

## Analysis of Phenolic Antioxidants in Food

Ingredients in foods form oxides when exposed to atmospheric oxygen, resulting in quality deterioration. A variety of food additives are used to prevent this oxidation. This Application News reports the results of HPLC analysis on phenolic antioxidants that are often used in fatty foods.

Four types of phenolic antioxidants are allowed to be added to foods in Japan. These are BHT (butylated

hydroxytoluene), BHA (butylated hydroxyanisole), NDGA (nordihydroguaiaretic acid), and PG (propyl gallate). They are permitted for use in oils and fats, butter, frozen seafood, and dried seafood products.

This Application News introduces examples of the analysis of nine phenolic antioxidants: the four permitted in Japan plus another five that are permitted for use in other countries.

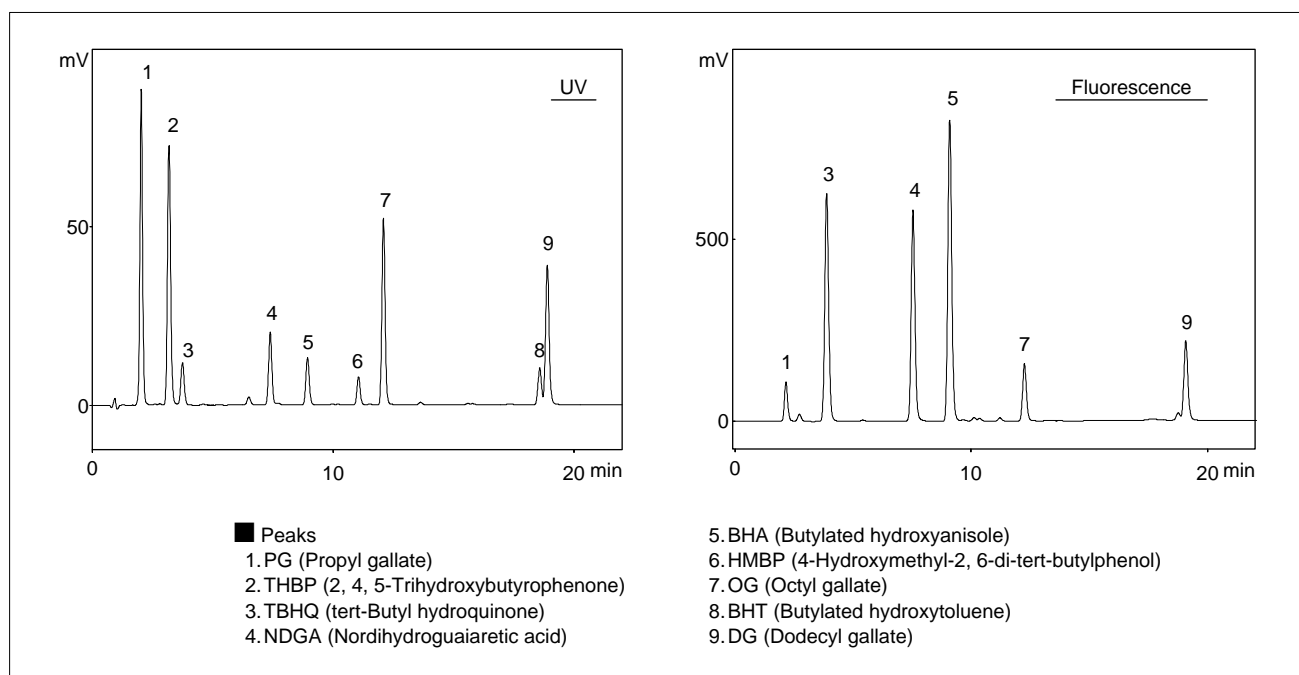
### ■ Simultaneous Analysis of Nine Antioxidants by Gradient Elution

Fig. 1 (left) shows the chromatogram from the simultaneous analysis of all nine phenolic antioxidants by gradient elution (UV detector) with injection of 10 $\mu$ L of sample. The sample was a methanol solution containing 25mg/L of each component.

Fig. 1 (right) shows the results measured with the fluorescence detector under identical analytical conditions. Compared to the UV detector, the fluorescence detector achieved superior sensitivity and selectivity for six of the components: TBHQ, NDGA, OG, PG, DG, and BHA. The sensitivity for three of the components – TBHQ, NDGA, and BHA – could be further enhanced by switching to a shorter fluorescence wavelength.

**Table 1 Analytical Conditions**

Column	:Shim-pack FC-ODS (75mmL.×4.6mmI.D.)
Mobile Phase	:A:5% Acetic Acid-water B:Methanol/Acetonitrile = 1/1 (v/v) B (40%)→B (80%)/15min Linear Gradient
Flow Rate	:1.0mL/min
Column Temp.	:40°C
Detection	:SPD-10AVP at 280nm RF-10AXL Ex at 275nm, Em at 365nm



**Fig. 1 Chromatograms of Mixture of Nine Antioxidant Standards by Gradient Elution (25 mg/L of each component, 10  $\mu$ L injected)**  
 Left: SPD-10AVP at 280 nm  
 Right: RF-10AXL; Ex at 275 nm, Em at 365 nm

## ■ Analysis of Butter

Fig. 3 shows the results of the analysis of butter, after pretreatment according to the procedure in Fig. 2. The analytical conditions are identical to Table 1 (UV detection at 280 nm). The lower chromatogram shows the analysis results for butter alone. The upper chromatogram shows the results for butter after the addition of nine phenolic antioxidants to a concentration of 20 mg/L during pretreatment.

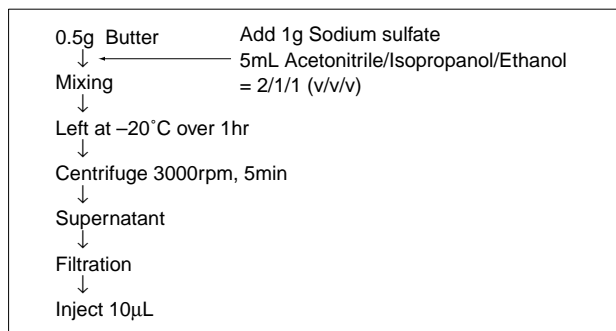


Fig. 2 Pretreatment of Butter

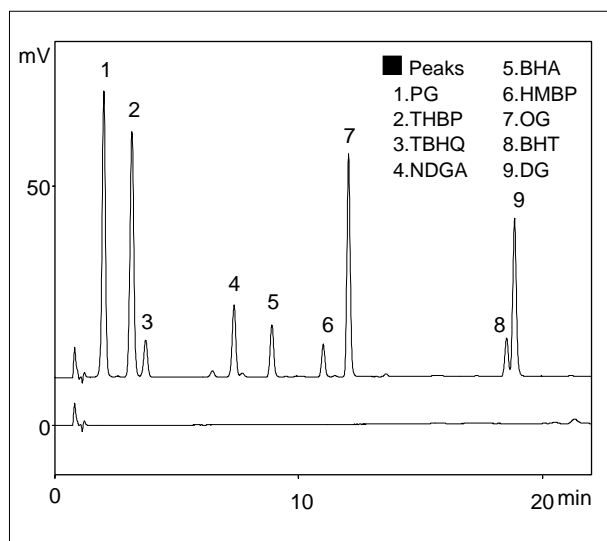


Fig. 3 Analysis of Butter  
Upper: Butter spiked 20 mg/L each component  
Lower: Butter alone

## ■ Analysis of Five Antioxidants by Isocratic Elution

Fig. 4 shows the analysis results for five antioxidants by isocratic elution. The left and right chromatograms were measured by the UV detector and the fluorescence detector, respectively. Table 2 shows the analytical conditions. The sample was a methanol solution containing 25mg/L of each component, and 10µL of the sample was injected. Increasing the organic solvent concentration permits the analysis of HMBP, OG, BHT, and DG. This method is suitable for routine analysis of specific components.

Table 2 Analytical Conditions

Column	:Shim-pack FC-ODS(75mmL.×4.6mmI.D.)
Mobile Phase	:5% Acetic Acid-water/Methanol/Acetonitrile =6/2/2 (v/v/v)
Flow Rate	1.0 mL/min
Column Temp.	:40°C
Detection	:SPD-10A <sub>VP</sub> at 280nm RF-10A <sub>XL</sub> Ex at 275nm, Em at 365nm

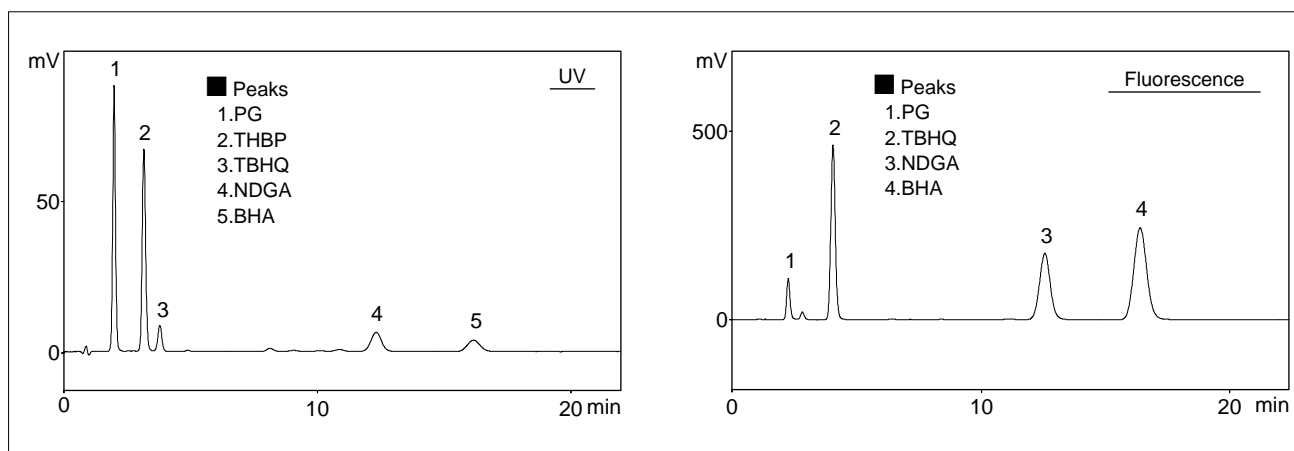


Fig. 4 Chromatograms of a Mixture of Five Antioxidant Standards by Isocratic Elution  
Left: SPD-10A<sub>VP</sub> at 280 nm  
Right: RF-10A<sub>XL</sub>; Ex at 275 nm, Em at 365 nm

## References

- 1) Standard Methods of Analysis in Health science 2000 (in Japanese)
- 2) Analysis of Additives in Food, Revision 2 (in Japanese)



SHIMADZU CORPORATION, International Marketing Division

3, Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax: 81(3)3219-5710  
Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-06321-10A-1K