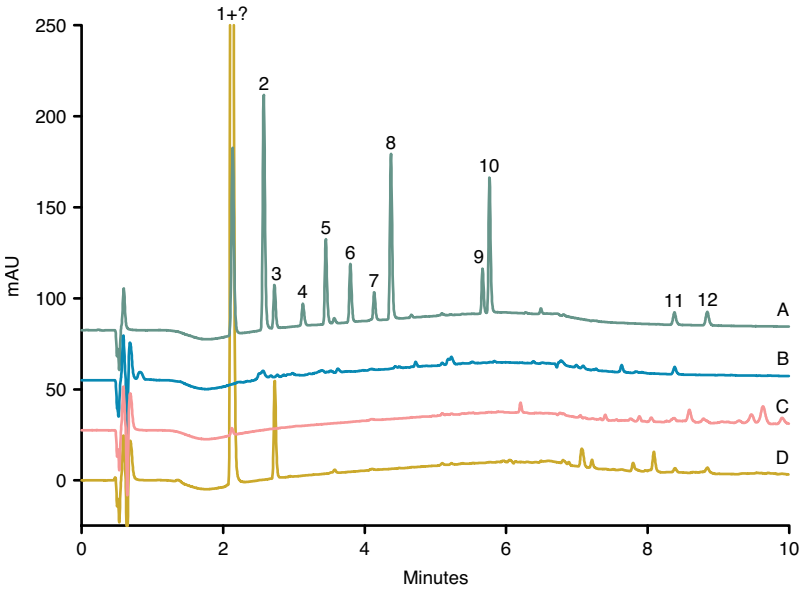

Figure 1: Antioxidants using Accucore C18. A: Standards 50 µg/mL in acetonitrile. B: Olive oil extract. C: Butter fat extract. D: Margarine oil extract. Peaks 1: Propyl gallate, 2: THBP, 3: TBHQ, 4: Ethoxyquin, 5: NDGA, 6: BHA, 7: Ionix-100, 8: Octyl gallate, 9: BHT, 10: Lauryl gallate, 11: Vitamin E, 12: Vitamin E acetate.

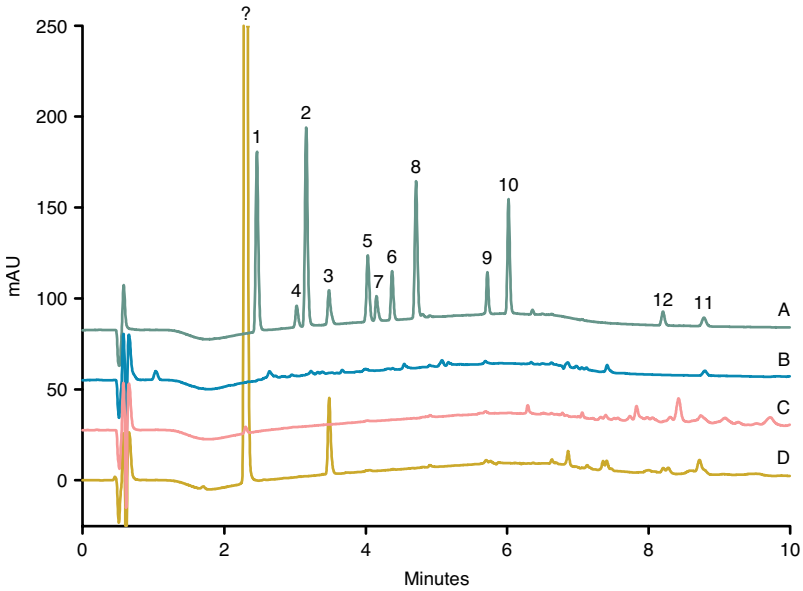

Figure 2: Antioxidants using Accucore Polar Premium shows alternate selectivity. A: Standards 50 µg/mL in acetonitrile. B: Olive oil extract. C: Butter fat extract. D: Margarine oil extract. Peaks 1: Propyl gallate, 2: THBP, 3: TBHQ, 4: Ethoxyquin, 5: NDGA, 6: BHA, 7: Ionix-100, 8: Octyl gallate, 9: BHT, 10: Lauryl gallate, 11: Vitamin E, 12: Vitamin E acetate.

Conclusion

- The Accucore C18 and Accucore Polar Premium columns provide excellent performance for AOAC Official Method 983.15 for antioxidants in edible oils.
- Food is a complex matrix, and in order to manage matrix interferences, it is beneficial to have more than one column type qualified for a method.

References:

[1] US Food and Drug Administration, 21CFR172 Subpart B (Preservatives).

[2] D.L. Madhavi, S.S. Deshpande, D.K. Salunkhe, "Food Antioxidants: Technological, Toxicological and Health Perspectives," p. 160-190, Marcel Dekker, Inc. NY (**1996**).

[3] AOAC Official Methods of Analysis, Method 983.15, Phenolic Antioxidants in Oils, Fats and Butter Oil (**1994**).

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