■ Analysis of Resveratrol

Resveratrol is a type of polyphenol that has been attracting attention as a natural antioxidant. Although all polyphenols have antioxidant effects, resveratrol is attracting special attention because its biological activities are well-characterized, and many reports claim that it is valuable to human health and exhibits anti-aging effects. While an intrinsic component of plants, resveratrol is one of the antibacterial substances synthesized by vegetables, known as phytoalexins. Resveratrol is produced when plants are infected by fungi or damaged, in order to defend themselves against fungi or damage. Resveratrol is attracting attention in the United States, as it is contained in grape skin and peanut seed coats, has a strong antioxidant effect, and is effective in preventing cancers and lifestyle diseases.

The following describes an example analysis of resveratrol.

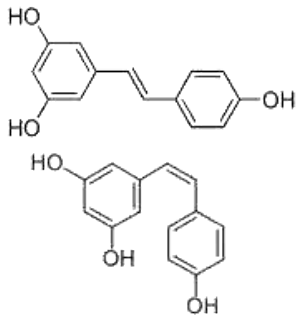
◆ Analysis of Resveratrol ◆

■Sample: Resveratrol

[Structural formula]

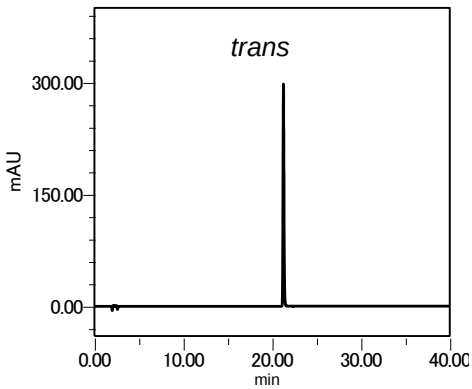
trans-Resveratrol
MW : 228.24

cis-Resveratrol
MW : 228.24



Standard solutions: Concentrations from 0.05-50 mg/L, prepared with methanol

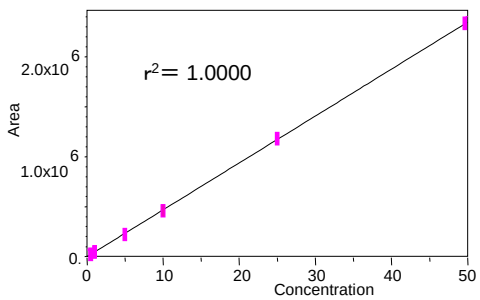
■ Analysis result of standard sample



trans-Resveratrol (Concentration: 50 mg/L)

■ Linearity

[Standard Curve]



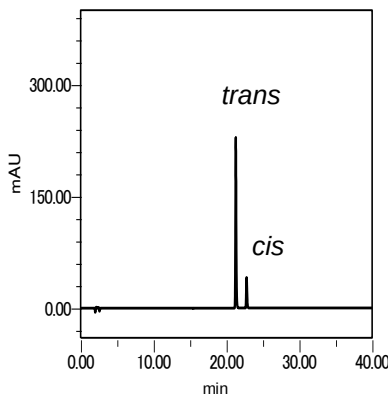
trans-Resveratol

A fairly linear standard curve was obtained in the concentration range between 0.05-50 mg/L.

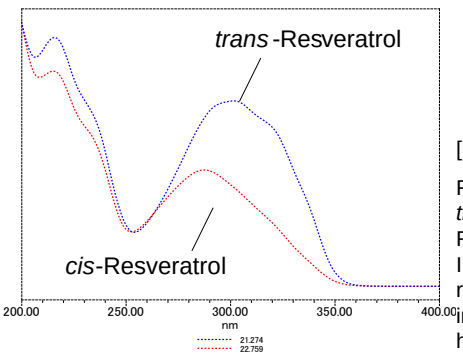
[LC Conditions]

Column	HITACHI LaChrom C18 (5 µm) 4.6 mm I.D. × 150 mm
Eluent	(A)0.1% H_3PO_4 (pH 2.2) (B)CH_3CN *Gradient: (0 min) B10% → (10 min) B10% → (30 min) B70%→ (40 min) B70% → (40.1 min) B10% → (55 min) B10%
Flow rate	1.0 mL/min
Column temperature	40°C
Detection	DAD 303 nm
Injection volume	10 µL

■ *trans*-Resveratrol standard sample after UV irradiation



trans-Resveratrol
(Concentration : 50 mg/L)



Spectrum overlay of the obtained peaks

[System configuration]
5110 Pump
5210 AutoSampler
5310 Column Oven
5430 DAD
Empower2 Data Processing System

[Identification of *cis*-resveratrol]

Resveratrol has two isomers: *trans* and *cis*. *trans*-Resveratrol is converted to *cis*-Resveratrol by UV or heat.
In this analysis, 10 mL of 50 mg/L *trans*-resveratrol was placed in a sample bottle, and irradiated with a UV disinfection lamp for 1 hour. The eluting position and spectrum of the *cis*-resveratrol formed by irradiation were identified.

■ Analysis of Resveratrol

■ Analysis example of a standard sample： Commercial red wine

[Pretreatment method of the sample]

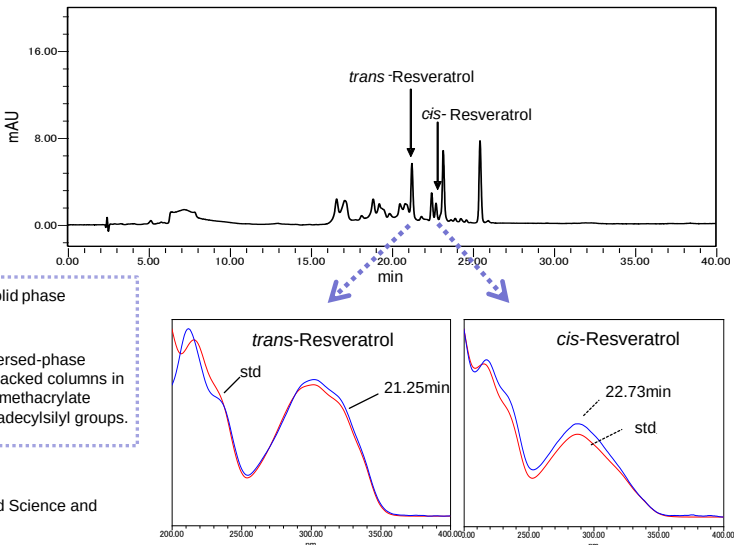
NOBIAS RP-OD1W *1

- Pretreatment
  - Methanol, 10 mL
  - 1/30 mol/L phosphoric acid buffer solution (pH7) 50 mL
- Sample loading — Red wine, 10 mL; white wine, 50 mL
  - Wine: 50 mL
  - H_2O, 30mL
  - Adjusted to pH 7.0 with 1N NaOH
  - The volume is fixed with H_2O at 100 mL
- Drying
  - Drying with nitrogen gas
- Extraction
  - After elution with 5 mL of ethyl acetate, the volume was fixed with ethyl acetate at 5 mL
  - Filtering through a 0.45 µm filter
- HPLC

*1 NOBIAS RP-OD1W：Solid phase extraction columns

These polymer-based reversed-phase columns are solid phase packed columns in structure, with hydrophilic methacrylate substrate bonded with octadecylsilyl groups.

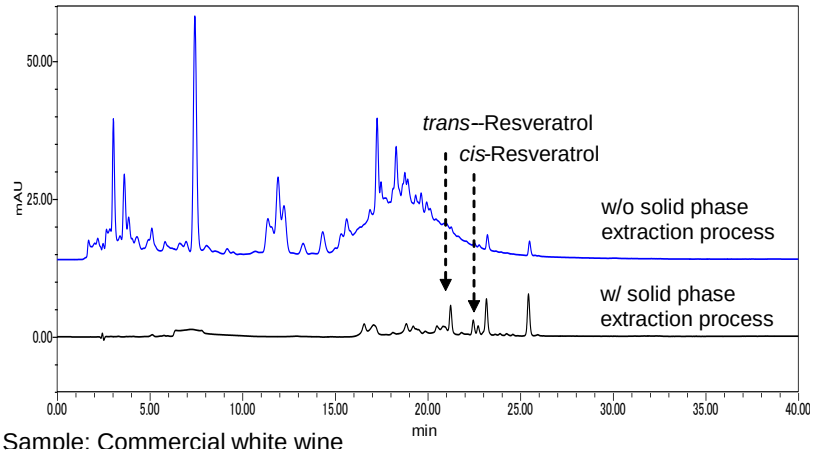
[Commercial red wine]



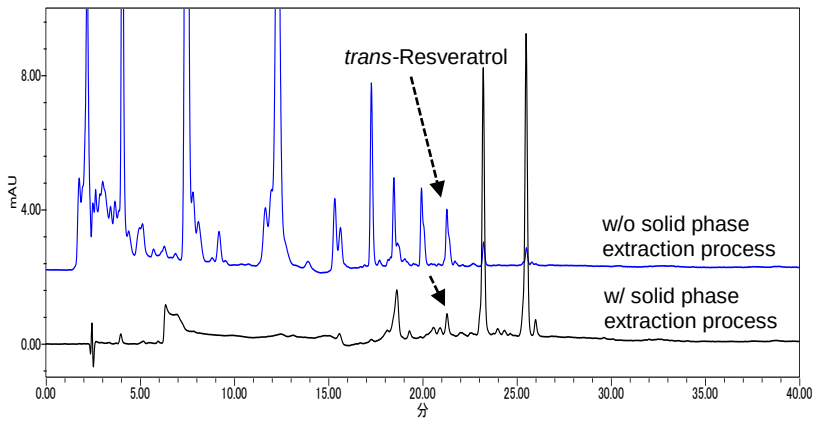
Reference: New Food Analysis Method〔Ⅱ〕The Japanese Society for Food Science and Technology and Japanese Society for Food Analysis
Published on November 15, 2006, by Korin Publishing Co.

■Comparison of the sample with and without a solid phase extraction process

(1) Sample: Commercial red wine



(2) Sample: Commercial white wine



[Comparison with the spectrum of a standard Sample]

[w/ solid phase extraction process]
Treated as described in the process flow above.

[w/o solid phase extraction process]
After adjustment to pH 7.0, as described in the process flow above, filtered with a 0.45 µm filter.

A sample with solid phase-extracted wine and a sample with non-processed wine were measured and the resulting chromatograms were compared. As wine includes various components, a peak for resveratrol in a sample with non-processed red wine cannot be identified. This result proves that pretreatment with solid phase extraction is effective.

Both red wine and white wine exhibited recovery rates of 94.0%, by standard addition.

Concentration of resveratrol in each wine sample:
Red wine：1.1 mg/L
White wine：0.24 mg/L

Resveratrol is contained in large amounts in grape skin. It was confirmed that red wine, when brewed from grapes retaining their skins, has a higher concentration of resveratrol.

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.
The system is for research use only, and is not intended for any animal or human therapeutic or diagnostic use..