

This note describes a simultaneous determination method for 9 phenolic antioxidants using a binary gradient HPLC system.

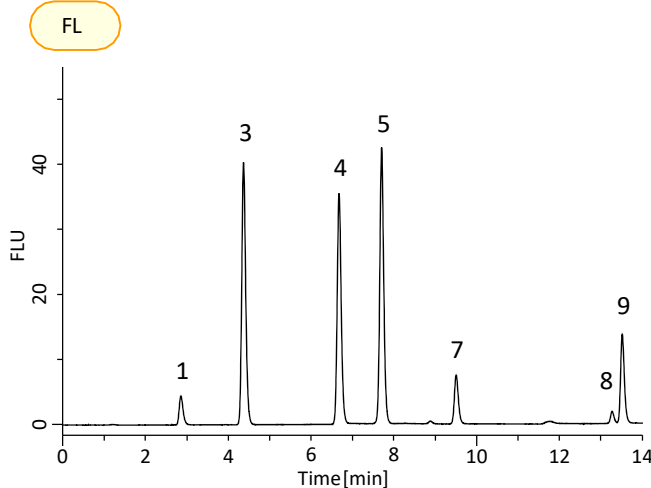
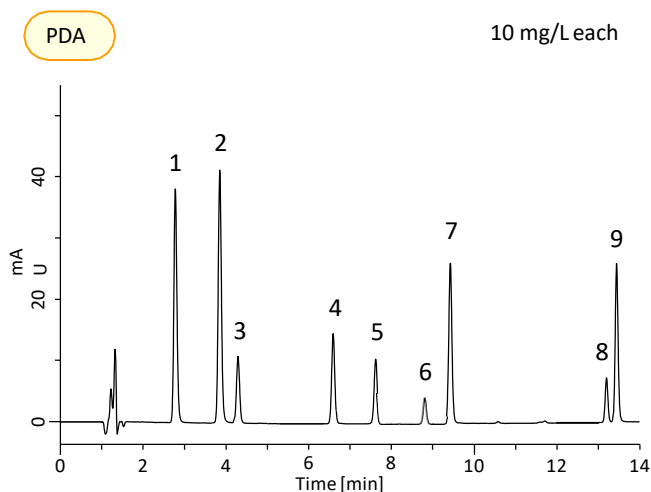
Among them, propyl gallate (PG), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are permitted as food additive in Japan. Meanwhile, 2,4,5-trihydroxybutyrophenone (THBP), *tert*-butylhydroquinone (TBHQ), 4-hydroxymethyl-2,6-di-

Chromatograms obtained from standard solution

phenol (HMBP), octyl gallate (OG), and dodecyl gallate (DG) are used in other countries.

All of the phenolic antioxidants can be determined with a photodiode array (PDA) detector. Fluorescence detection also can be utilized except for THBP and HMBP. In this note, a PDA detector and a fluorescence detector were connected in series, and the chromatograms obtained with both detectors were shown.

(K.Suzuki)

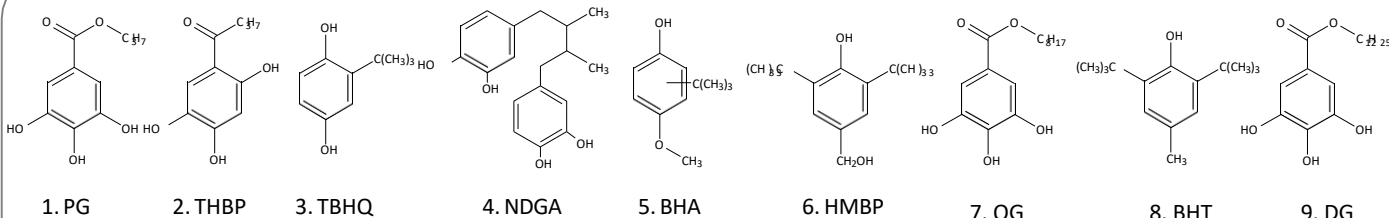


1. Propyl gallate (PG)
2. 2,4,5-Trihydroxybutyrophenone (THBP)
3. *tert*-Butylhydroquinone (TBHQ)
4. Nordihydroguaiaretic acid (NDGA)
5. Butylated Hydroxyanisole (BHA)
6. 4-Hydroxymethyl-2,6-di-*tert*-butylphenol (HMBP)
7. Octyl gallate (OG)
8. Butylated hydroxytoluene (BHT)
9. Dodecyl gallate (DG)

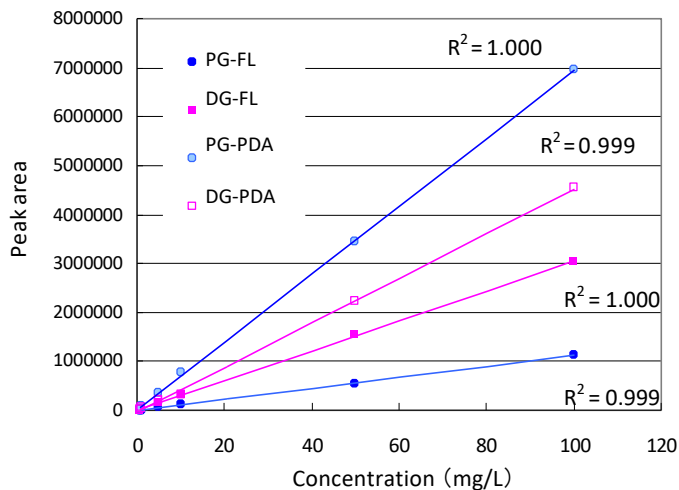
HPLC Conditions

Column	: Inertsil ODS-SP (5 μ m, 150 x 4.6 mm I.D.) Cat.No. 5020-02745
Eluent	: A) CH ₃ OH B) CH ₃ CN C) 5% Acetic acid A/B/C = 20/20/60 - 15 min - 50/50/0 (equilibration 10 min), v/v/v (Mixed by a gradient mixer)
Flow rate	: 1.5 mL/min
Col. Temp.	: 40 °C
Detection	: PDA 280 nm FL Ex. 275 nm, Em. 365 nm
Inj. Volume	: 10 μ L

Chemical Structures



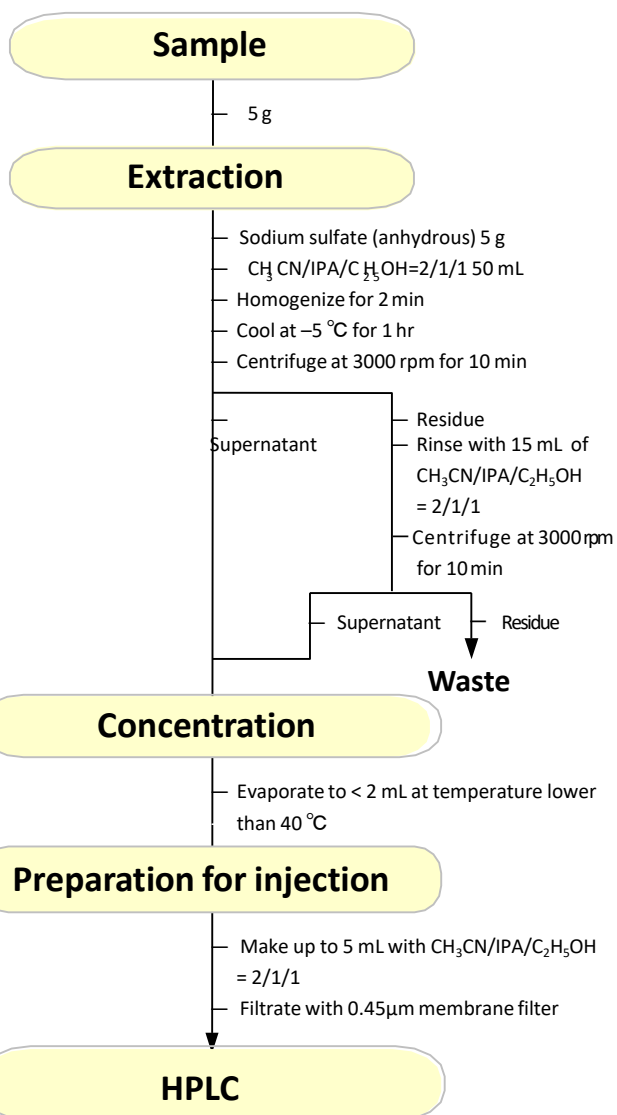
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.



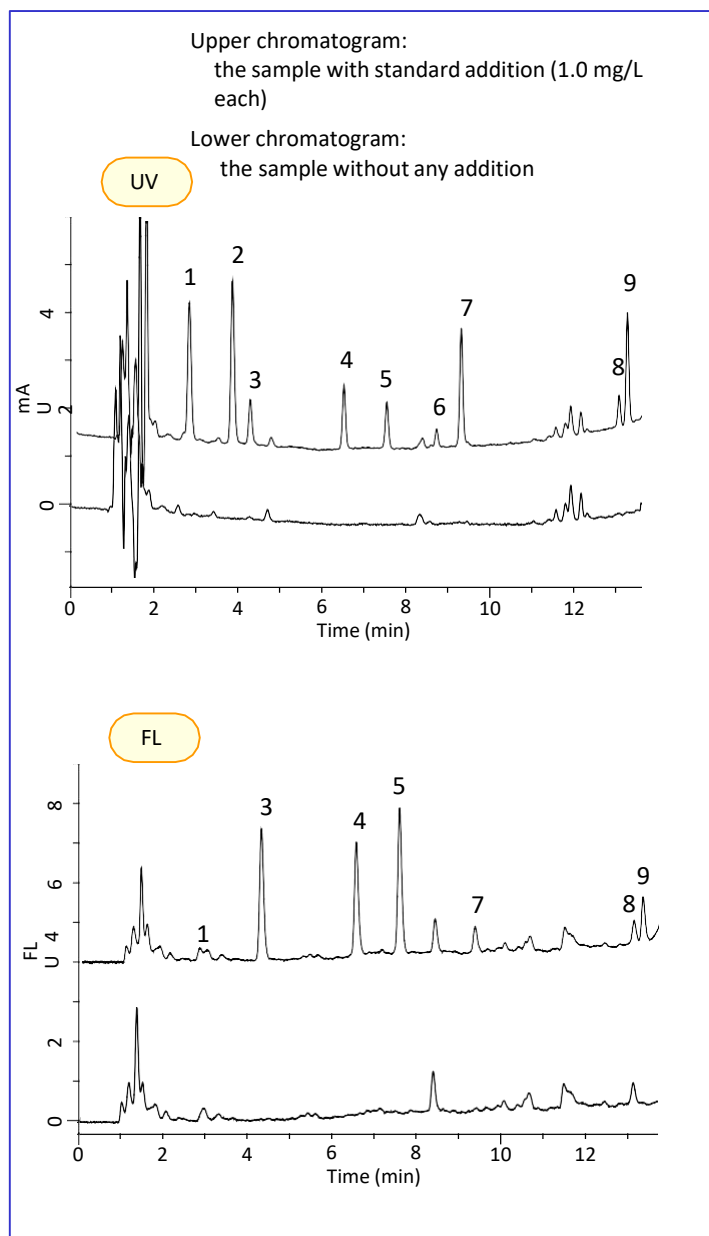
	R ²	
	PDA	FL
1. PG	: 1.000	0.999
2. THBP	: 1.000	—
3. BHQ	: 1.000	0.997
4. NDGA	: 1.000	0.998
5. BHA	: 1.000	0.999
6. HMBP	: 1.000	—
7. OG	: 1.000	0.999
8. BHT	: 0.998	0.999
9. DG	: 0.999	1.000

The calibration curves and their correlation coefficients

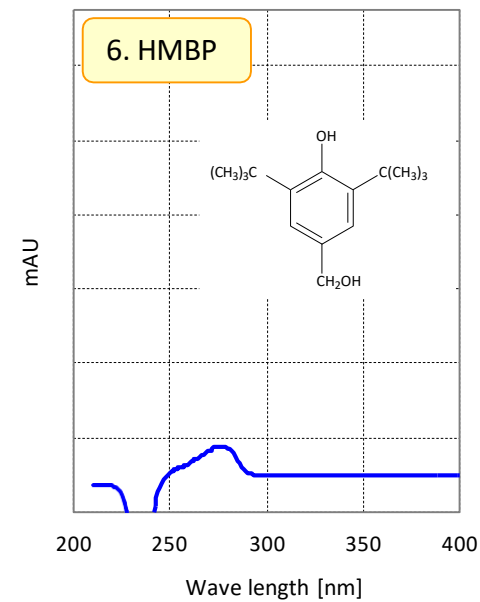
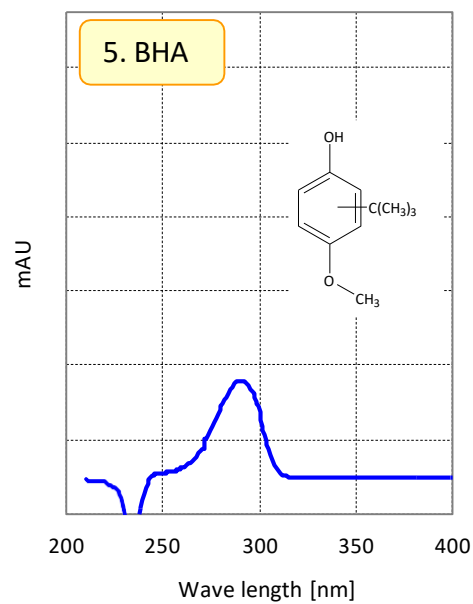
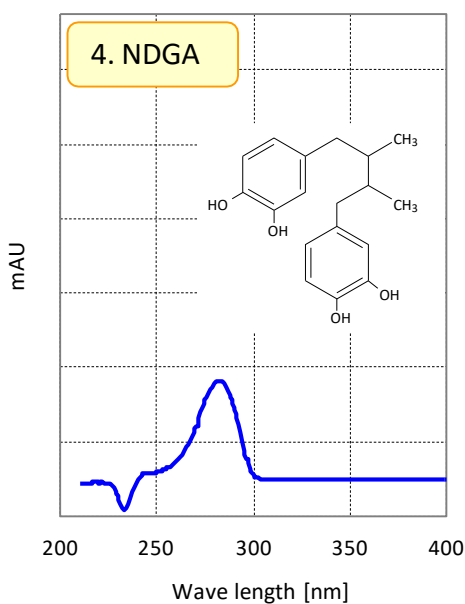
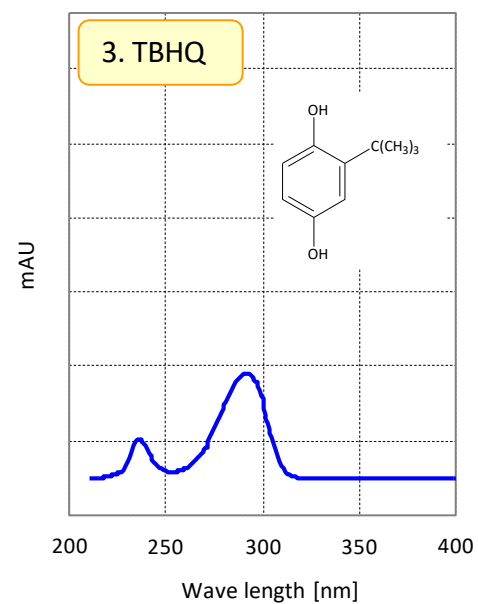
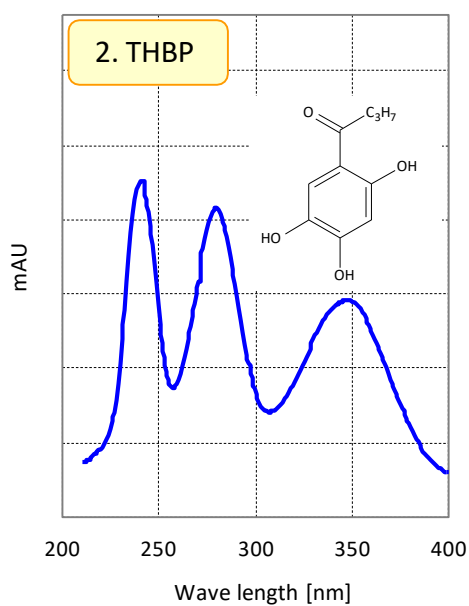
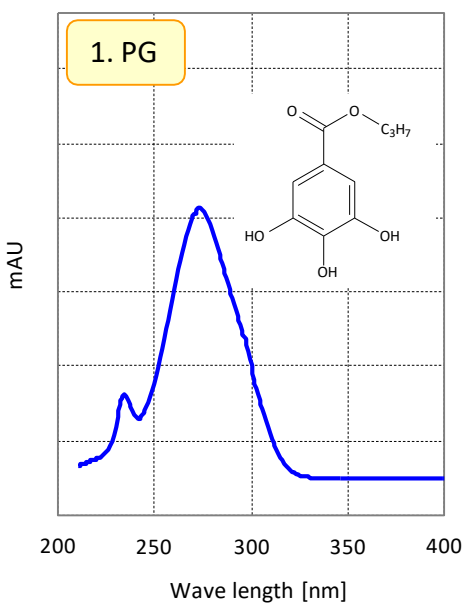
An example of sample pretreatment

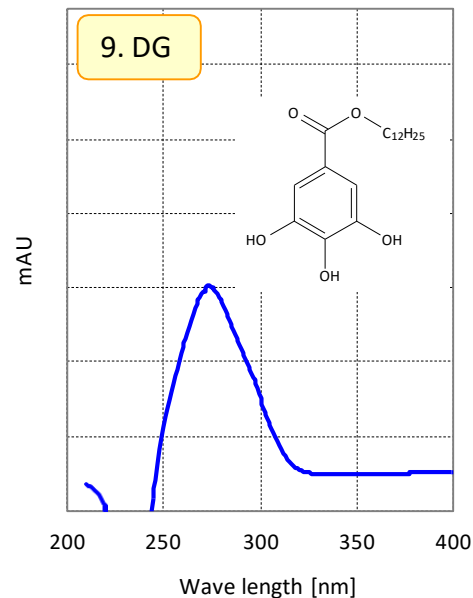
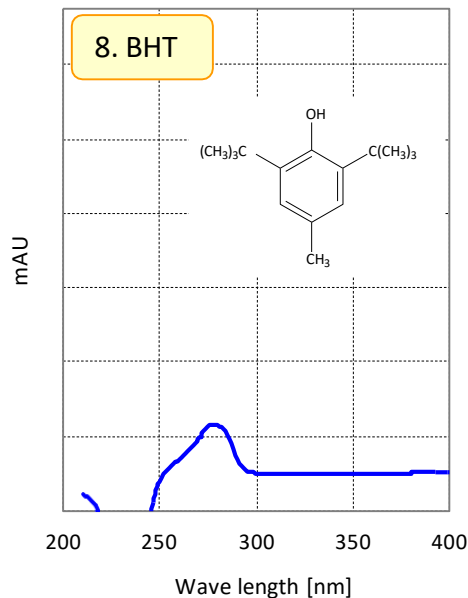
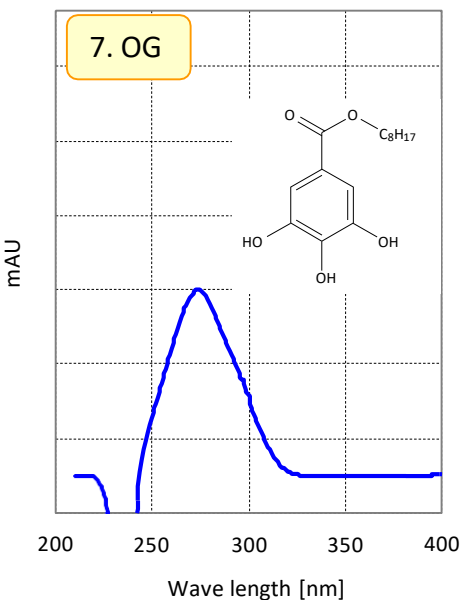


Chromatograms obtained from food sample (margarine)



The adsorption spectra obtained with PDA detector





* The absorbance scale of each spectrum was all the same.

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