

Application News

High Performance Liquid Chromatography

No.L400

Analysis of Ginkgolic Acids in Ginkgo Biloba Extract

Ginkgo leaf extract (ginkgo biloba extract), which contains active ingredients extracted from ginkgo biloba leaves, is reported to be effective in improving cerebral and peripheral blood circulation deficiencies. It is used in Japan and the United States in the form of a dietary supplement, and in Germany, France, and other European countries as a prescription medication. However, alkylphenols which are present

in the ginkgolic acids contained in ginkgo leaves are known to cause allergic reactions. For this reason, the United States Pharmacopeia (USP) has established an upper limit for ginkgolic acid content in ginkgo biloba extract.

Here we introduce an example of analysis of ginkgolic acids contained in ginkgo biloba leaves.

■ Analysis of Standard Solution

The ginkgolic acids in ginkgo biloba leaves that were analyzed include ginkgolic acid C13:0 (hereafter, GA C13:0), GA C15:0, GA C15:1, and GA C17:1. Fig. 1 shows the structural formula of these 4 substances. Due to the high hydrophobicity of these ginkgolic acids, the Shim-pack CLC-C8 in which the silica gel is modified with an octyl group (C8) was used, and chromatography was conducted using gradient elution. For detection, the SPD-M20A photodiode array detector was used. Table 1 shows the analytical conditions used. Fig. 2 shows the spectrum of GA C17:1, and Fig. 3 shows a chromatogram of a standard mixture of 4 ginkgolic acids.

Table 1 Analytical Conditions

Column	: Shim-pack CLC-C8 (250 mm L. × 4.6 mm I.D.)
Mobile Phase	: A; 0.01 % Phosphoric acid (85 %) - Water B; 0.01 % Phosphoric acid (85 %) - Acetonitrile
Time Program	: B Conc. 80 % (0 min) → 90 % (15-18 min) → 80 % (18.01-25 min)
Flow Rate	: 1.0 mL/min
Column Temp.	: 35 °C
Injection Volume	: 20 µL
Detection	: SPD-M20A at 311 nm

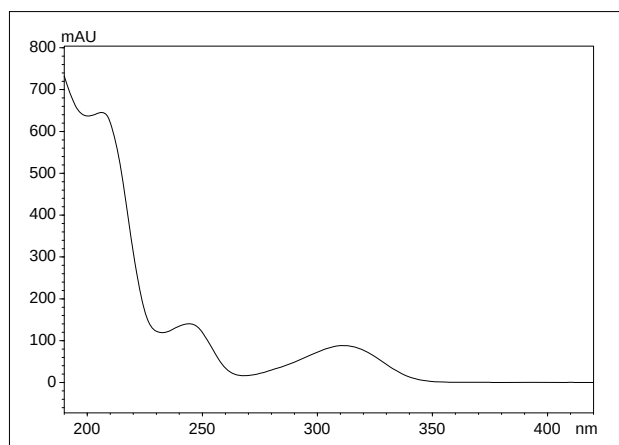
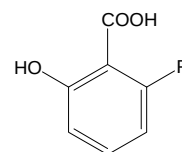


Fig. 2 UV Spectrum of Ginkgolic Acid C17:1



Compound		R
GA C13:0	Ginkgolic Acid C13:0	C ₁₃ H ₂₇
GA C15:0	Ginkgolic Acid C15:0	C ₁₅ H ₃₁
GA C15:1	Ginkgolic Acid C15:1	C ₁₅ H ₂₉
GA C17:1	Ginkgolic Acid C17:1	C ₁₇ H ₃₃

Fig. 1 Structures of Ginkgolic Acids

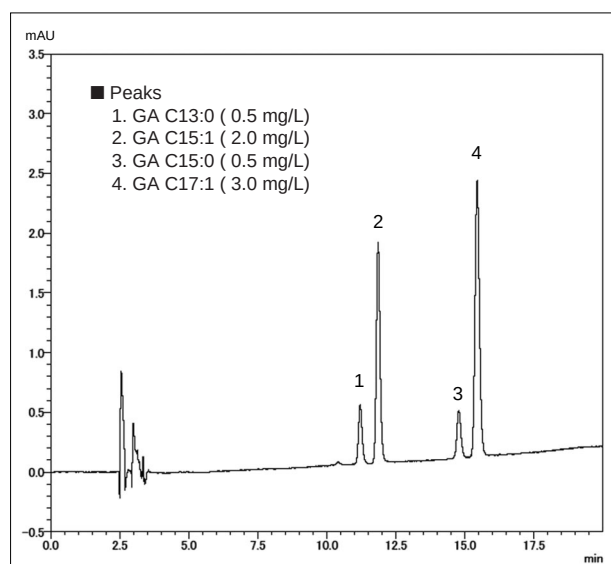


Fig. 3 Chromatogram of a Standard Mixture of 4 Ginkgolic Acids

■ Linearity and Repeatability

Fig. 4 shows the linearity of each substance within the concentration range of 0.02 - 4.0 mg/L, and the peak

area repeatability based on 6 successive analyses (using the concentrations recorded in the figure).

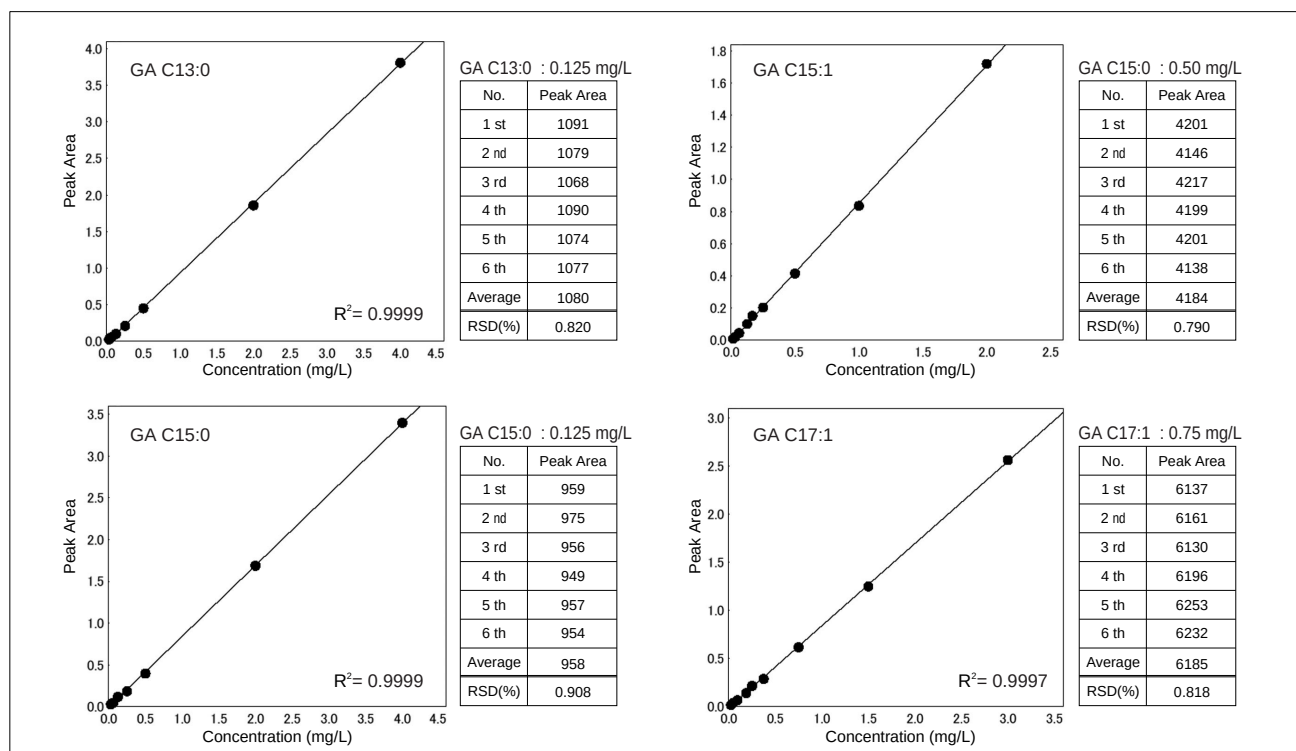


Fig.4 Linearity and Repeatability

■ Analysis of Ginkgo Biloba Extract Supplement

Analysis of the ginkgo biloba extract was conducted after performing sample pretreatment of the commercial supplement containing the extract as shown in Fig. 5. Fig. 6 shows the chromatogram. Since

ginkgolic acid was barely detected in this supplement, the chromatogram also shows the results of analysis of the prepared sample solution spiked with ginkgolic acid standard.

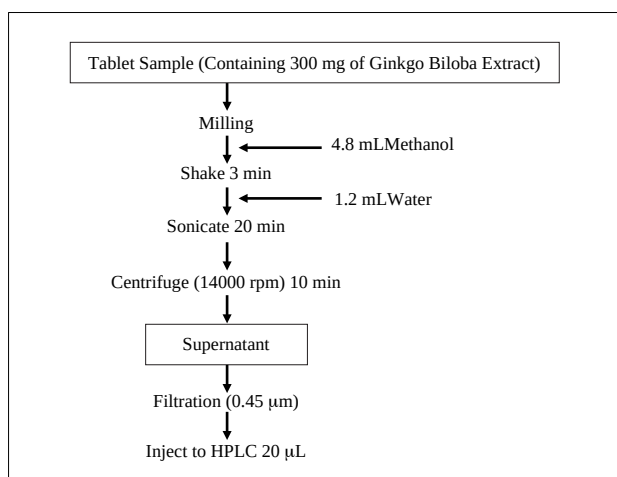


Fig. 5 Sample Preparation

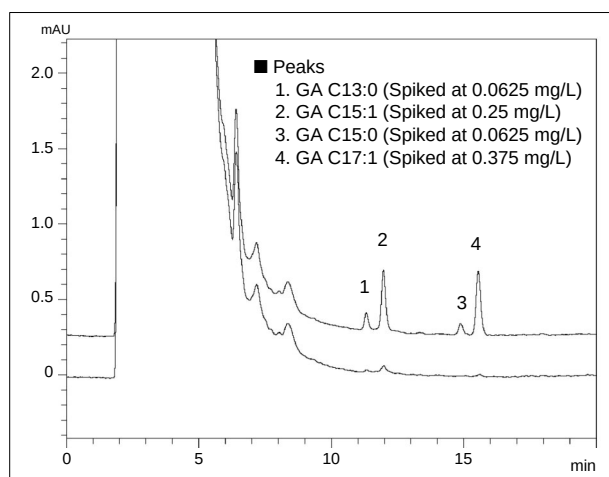


Fig. 6 Chromatogram of Ginkgo Biloba Extract Supplement (Upper: Spiked, Lower: Not Spiked)

[Reference]

United States Pharmacopeia (USP32-NF27)