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Monitoring Antioxidant Additives in Foods, Using HPLC or Capillary GC and a New Reference Standards Kit

E. Doughty, K. Kiefer

Supelco now offers an antioxidant standards kit containing all 9 antioxidants listed in Association of Official Analytical Chemists (AOAC) Official Method 983.15:* Phenolic Antioxidants in Oils, Fats, and Butter Oil, *plus ethoxyquin, an additional commonly used antioxidant. These compounds are separated well, in less than 13 minutes, on a SUPELCOSIL LC-18 HPLC column, under the conditions described in the AOAC method. Several of the antioxidants also can be resolved on a SAC-5 capillary GC column.

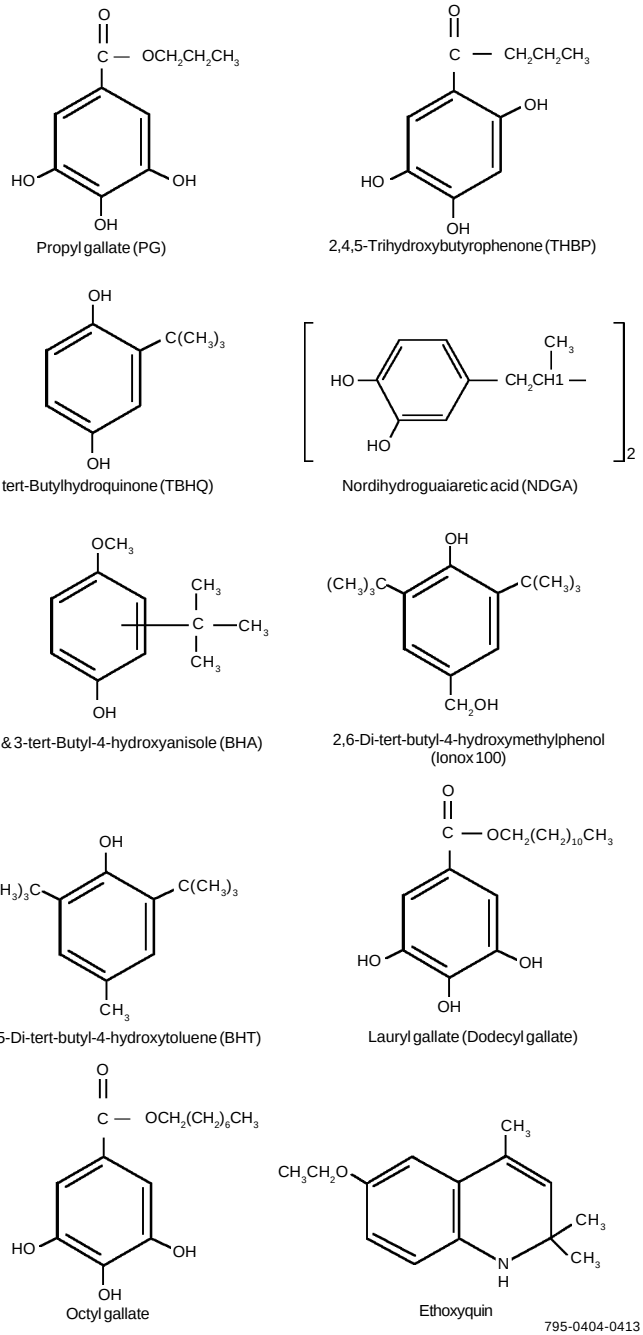
Rancidity caused by the oxidation of unsaturated fats in foods is a major problem for the food industry. With the trace metals present in foods acting as catalysts, the unsaturated fats react with oxygen, producing free radicals and peroxides. These compounds can destroy many vitamins, reducing the nutritional value of the food, destroy pigments, bleaching the food, and cause off-odors and off-flavors. While the portion of fat undergoing such changes can be small, the resulting off-odors are strong, and very much out of proportion to the amount of oil or fat involved.

Antioxidants are added to foods and other products to prevent rancidity. Although the exact mechanism(s) is (are) not clear, antioxidants react with free radicals and peroxides, slowing the development of rancidity. Certain other additives greatly enhance the effectiveness of antioxidants. Metal scavenger and chelating agents, such as citric acid and citrates, tie up the trace metals and greatly reduce their catalytic activity. Synergism between antioxidants also has been noted, and many commercial antioxidant mixes are formulated to contain mixtures of antioxidants. The most common of these mixtures contain both butyl-hydroxyanisole (BHA) and butyl-hydroxytoluene (BHT).

In order to assure consistent product quality, these additives must be monitored. In the past, food analysts had to go to several vendors to obtain good quality standards of all of the antioxidants they might be required to monitor. Now, Supelco offers an antioxidant standards kit, containing all 9 of the antioxidants listed in Association of Official Analytical Chemists (AOAC) Official Method 983.15: *Phenolic Antioxidants in Oils, Fats, and Butter Oil,* (Figure A) plus ethoxyquin, an additional antioxidant commonly used in spices and other food products, and in cosmetics.

The AOAC has granted final approval of Method 983.15, and has included this method in the 1995 (16th) edition of the *Official Methods of Analysis* of AOAC International (1). The method describes a linear gradient HPLC analysis for resolving the nine antioxidants shown in Figure A. Figure B shows a chromatogram generated on a 15cm x 4.6mm SUPELCOSIL™ LC-18 HPLC column, using the conditions described in the AOAC method. All 9 analytes were well

Figure A. Phenolic Antioxidants Commonly Used to Preserve Food Quality



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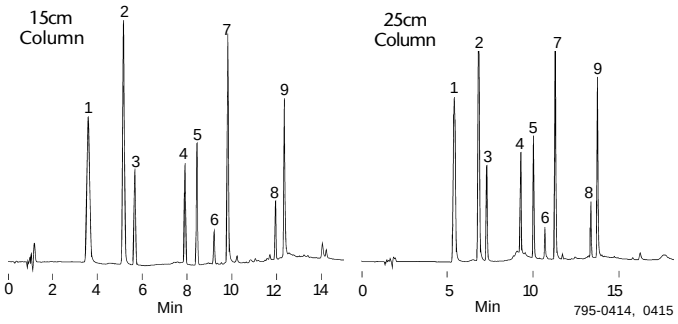
resolved in less than 13 minutes. Ethoxyquin is not detected at the 280nm UV wavelength indicated by the AOAC method, and thus is not included here. Figure B also shows the analysis on a 25cm SUPELCOSIL LC-18 column. The analytes again are well resolved, and analysis time is only slightly prolonged.

In most food products, only 1 or 2 antioxidants are used. In some cases, gas chromatography can be used to monitor the antioxidants. (Many of the antioxidants in Figure A give a poor response to FID detection, however.) Figure C shows the chromatogram generated from a mix of 7 antioxidants, including ethoxyquin, separated on a 30m x 0.25mm x 0.25µm phase film SAC™-5 capillary GC column under isothermal (200°C) conditions. Resolution is good. Ethoxyquin was eluted at approximately 7 minutes. Note that at

Figure B. Antioxidants in AOAC Method 983.15 by HPLC

Column: **SUPELCOSIL LC-18, 15cm or 25cm x 4.6mm ID, 5µm particles**
Cat. No.: **58230-U** (15cm column), **5-8298** (25cm column)
Mobile Phase: A = 5% acetic acid in DI water
B = acetonitrile:methanol, 50:50
30% B to 100% B in 10 min, hold 10 min
Flow Rate: 2mL/min
Temp.: ambient
Det.: UV, 280nm
Inj.: 10µL, 20µg/mL each analyte

1. Propyl gallate
2. 2,4,5-Trihydroxybutyrophenone
3. tert-Butylhydroquinone
4. Nordihydroguaiaretic acid
5. 2- & 3-tert-Butyl-4-hydroxyanisole
6. 2,6-Di-tert-butyl-4-hydroxymethylphenol (Ionox 100)
7. Octyl gallate
8. 3,5-Di-tert-butyl-4-hydroxytoluene
9. Lauryl gallate



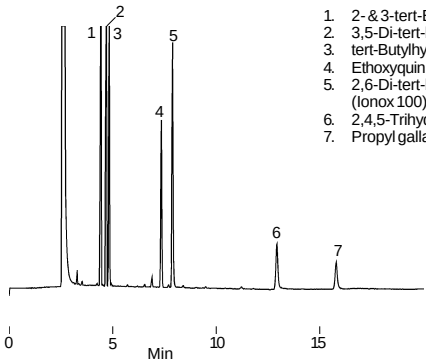
200°C, an analysis time of more than 15 minutes was needed to elute propyl gallate. Lauryl gallate and octyl gallate cannot be monitored under these conditions – a very high oven temperature is required to elute these high boiling/low volatility compounds from the capillary column.

If your analyses include monitoring antioxidants, this new standards kit should meet all your routine needs, and will prepare you for identifying unanticipated additional antioxidants in your samples. Note that the ethoxyquin standard is qualitative. Because of its highly reactive nature, we cannot assure a purity level of more than 70% for this compound. Each antioxidant is packaged neat, under nitrogen.

Figure C. Antioxidants with Lower Boiling Points Can Be Monitored by GC

Column: **SAC-5, 30m x 0.25mm, 0.25µm film**
Cat. No.: **24156**
Oven: 200°C
Carrier: helium, 30cm/sec (set at 200°C)
Det.: FID (250°C)
Inj.: 2µL, 200µg/mL each analyte, split 100:1 (250°C)

1. 2- & 3-tert-Butyl-4-hydroxyanisole
2. 3,5-Di-tert-butyl-4-hydroxytoluene
3. tert-Butylhydroquinone
4. Ethoxyquin
5. 2,6-Di-tert-butyl-4-hydroxymethylphenol (Ionox 100)
6. 2,4,5-Trihydroxybutyrophenone
7. Propyl gallate



Ordering Information:

Description	Cat. No.
Phenolic Antioxidants Kit	**47192**

Individually packaged under nitrogen, neat, in quantities listed below.

Component	CAS No.	Qty. (mg)
Propyl gallate (PG)	121-79-9	500
2,4,5-Trihydroxybutyrophenone (THBP)	1421-63-2	500
tert-Butylhydroquinone (TBHQ)	1948-33-0	500
Nordihydroguaiaretic acid (NDGA)	500-38-9	100
2- & 3-tert-Butyl-4-hydroxyanisole (BHA)	25013-16-5	500
2,6-Di-tert-butyl-4-hydroxymethylphenol (Ionox 100)	88-26-6	100
3,5-Di-tert-butyl-4-hydroxytoluene (BHT)	128-37-0	500
Lauryl gallate (Dodecyl gallate)	1166-52-5	500
Octyl gallate	1034-01-1	500
Ethoxyquin*	91-53-2	500

*Qualitative standard. Purity >70% cannot be assured.

Description	Cat. No.
SUPELCOSIL LC-18 HPLC Columns	
15cm x 4.6mm, 5µm particles	**58230-U**
25cm x 4.6mm, 5µm particles	**58298**
SAC-5 Capillary GC Column	
30m x 0.25mm, 0.25µm film	**24156**

Reference

1. *Official Methods of Analysis* (16th ed.), Method 983.15 Association of Official Analytical Chemists, Arlington, VA USA (1995).

Reference not available from Supelco.