

# Application News

## High Performance Liquid Chromatography

## No.L405

### Analysis of Flavonoids in Ginkgo Biloba Extract

Flavonoids are a kind of polyphenol, and the name also refers to a class of plant metabolites.

Recently, there has been much research on flavonoids with regard to their physiological activity, and in particular, their anti-oxidative effects have been reported.

Ginkgo biloba leaves are said to contain as many as

20 types of flavonoids, and among these are quercetin, kaempferol and isorhamnetin, 3 types that are present in large quantities.

Here we introduce an example of the analysis of these 3 flavonoids present in ginkgo biloba leaves using the SPD-M20A photodiode array detector.

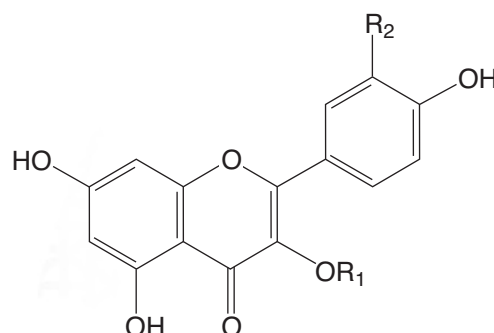
#### ■ Analysis of Standard Solution

Fig. 1 shows the structures of the 3 flavonoids (quercetin, kaempferol and isorhamnetin) analyzed here.

Fig. 2 shows the UV-VIS absorption spectrum of quercetin, indicating that all of these compounds display maximum absorption in the vicinities of 250-260 nm and 370 nm. Fig. 3 shows a chromatogram of the 3 flavonoids in a standard solution at 370 nm, and Table 1 shows the analytical conditions used. For detection, the SPD-M20A photodiode array detector was used.

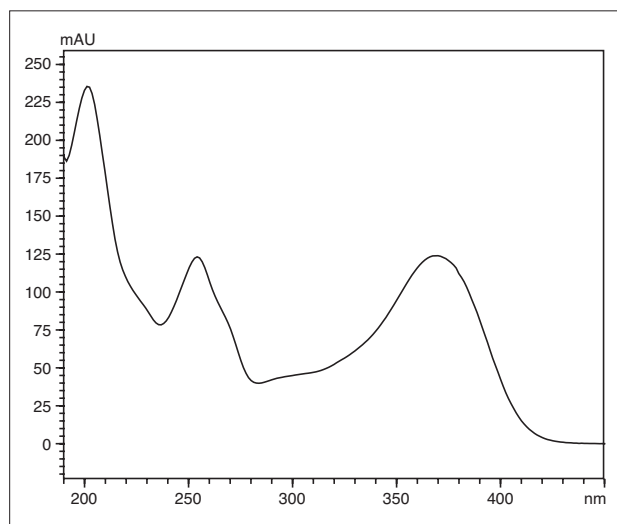
**Table 1 Analytical Conditions**

Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D. )
Mobile Phase	: A; 1.0 % Phosphoric acid (85 % ) aq. : B; Acetonitrile B Conc. 30 % (0 -12 min) 90 % (12.01-15 min) : → 30 % (15.01-20 min)
Flow Rate	: 1.5 mL/min
Column Temp.	: 60 °C
Injection Volume	: 10 μL
Detection	: SPD-M20A at 370 nm

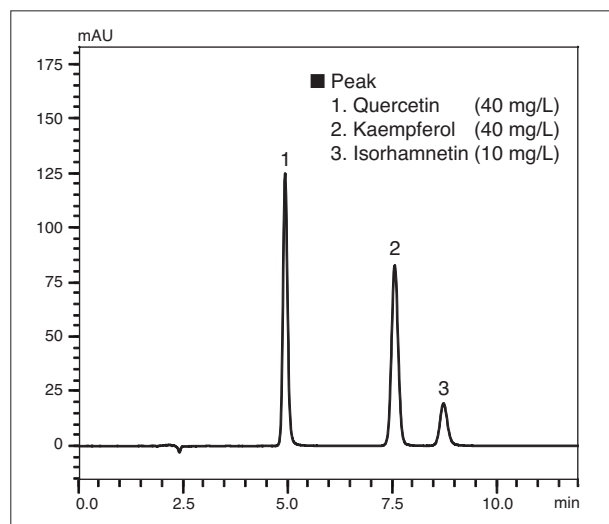


Compound	R <sub>1</sub>	R <sub>2</sub>
Kaempferol	H	H
Quercetin	H	OH
Isorhamnetin	H	OCH <sub>3</sub>

**Fig. 1 Structures of 3 Flavonoids**



**Fig. 2 UV-VIS Spectrum of Quercetin**



**Fig. 3 Chromatogram of a Standard Mixture of 3 Flavonoids**

### ■ Linearity

Fig. 4 shows the calibration curves for quercetin and kaempferol using a concentration range of 2-40 mg/L, and isorhamnetin with a concentration range of

0.5-10 mg/L. Excellent linearity was obtained for all the substances, with  $R^2$  values greater than 0.9999.

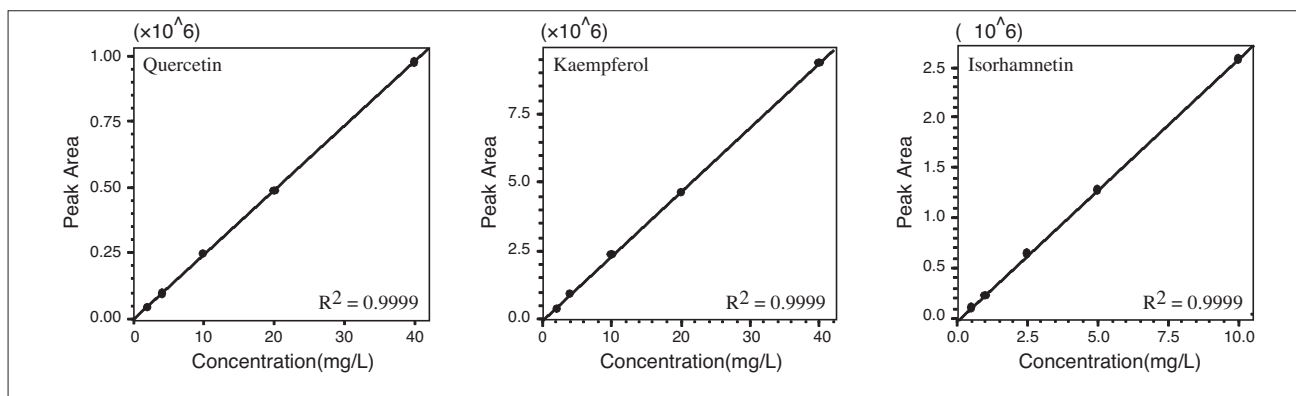


Fig. 4 Linearity

### ■ Analysis of Ginkgo Biloba Extract Supplement

We conducted analysis of a commercially available ginkgo biloba leaf extract (tablet) after preparing the sample according to the procedure shown in Fig. 5.

Fig. 6 shows the obtained chromatogram. Fig. 7 shows the respective spectra of the flavonoids in the sample overlaid with the corresponding standard spectra.

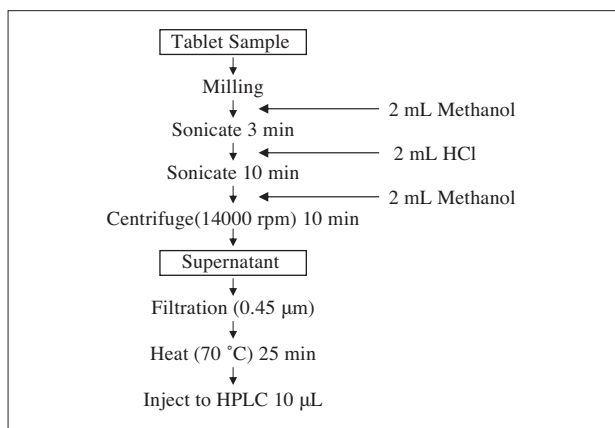


Fig. 5 Sample Preparation

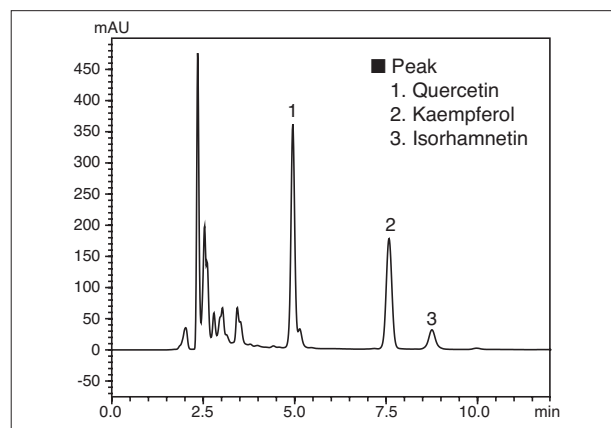


Fig. 6 Chromatogram of Ginkgo Biloba Dietary Supplement

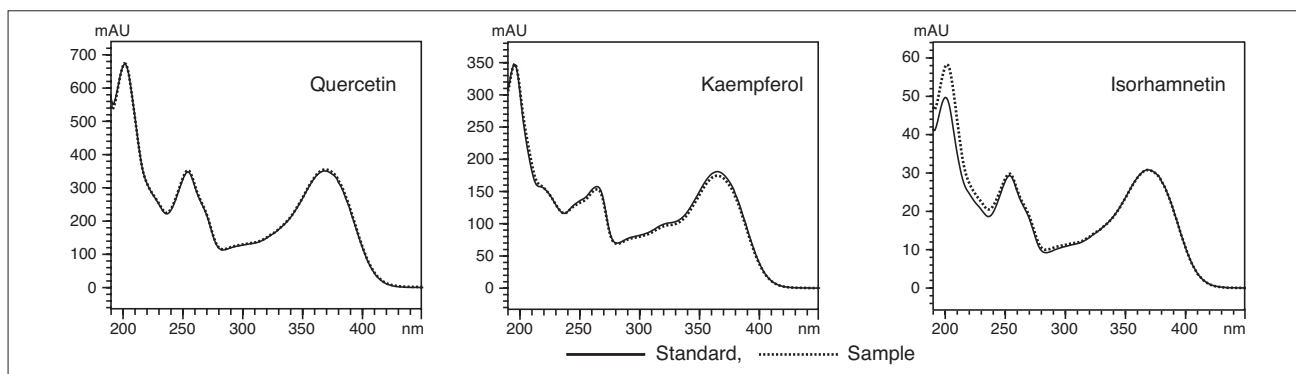


Fig. 7 UV-VIS Spectra of 3 Flavonoids in Ginkgo Biloba Dietary Supplement

#### [References]

US Pharmacopeia (USP32-NF27)