Abstract

Dietary supplements are often natural products consisting of complex mixtures of variable composition, making it difficult to extend the toxicological evaluation results for a single reference sample to similar products in the market. The NTP has begun to assess the chemical and biological parameters needed to establish sufficient similarity between natural products and GbE was selected as the first test article for this program. We evaluated the hydrolyzed and unhydrolyzed chemical similarity of 31 GbE samples procured from 17 suppliers using non-targeted HPLC-UVE/ELSD. 25 GbE chromatograms had 15 to 20 peaks in 4 categories: terpenoid lactones (TL), flavonoids, ginkgolides, flavonol aglycones (FA), and glycosides (GbE). Six samples yielded ≤ 1 peak. Targeted analysis for 12 constituents was performed on all 31 GbE’s. Targets included FA (quercetin, kaempferol,isorhamnetin), rutin, and TL (lilialide, ginkgolides A, B, C, and J) analyzed by HPLC-UVE/ELSD, ginkgolide (GT) by HPLC-Fluorescence, and GbE and FA by UVE-MS/MS. Total TL ≤ 20%, where in unhydrolyzed 6 samples, total FA was ≤ 11.8%. FA were found in some unhydrolyzed samples indicating potential adulteration. Individual TL and FA varied widely between samples. GbE (≤ 0.14%) ≤ 0.22%) was found in 23/31 samples. Differences between samples were related to the amounts of each constituent present and the presence or absence of aglycones indicating potential adulteration.

Background

GbE is a constituent of many commercially available herbal dietary supplements and is sold by a large number of vendors worldwide. GbE is an antheric extract of Ginkgo biloba leaves with a complex composition. Approximately 30-35% of the constituents of GbE are known. Standardized extracts contain 24% flavonoid glycosides, which upon hydrolysis consist primarily of quercetin, kaempferol, andisorhamnetin, and 6% terpenoids, which are primarily ginkgolides A, B, C, and J, and bilobaide. In some GbE products pure flavonoid aglycones are added to a standard extract to meet the 24% requirement, while others are mixtures of source materials adjusted to give the appropriate 24% flavonoid. Commercially available GbE products in the US have levels of flavonoid glycosides ranging from 340–380 mg/tpg, terpenoid lactones from 40–110 mg/tpg and glycosides from ≤500–900,000 µg/tpg. The variable composition of GbE makes it difficult to extend the results of research on a single reference sample to similar products in the market. The NTP has begun to assess the chemical and biological parameters needed to establish sufficient similarity for GbE dietary supplements as compared to an authentic reference standard, and GbE was selected as the first test article this program.

Objectives

• Evaluate the use of non-targeted analysis to screen GbE samples from multiple suppliers. Compare non-targeted results to results for a NIST standard reference material (SRM).
• Use targeted analysis of 12 marker compounds to confirm the qualitative results from the non-targeted analysis. Compare targeted results to results for a NIST standard reference material.
• Authorize 17 samples from suppliers using HP-TLC (Alkemists). Compare targeted results to results for a NIST standard reference material.
• Compare NPT tested lot to NIST SRM and commercially available GbE.

Methods

Non-Targeted LC/UVE/ELSD Analysis

• Samples were analyzed on a Shimadzu LC-20AD/HT liquid chromatographic coupled to an ELSD (Atech Fluorescence Detector). The mobile phase was methanol:water (90:10 v/v) with 0.02% formic acid (A) and methanol:water (50:50 v/v) with 0.02% formic acid (B) with a flow rate of 1.0 ml/min and the gradient following: 89% B, 15% A, then to 62% A, 38% B in 20 minutes, then to 34% A, 66% B in 20 minutes, then to 15% A, 85% B in 20 minutes, hold for 10 minutes, then to 89% B, 15% A, in 2 minutes, hold 8 minutes. Total run time was 60 minutes.

Targeted LC/UVE/ELSD Analysis

• Samples were analyzed on a Shimadzu LC-20AD/HT liquid chromatographic coupled with an Atech Fluorescence Detector. The mobile phase was methanol:water (90:10 v/v) with 0.02% formic acid (A) and methanol:water (50:50 v/v) with 0.02% formic acid (B) with a flow rate of 1.0 ml/min and the gradient following: 61% B, 39% A, then to 50% A, 50% B in 20 minutes, then to 34% A, 66% B in 20 minutes, then to 15% A, 85% B in 5 minutes, hold for 10 minutes, then to 89% B, 15% A, in 2 minutes, hold 8 minutes. Total run time was 60 minutes.

Targeted LC/MS/MS Analysis

• Samples were analyzed on a Shimadzu LC-20AD/HT liquid chromatographic coupled with an ABSciex Triple Quadrupole Mass Spectrometer using an electrospray ionization LC-MS/MS operating conditions. Transitions monitored were 345–351 for ginkgolide A (353) and 337–333 for ginkgolide B (371). Mobile phase was methanol:water (90:10 v/v) with 0.1% formic acid (A) and methanol:water (50:50 v/v) with 0.1% formic acid (B). The flow rate was 3 ml/min, and the gradient following: 61% B, 39% A, then to 50% A, 50% B in 20 minutes, then to 34% A, 66% B in 20 minutes, then to 15% A, 85% B in 5 minutes, hold for 10 minutes, then to 89% B, 15% A, in 2 minutes, hold 8 minutes. Total run time was 60 minutes.

Targeted LC/Fluorescence Analysis

• Samples were analyzed on a Shimadzu LC-20AD/HT liquid chromatographic coupled to a Shimadzu RF-5300 determination with an excitation wavelength of 265 nm and an emission wavelength of 395 nm. The mobile phase was methanol:water (90:10 v/v) with 0.02% formic acid (A) and methanol:water (50:50 v/v) with 0.02% formic acid (B). The flow rate was 3 ml/min, and the gradient following: 61% B, 39% A, then to 50% A, 50% B in 20 minutes, then to 34% A, 66% B in 12 minutes, then to 15% A, 85% B in 1 minute, hold 8 minutes. Total run time was 60 minutes.

Results

• Representative GbE Samples

• Authentication Representative Samples

Figure 1 – Representative GbE Samples

Figure 4 – Authentication Representative Samples

Table 1 – Authentication Results

References


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