Food & Flavor

Applications note

cat.# 59582

Optimize Detection of Synthetic and Natural Antioxidants in Food

Foods containing fats and oils are prone to lipid oxidation, which causes off-flavors and limits shelf life. To inhibit the oxidation process, food preservatives, known as antioxidants, are often added. Antioxidants retard oxidative rancidity caused by the atmosphere and delay the discoloration of meats, meat products, fruits, and vegetables. Commonly used antioxidants include phenolic compounds such as BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene), PG (propyl gallate), and TBHQ (tert-butyl-hydrquinone). Recent attention has focused on natural antioxidants, such as tocopherols and tocotrienols, because of their dual function in preserving foods and promoting general health. However, regulations require the monitoring of these antioxidants.

Phenolic Antioxidants

Primary antioxidants, including BHA, BHT, TBHQ, and PG, terminate the free radical chains susceptible to lipid oxidation. Secondary antioxidants, including DLTDP (dilaurylthiodipropionate), decompose the lipid hydroperoxides into stable end products[1]. The Food and Drug Administration (FDA) has specified regulations on phenolic antioxidant addition because many are toxic above certain levels. The Generally Recognized as Safe (GRAS) limit for direct addition of phenolic antioxidants to food is 0.02% (200ppm), based on the fat content of the food[1]. If added to food packaging, which is considered an indirect addition to food, the maximum allowable limit is 0.005% (50ppm) in the food item[1]. This limit applies to a maximum concentration allowable for antioxidants used alone or a total concentration for combinations of antioxidants.

Several methods have been developed for the analysis of regulated antioxidants in food[1,2,3]. In the Association of Analytical Chemists (AOAC) Official Method 968.17, BHA and BHT are extracted from cereal samples using CS_2, and detected by GC/FID[2]. BHA, BHT, DLTDP, HMBP (4-hydroxymethyl-2,6-di-tert-butyrophenol), and PG are extracted from lard samples using vacuum sublimation and then analyzed on a dual packed column system: Apiezon for detection of DLTDP, and GE-XE-60 for detection of the other antioxidants[1]. Although antioxidants can be analyzed on these non-polar columns, simultaneous detection requires the selectivity of a more polar column. A stationary phase with intermediate polarity provides resolution of all analytes on one column.

The selectivity and thermal stability of the intermediate polarity Rtx®-50 (Crossbond® 50% methyl - 50% phenyl polysiloxane) capillary column provides exceptional resolution and peak shapes. **Figure 1** shows the analysis of seven regulated antioxidants on an Rtx®-50 capillary column. The GRAS limit can be easily detected on an Rtx®-50 column using the direct injection mode. Baseline resolution of all components is achieved in less than 20 minutes by taking advantage of the column's 310°C thermal stability.

Tocopherol Antioxidants

Tocopherol antioxidants are primary antioxidants that quench the free radicals created during oxidation of unsaturated bonds in fats[4]. They can be created synthetically or extracted from natural sources such as nuts, seed oils, or soybeans. Biological activity decreases and antioxidant potency increases in the order of α-, β-, γ-, δ-tocopherols. Therefore, concentrates used in food antioxidants contain high levels of γ- and δ-tocopherols, and smaller amounts of α- and β-tocopherols. Tocopherol acetate, a stable form of Vitamin E, is also added to food products. Although tocopherol acetate is not an antioxidant itself, in an acidic environment it slowly hydrolyzes and tocopherol is released[4].

The FDA regulation for general usage of tocopherols allows discretion for good manufacturing practice, or using the amount required for technical effect[5,6]. The U.S. Department of Agriculture (USDA) regulations are more specific. The maximum limit for addition of tocopherols to animal and/or vegetable fat is 0.03% (300ppm) and 0.01-0.02% (100-200ppm) for poultry fats[7,8].

Because tocopherols are found in a variety of sample matrices, labs may be required to perform some type of sample preparation. Tocopherols and

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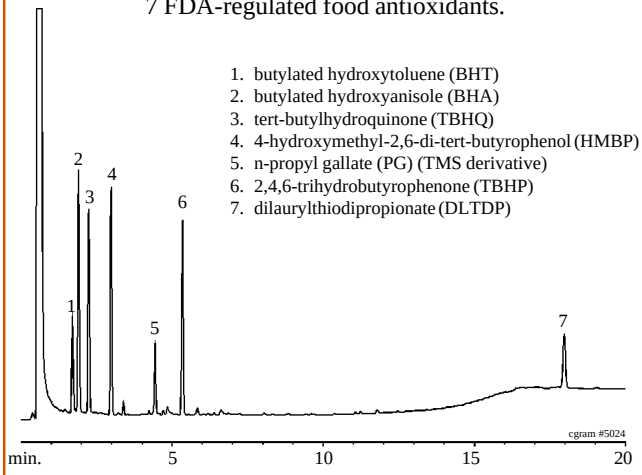
Figure 1

The Rtx®-50 capillary column provides rapid analysis of 7 FDA-regulated food antioxidants.

1. butylated hydroxytoluene (BHT)
2. butylated hydroxyanisole (BHA)
3. tert-butylhydroquinone (TBHQ)
4. 4-hydroxymethyl-2,6-di-tert-butyrophenol (HMBP)
5. n-propyl gallate (PG) (TMS derivative)
6. 2,4,6-trihydrobutyrophenone (TBHP)
7. dilaurylthiodipropionate (DLTDP)

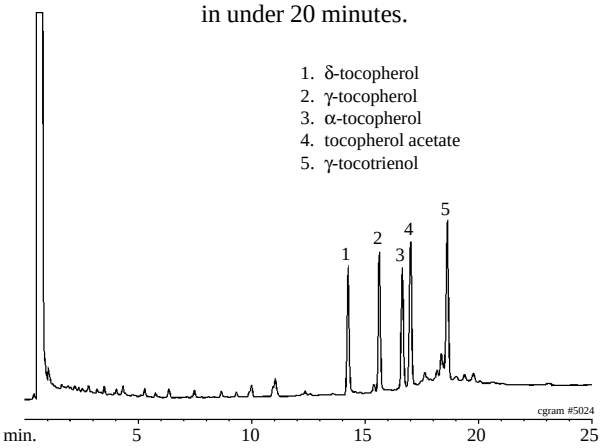


30m, 0.53mm ID, 0.50µm Rtx®-50 (cat.# 10540)
1.0µl direct injection of 7 regulated food antioxidants using a Uniliner® sleeve.
On-column concentration: 100ppm; **Oven temp.:** 165°C (hold 1 min.) to 310°C @ 10°C/min. (hold 10 min.); **Inj./det. temp.:** 290°C/310°C; **Detector:** FID; **Carrier gas:** helium; **Linear velocity:** 89cm/sec., set @ 100°C; **Detector sensitivity:** 8 x 10^{-11} AFS; **Column flow:** 11.7cc/min.

Figure 2

Underivatized tocopherol antioxidants can be successfully analyzed on the Rtx®-50 capillary column in under 20 minutes.

1. δ-tocopherol
2. γ-tocopherol
3. α-tocopherol
4. tocopherol acetate
5. γ-tocotrienol



30m, 0.53mm ID, 0.50µm Rtx®-50 (cat.# 10540)
1.0µl direct injection of Vitamin E food antioxidants.
On-column concentration: 50ppm; **Oven temp.:** 200°C to 290°C @ 5°C/min. (hold 15 min.); **Inj./det. temp.:** 290°C/310°C; **Detector:** FID; **Carrier gas:** helium; **Linear velocity:** 89cm/sec., set @ 100°C; **Detector sensitivity:** 8 x 10^{-11} AFS; **Column flow:** 11.7cc/min.

other sterols are extracted from the unsaponified fraction of animal and vegetable fats and oils, and marine oils as per the American Oil Chemists Society (AOCS) methods Ca 6a-40 and Ca 6-53, respectively[3]. AOCS methods outline derivatization procedures for tocopherols using either butyric anhydride (AOCS Ce 3-74) or Sylon BFT (AOCS Ce 7-87)[3]. However, the inertness of capillary columns allows tocopherols to be analyzed in their free form, eliminating the derivatization step. **Figure 2** shows the analysis of underivatized α-, γ-, and δ-tocopherols, and tocopheryl acetate on a 30m, 0.53mm ID, 0.5µm Rtx®-50 column. All components exhibit good peak shape and are baseline resolved in less than 20 minutes.

Conclusion

The Rtx®-50 column is an excellent choice for both phenolic and tocopherol antioxidant analysis. The inertness and high thermal stability of the Rtx®-50 column permits the analysis of all components, with the exception of propyl gallate, in their free form. All components are well resolved to provide exceptional qualitative and quantitative accuracy. The 310°C maximum operating temperature of the Rtx®-50 column reduces analysis time while maintaining a stable baseline during temperature programming.

References

1. AOAC, Food Additives, Analytical Manual: Volumes I and II, 1992
2. AOAC, Official Methods of Analysis, Volumes I and II, 1990
3. AOCS, Official Methods and Recommended Practices, 1994
4. Hudson, B.F.J., *Food Antioxidants*, 1990
5. Federal Register, 21 CFR 182.3890
6. Federal Register, 2 CFR 165.175, 166.110, 164.110
7. Federal Register, 9 CFR 318.7
8. Federal Register, 9 CFR 381.147

Product Listing

Rtx®-50 Column

ID	df (µm)	30-meter
0.53mm	0.50	10540

RESTEK

ISO 9001 REGISTERED

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