

GL Sciences Inc.

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

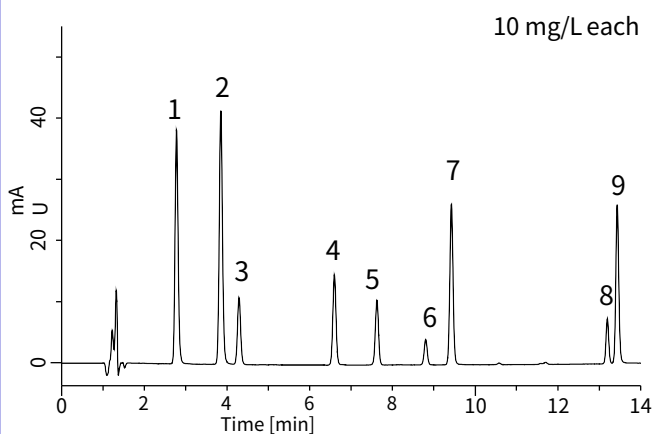
to use another column in which stationary phase is modified with different functional groups.

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.

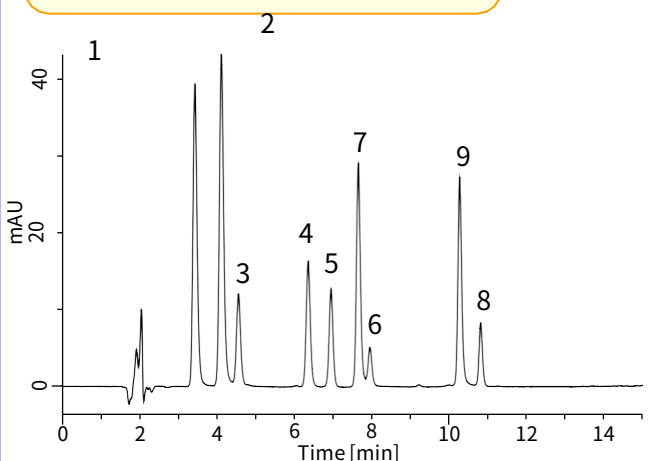
(K.Suzuki)

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. 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)

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

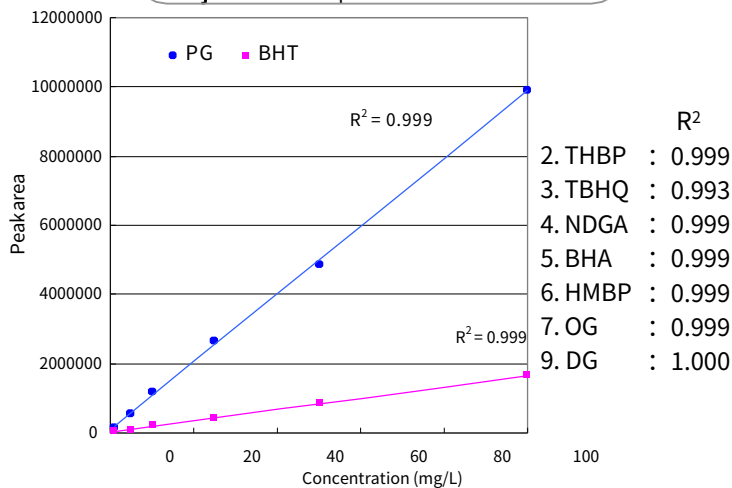
— 50/50/0 (Equilibration for 10 min), v/v/v

(Mixed by a gradient mixer)

Col. Temp. : 40 °C

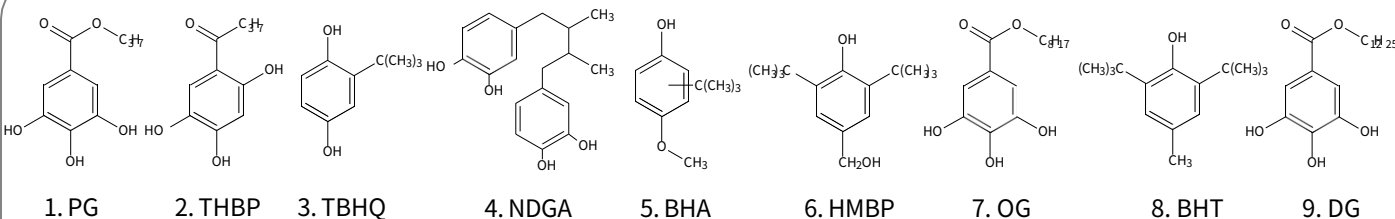
Detection : PDA 280 nm

Inj. Volume : 10 μ L



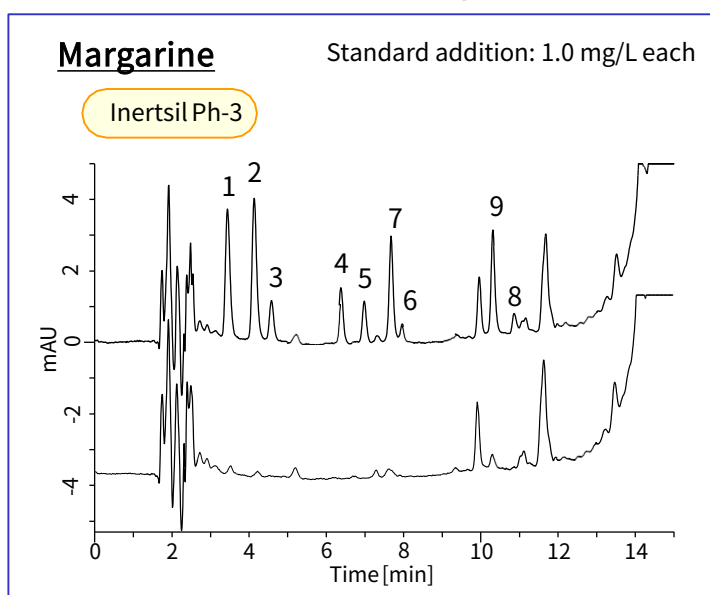
Calibration curves and correlation coefficients (Column: Inertsil Ph-3)

Chemical Structures



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

A chromatogram obtained from food sample



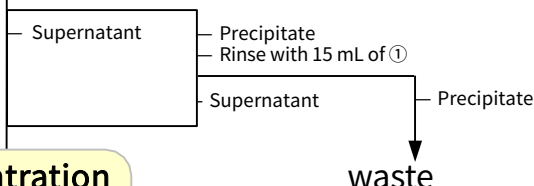
Sample

<Sample Pretreatment Method>

-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



Concentration

- Evaporate *in vacuo* to < 2 mL at lower than 40°C
- Make up to 5 mL with ①
- Filtrate with $0.45\text{-}\mu\text{m}$ membrane filter (GL CHROMATO DISK)

HPLC

GL Sciences disclaims any and all responsibility for any injury or damage which may be caused by this data directly or indirectly. We reserve the right to amend this information or data at any time and without any prior announcement.

GL Sciences Inc. Japan

22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@glsc.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA

4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.

Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited

Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn



International Distributors

Visit our Website at www.glsciences.com/distributors