

Detection of Fortification of Ginkgo Products Using Nanoelectrospray Ionization Mass

Spectrometry: Supplemental Data

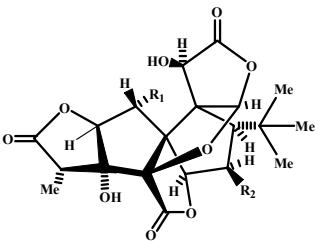
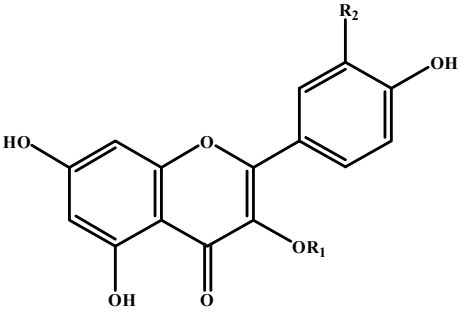
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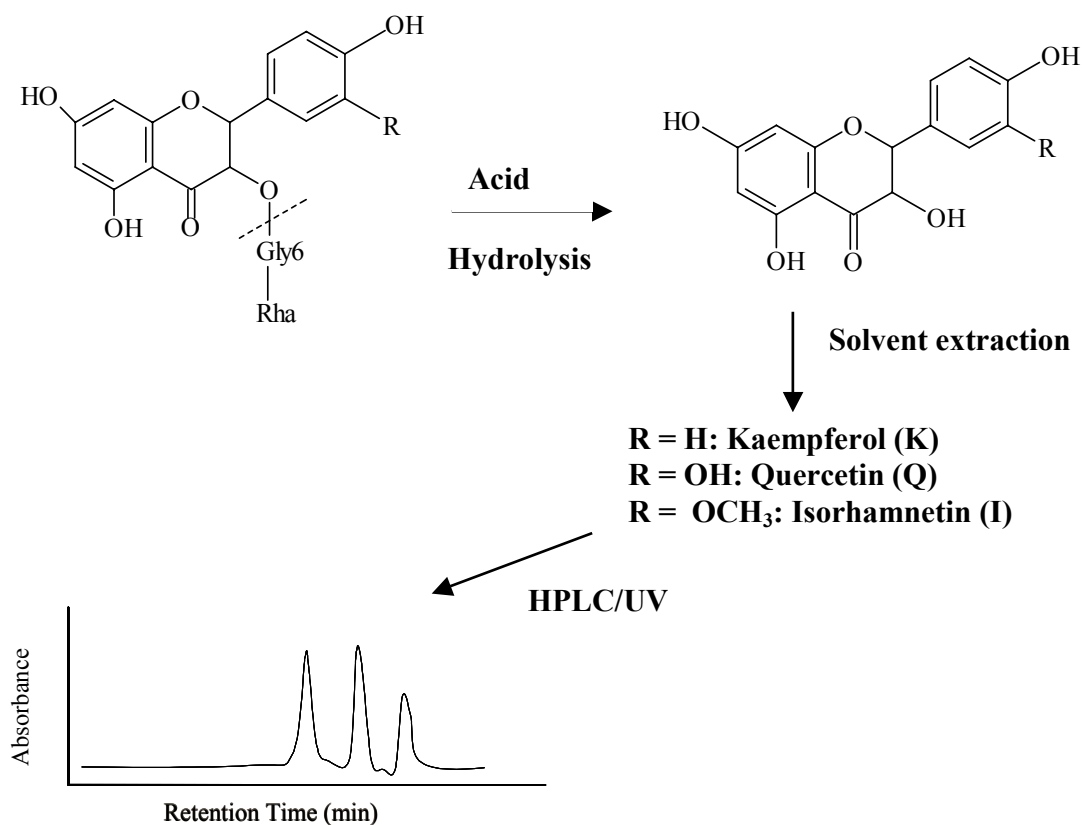
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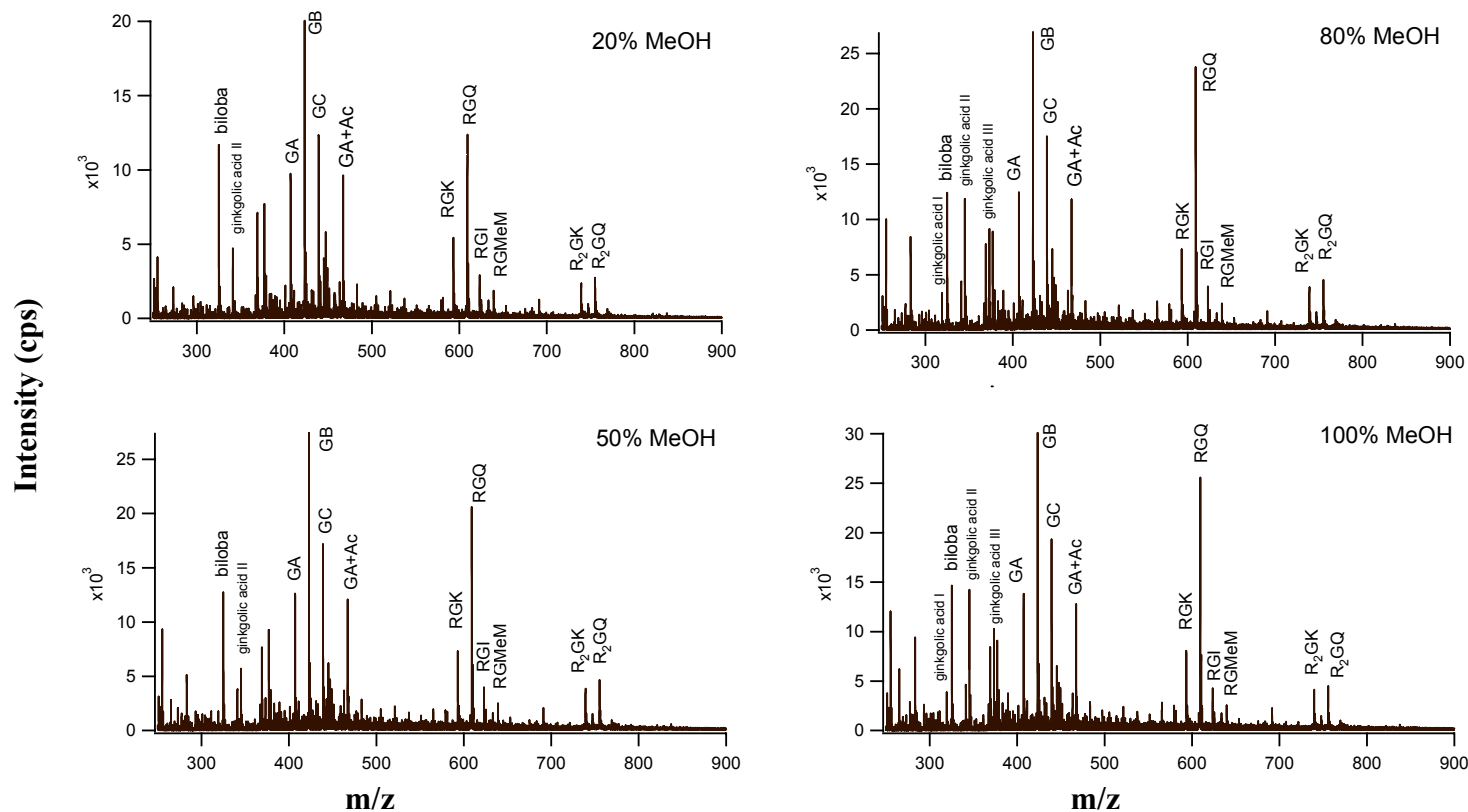
Supplemental Figure 1. Structures and molecular weights of the target compounds in Ginkgo

Structures	Name of the components	MW
	Terpenes: Ginkgolide A (GA) R ₁ = R ₂ = H Ginkgolide B (GB) R ₁ = OH, R ₂ = H Ginkgolide C (GC) R ₁ = R ₂ = OH	408 424 440
	Bilobalide	326
		Flavonol Glycosides: Kaempferol I (K) R ₁ =H, R ₂ =H Quercetin I (Q) R ₁ =H, R ₂ =OH Isorhamnetin I (I) R ₁ =H, R ₂ =OCH ₃
3-O-(6''-O-(α-L-rhamnosyl)-β-D-glycosyl) Kaempferol III (RGK) R ₁ =glc-rha, R ₂ =H		594
3-O-(6''-O-(α-L-rhamnosyl)-β-D-glycosyl) Quercetin III (RGQ) R ₁ =glc-rha, R ₂ =OH		610
3-O-(2''-O, 6''-O-bis(α-L-rhamnosyl)-β-D-glycosyl) Kaempferol IV (R₂GK) R ₁ =glc-(rha) ₂ , R ₂ =H		740
3-O-(2''-O, 6''-O-bis(α-L-rhamnosyl)-β-D-glycosyl) Quercetin IV (R₂GQ) R ₁ =glc-(rha) ₂ , R ₂ =OH		756
Ginkgolic acids: Ginkgolic acid I R=(CH ₂) ₁₂ CH ₃ Ginkgolic acid II R=(CH ₂) ₁₄ CH ₃ Ginkgolic acid III R=(CH ₂) ₇ -CH=CH-(CH ₂) ₅ CH ₃		320 348 374



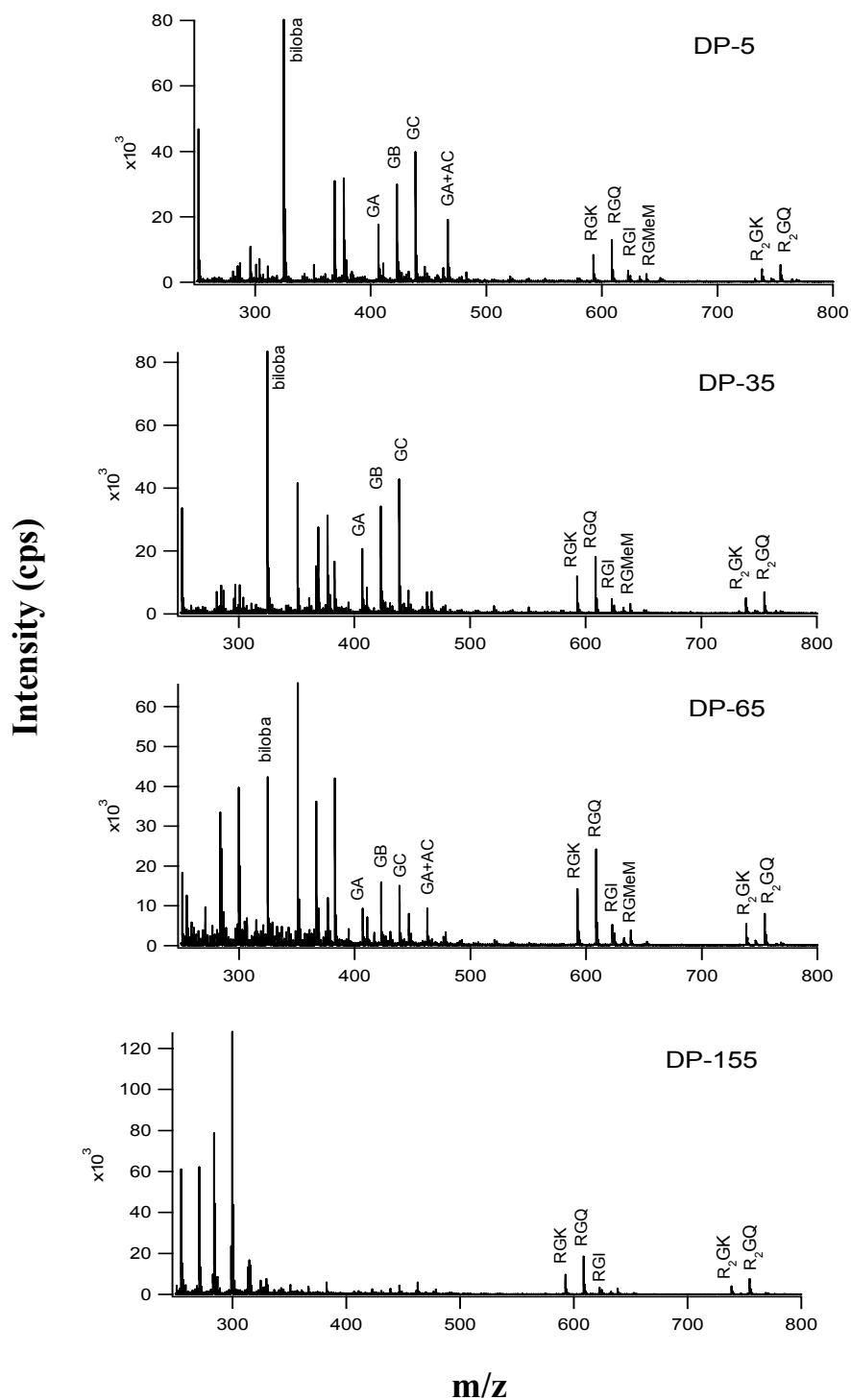
Supplemental Figure 2. Conventional HPLC analysis of flavonoids after acid hydrolysis of flavonol glycosides.

Conventionally, the flavonol glycosides are hydrolyzed with acid to flavonoids quercetin (Q), kaempferol (K), and isorhamnetin (I), which are then separated by reverse phase high performance liquid chromatography (HPLC) followed by UV absorbance or fluorescence detection. The contents of flavonol glycosides are inferred from the measurement of the three flavonoids (Q, K and I). These steps are schematically shown in Supplemental Figure 2.

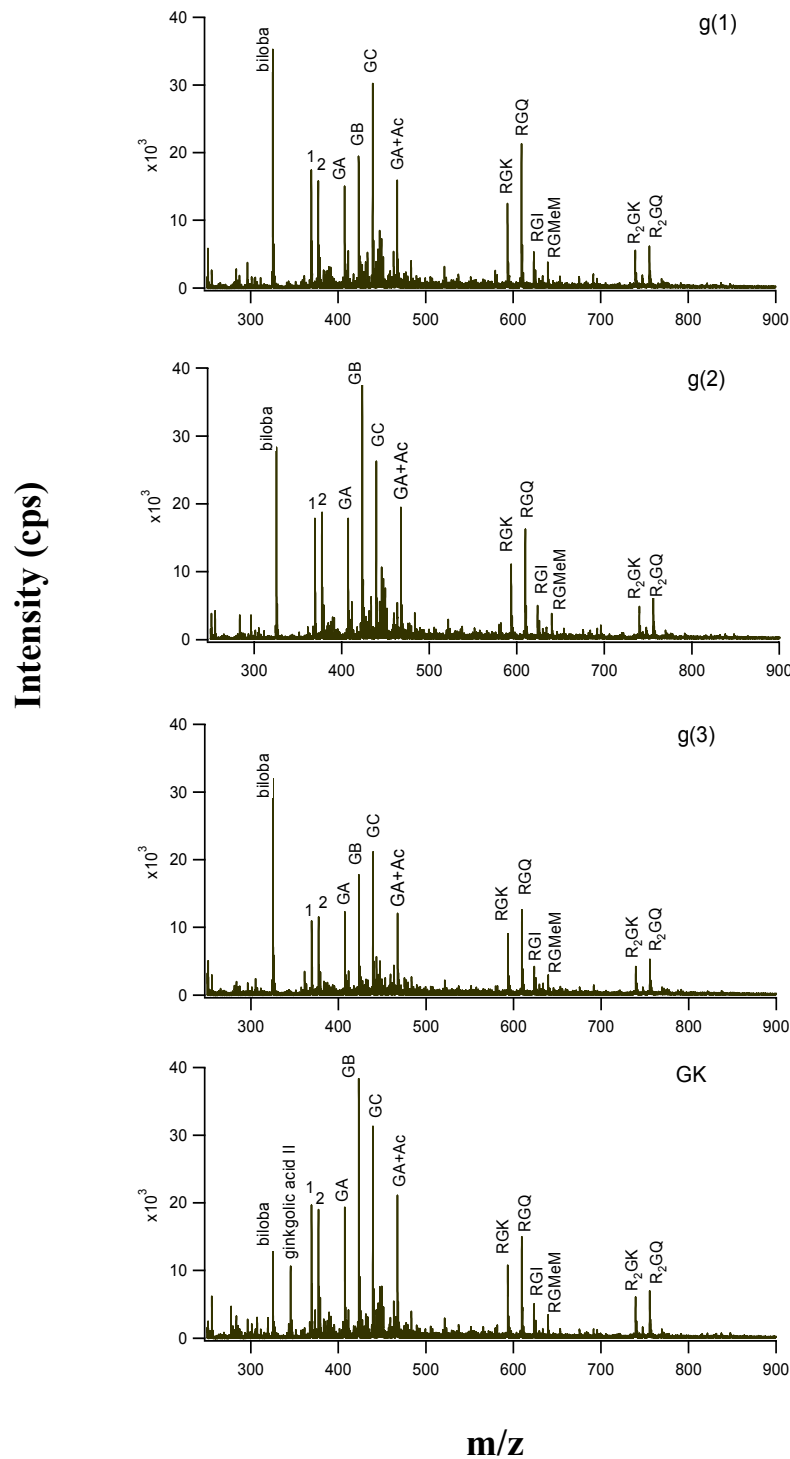


Supplemental Figure 3. Effect of methanol on the extraction of ginkgo compounds.

Effect of methanol on the extraction of the active and toxic compounds in ginkgo products was studied varying the percentage of methanol (20 % to 100%) in water. With increasing the percentage of methanol from 20 % to 100%, the amount of ginkgolic acids increased, while other components did not change significantly. Therefore, all the commercial samples (1 mg) were extracted using 1 mL of 100% methanol then diluted with aqueous ammonium acetate buffer to be 100 $\mu\text{g/mL}$ in 50% MeOH and 5 mM ammonium acetate for the subsequent nanoESI-MS analysis.

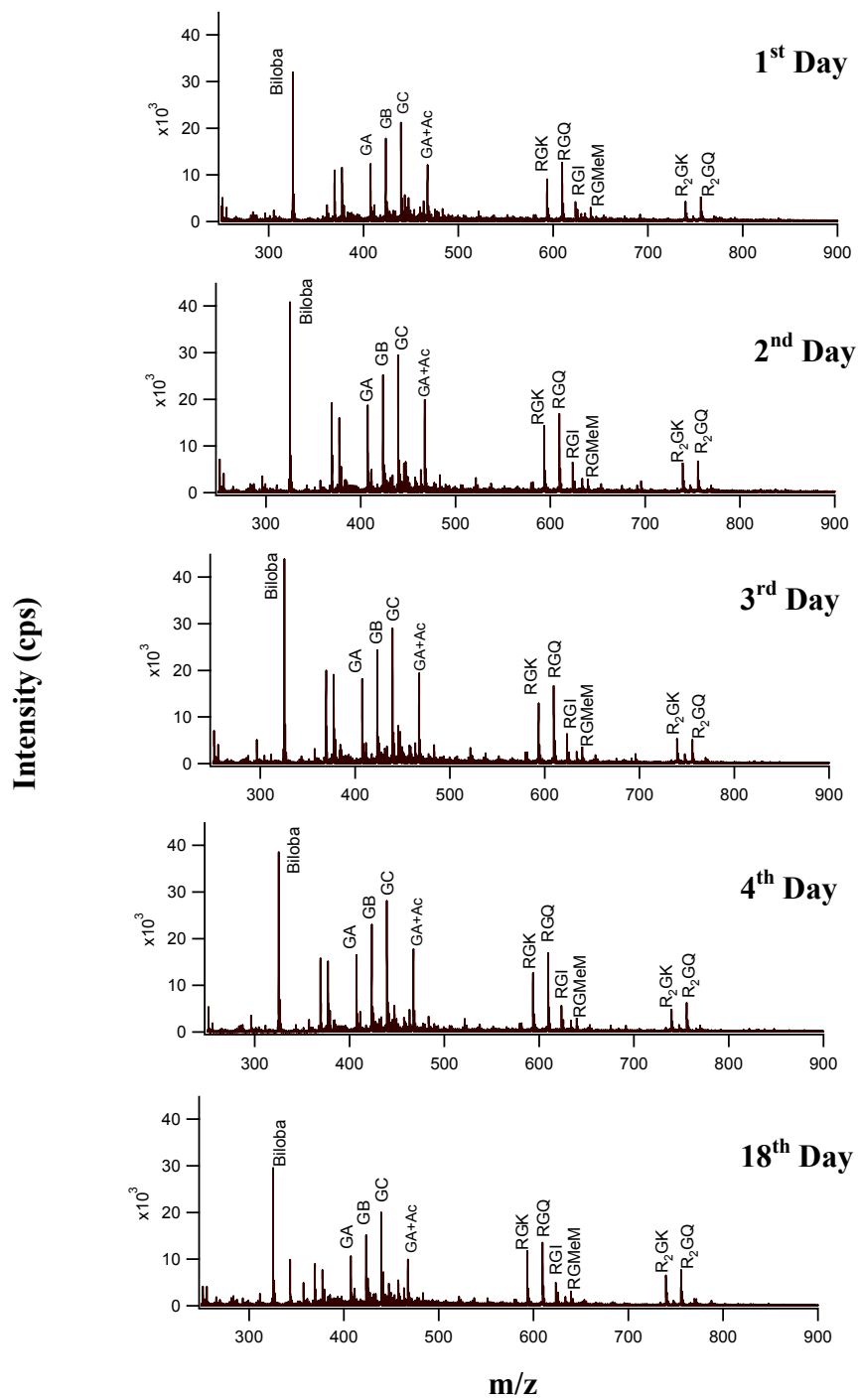


Supplemental Figure 4. Fragmentation of the mixed components in the ginkgo extract observed at different DP1. With the increase of DP1 from -5 to -155, fragmentation of these compounds increased. When DP1 was set at -5, minimum fragmentation of these components in the ginkgo extract was achieved. Therefore, DP1 at -5 was used for the rest of this study. The electrospray voltage at -900V was found to provide optimum ionization efficiency. Other instrumental parameters (such as second declustering potential DP2, focusing potential FP) were also optimized in the same manner. The DP 2 of -10 V and FP of -5 V were selected.



Supplemental Figure 5. Negative ESI-MS spectra of four standardized ginkgo leaf extracts, showing consistency between the standardized extracts.

GA, GB, and GC are ginkgolides A, B, and C, respectively. GA+Ac corresponds to an acetylated form of GA. RGK, RGQ, RGI, RGMMeM, R₂GK, R₂GQ, are intact flavonol glycosides. The structures and molecular weights of ginkgolides and flavonol glycosides are shown in Supplemental Figure 1. Peak 1 is an unidentified peak. Peak 2 represents GA-2CH₃ (m/z 377.0940, mass accuracy 0.18 ppm). Tandem mass spectrometry (MS/MS) of GA confirmed the peak 2.



Supplemental Figure 6. Representative mass spectra obtained from repeated analysis of a standardized extract over 18 days, showing the day-to-day reproducibility. The peak identities are the same as in Supplemental Figure 5.