

This note describes a determination method for phenolic antioxidants using an Inertsil Ph-3 column, in which phenyl groups chemically bonded directly to porous silica particles.

In a previous note (No.64), sufficient separation of the antioxidants was achieved by an ODS column coupled with gradient elution of mobile phase. However, if an interfering peak is detected near an analyte peak or an unknown peak is required to be identified, it is necessary

to use another column in which stationary phase is modified with different functional groups.

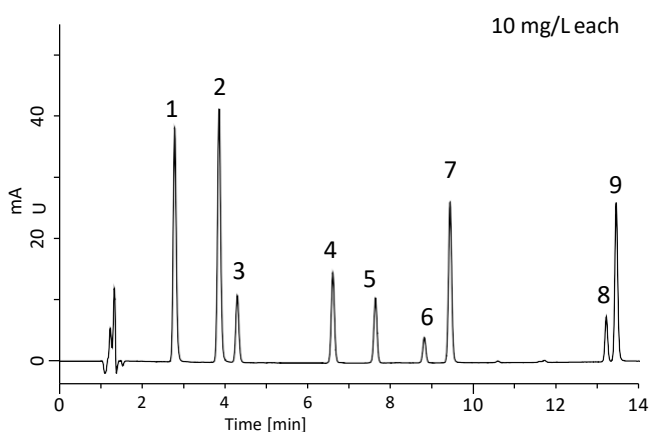
In this note, Inertsil Ph-3 was chosen among reversed-phase HPLC columns. As well as good separation of the antioxidants was obtained, elution order was significantly changed owing to the interaction between π electrons of the benzene rings bonded to the column particles and the aromatic analytes.

(K.Suzuki)

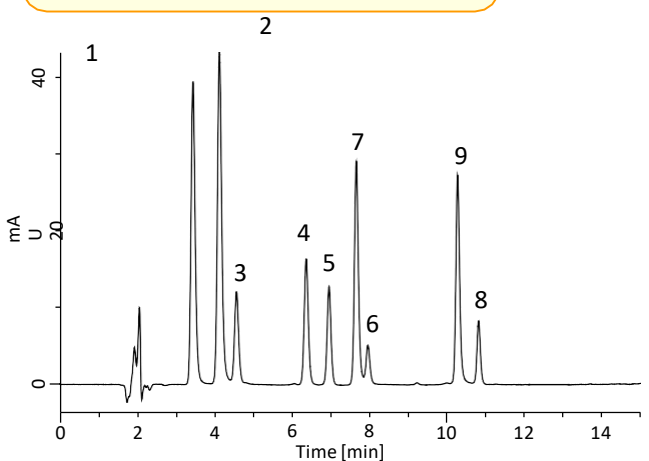
Chromatograms obtained from standard

solution

Inertsil ODS-SP (Flow rate: 1.5 mL/min)



Inertsil Ph-3 (Flow rate: 1.0 mL/min)

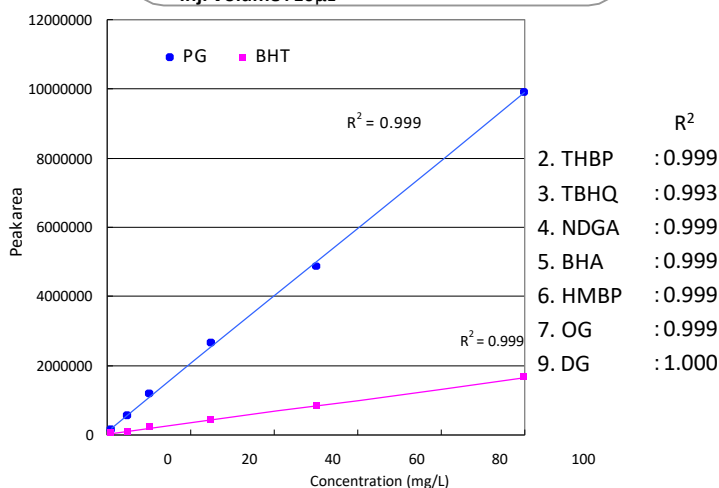


1. Propyl gallate (PG)
2. 2,4,5-Trihydroxybutyrophenone (THBP)
3. *tert*-Butylhydroquinone (TBHQ)
4. Nordihydroguaiaric acid (NDGA)
5. Butylated Hydroxyanisole (BHA)
6. 4-Hydroxymethyl-2,6-di-*tert*-butylphenol (HMBP)
7. Octyl gallate (OG)
8. Butylated hydroxytoluene (BHT)
9. Dodecyl gallate (DG)

Conditions

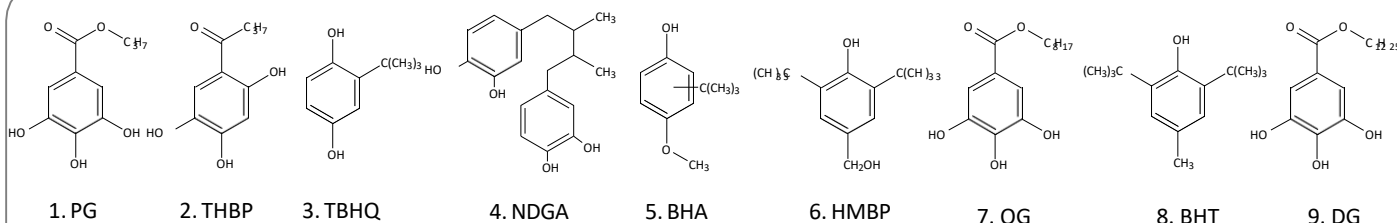
Column : (5 μ m, 150 x 4.6 mm I.D.)
Eluent : A) CH₃OH
 B) CH₃CN
 C) 5 % Acetic acid
 A/B/C = 20/20/60 — 15 min
 — 50/50/0 (Equilibration for 10 min), v/v/v
 (Mixed by a gradient mixer)

Col. Temp. : 40 °C
Detection : PDA 280 nm
Inj. Volume : 10 μ L



Calibration curves and correlation coefficients (Column: Inertsil Ph-3)

Chemical Structures

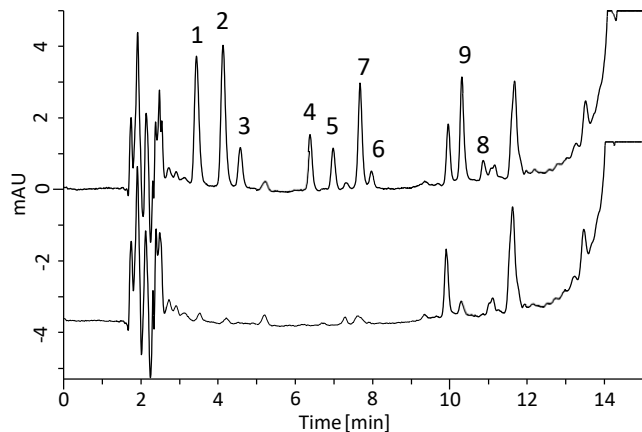


Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

A chromatogram obtained from food sample**Margarine**

Standard addition: 1.0 mg/L each

Inertsil Ph-3

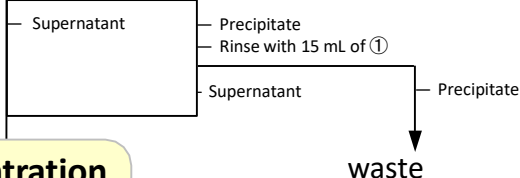
**Sample**

—5 g

Extraction

- Sodium sulfate anhydrous 5 g
- $\text{CH}_3\text{CN}/\text{IPA}/\text{C}_2\text{H}_5\text{OH} = 2/1/1$ (①) 50 mL
- Homogenize for 2 min
- Cool at -5°C for 1 hr

<Sample Pretreatment Method>

Concentration

- Evaporate *in vacuo* to < 2 mL at lower than 40°C
- Make up to 5 mL with ①
- Filtrate with 0.45- μm membrane filter (GL CHROMATO DISK)

HPLC

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