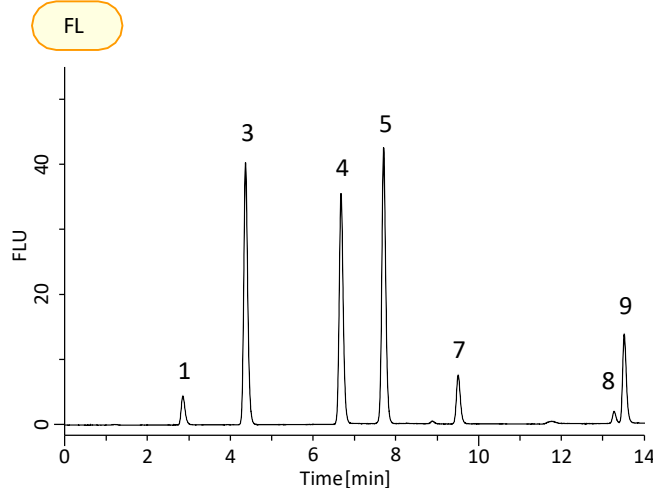
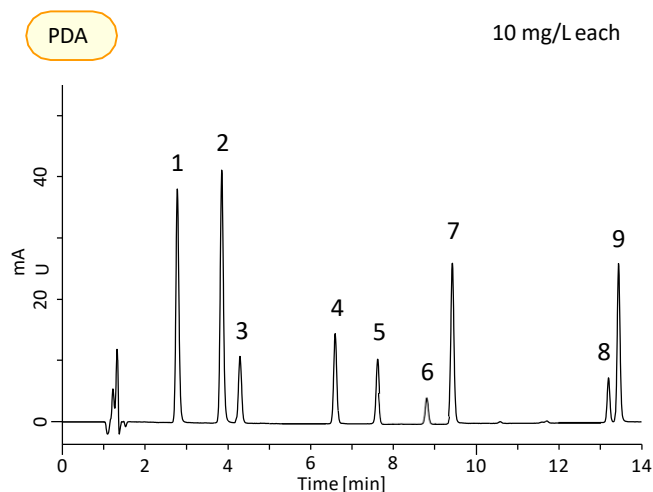


This note describes a simultaneous determination method for 9 phenolic antioxidants using a binary gradient HPLC system.

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.

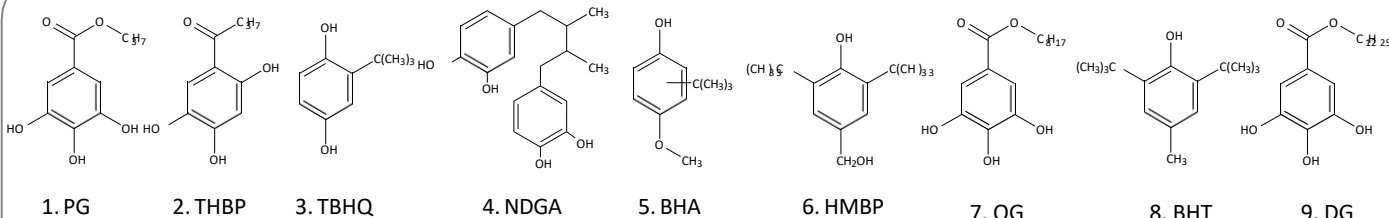
(K.Suzuki)

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)

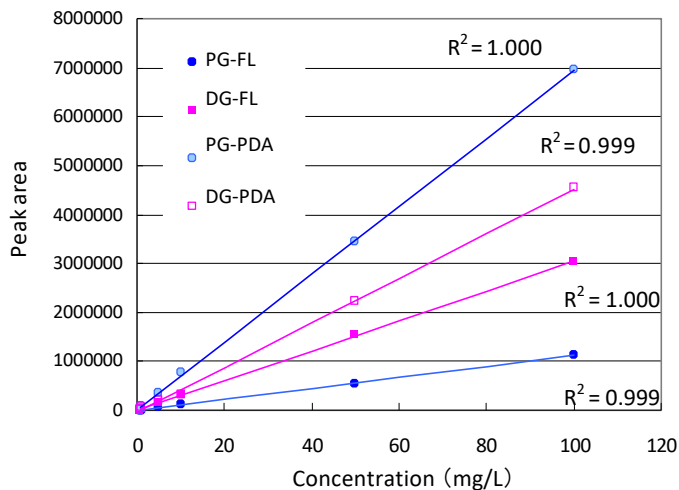
HPLC Conditions

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



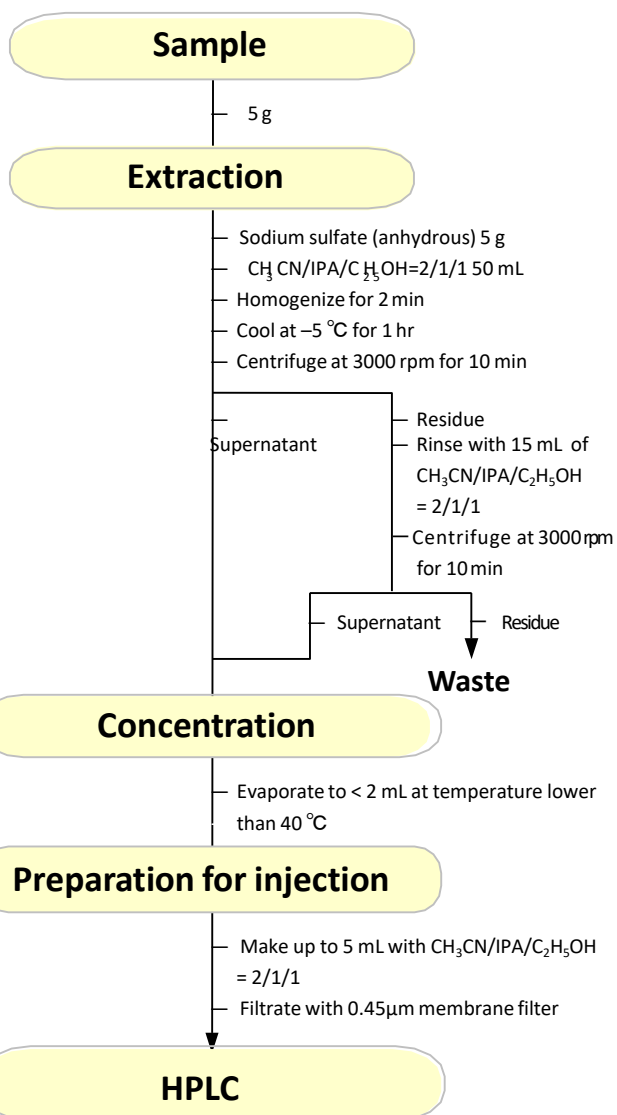
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.



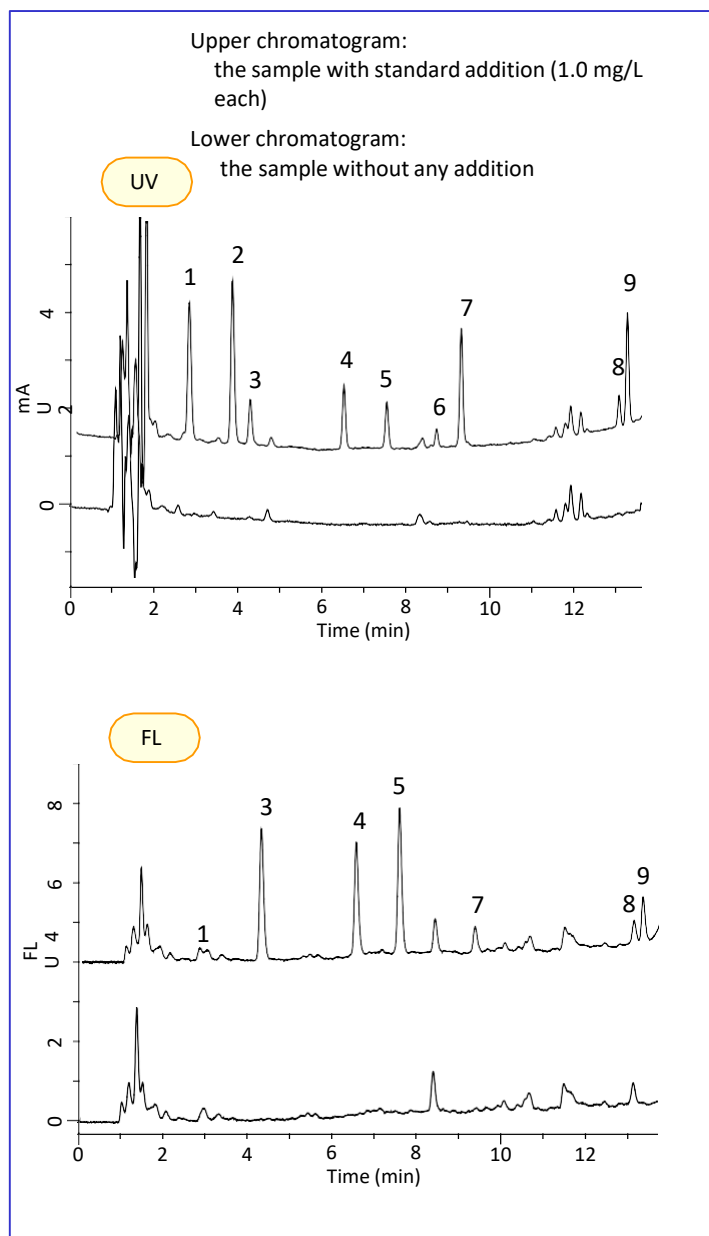
	R^2	
	PDA	FL
1. PG	1.000	0.999
2. THBP	1.000	—
3. BHQ	1.000	0.997
4. NDGA	1.000	0.998
5. BHA	1.000	0.999
6. HMBP	1.000	—
7. OG	1.000	0.999
8. BHT	0.998	0.999
9. DG	0.999	1.000

The calibration curves and their correlation coefficients

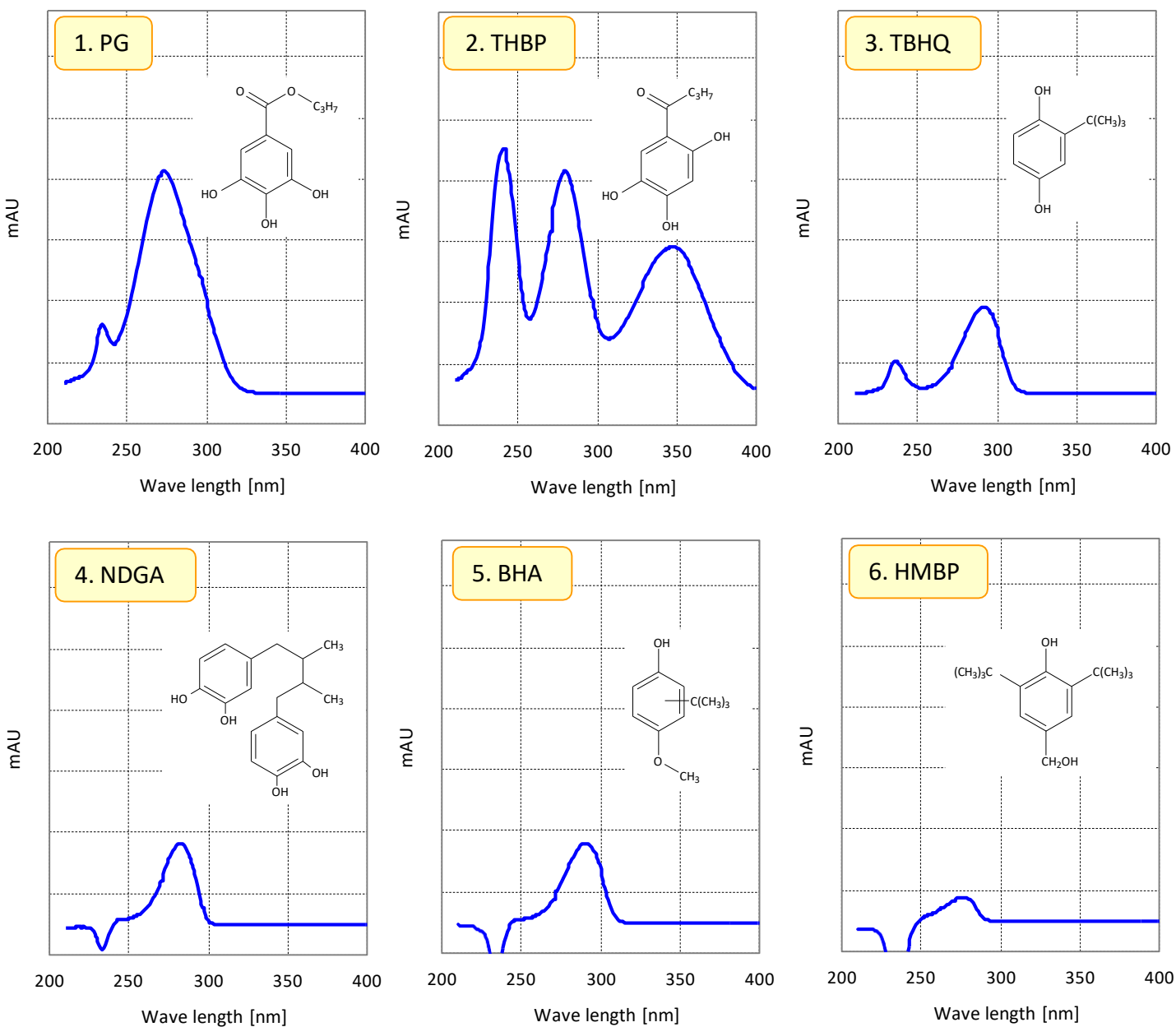
An example of sample pretreatment

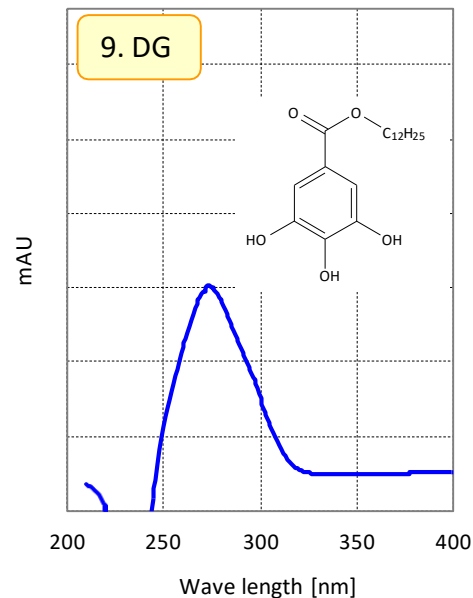
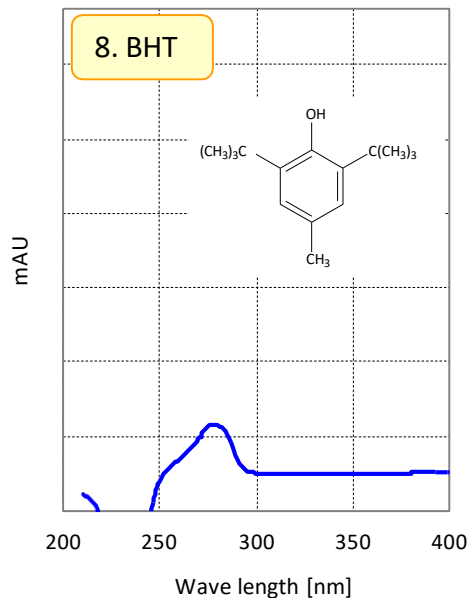
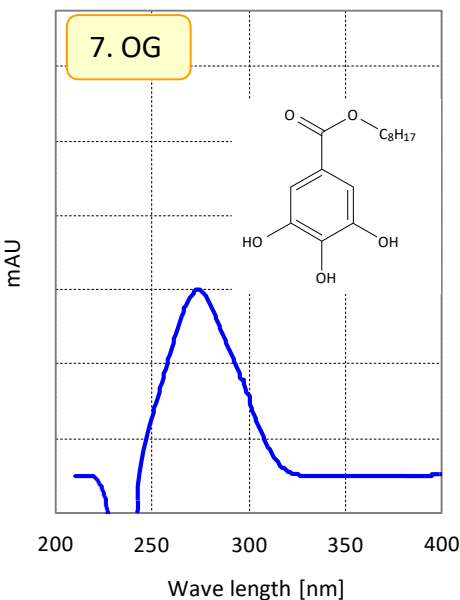


Chromatograms obtained from food sample (margarine)



The adsorption spectra obtained with PDA detector





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

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