Rapid Determination of Terpene Lactones in *Ginkgo Biloba* Commercial Products by HPLC with Evaporative Light-Scattering Detection

A rapid, sensitive, and reproducible high performance liquid chromatography gradient method has been developed for the measurement of ginkgolides A, B, C, and J, along with bilobalide, in a *Ginkgo biloba* commercial product. The separation was achieved in less than 14 min, employing a water–methanol–trifluoroacetic acid mobile phase and an evaporative light-scattering detector. No sample clean-up procedures were used with the methanol extraction of the *Ginkgo biloba* dietary supplement. The detection limit (S/N > 5) is less than 125 ng on-column for each terpene lactone on a reversed-phase C18 column. Both intra- and interday reproducibility were evaluated. Four brands of standardized *Ginkgo biloba* herbal supplements were assessed for their terpene lactone content. This method is applicable for analyzing a *Ginkgo biloba* dietary supplement in capsule, tablet, or liquid form.

**Experimental**

**Reagents and chemicals:** Methanol (high performance liquid chromatography [HPLC] grade) was purchased from Fisher Scientific Co. (Fair Lawn, New Jersey). Deionized water was obtained with an in-house U.S. Filter Service Deionization water system (Pittsburgh, Pennsylvania). Trifluoroacetic acid (99%) was acquired from Aldrich Chemicals Co. (St. Louis, Missouri). Bilobalide, ginkgolide J, ginkgolide C, ginkgolide A, and ginkgolide B standards were obtained from Alltech Associates, Inc. (Deerfield, Illinois). Several brands of *Ginkgo biloba* supplements were bought from a local department store. Supplies for the preparation of standards and samples included 5-mL Luer tip plastic syringes; 0.45-μm porosity polypropylene-encased syringe filters; 0.45-μm porosity, 47-mm nylon filter membranes; and 4-mL HPLC vials with caps (Alltech Associates).

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Chromatographic conditions: The HPLC system was assembled from an on-line degassing system (Alltech Associates), a model L-7100 quaternary gradient HPLC pump (Hitachi Instruments, San Jose, California), a model 7125 manual sample injector (Rheodyne, Cotati, California) with a 5-μL PEEK flex-connect sample loop (Alltech Associates), a model ELSD 2000 evaporative light-scattering detector (Alltech Associates), and a PeakSimple chromatography data system (SRI, Torrance, California). The detector was operated in the impactor off mode (no aerosol splitting). The drift tube temperature was 110 °C, and the nitrogen gas flow was 3.1 L/min.

A 100 mm × 4.6 mm, 3-μm d_p Alltima C18 column (Alltech Associates) was used for all separations, and they were done at ambient temperature. A binary mobile phase was used with mobile phase A being 0.1% trifluoroacetic acid in deionized water and mobile phase B consisting of 0.1% trifluoroacetic acid in methanol (6). The gradient was 10–55% B over 15 min. All runs were baseline-equilibrated before the initiation of the next run. The flow rate was 1.3 mL/min. All HPLC injections of standards and samples had an injection volume of 5 μL. All mobile phases were mixed, degassed, and filtered before use.

Standard solution preparation: 1.017 mg of bilobalide, 1.011 mg of ginkgolide J, 1.025 mg of ginkgolide C, 1.031 mg of ginkgolide A, and 1.004 mg of ginkgolide B were weighed accurately on a microscale and quantitatively transferred into a clean, dry 10-mL volumetric flask. Methanol was added to volume, and the compounds were dissolved. From this, a subsample was syringe-filtered through a 13-mm, 0.45-μm nylon-syringe filter into a 4-mL HPLC vial that was capped tightly and stored at 4 °C.

Sample solutions preparation: Four Ginkgo biloba herbal supplements were analyzed. Samples of the supplements in capsule form were prepared by emptying the content of single capsules into clean, dry, and tared 4-mL vials. Each vial was weighed accurately to determine the mass of the sample. Samples of the supplements in tablet form were prepared by thoroughly pulverizing single tablets with a mortar and pestle. The powders were transferred carefully into individual clean, dry, and tared 4-mL vials. Each vial was accurately weighed to determine the mass of the sample.

All solid samples were extracted as follows: 3 mL of methanol was added to a vial containing a solid sample. The vial was tightly capped and sonicated for 10 min. The content of the vial was allowed to settle for 10 min, and the supernatant was transferred by pipette into a 5-mL syringe fitted with a 13-mm diameter, 0.45-μm porosity nylon syringe filter. The liquid extract was filtered into a clean, dry, 10-mL volumetric flask. For each solid sample, the extraction was repeated two more times, and the combined extracts were brought to volume with methanol (7). The samples were capped tightly and stored at 4 °C.

The commercially prepared liquid Ginkgo biloba extract with 2 g of leaf extracted into 1 mL of deionized water and ethanol (1:1) was analyzed directly by HPLC with no sample preparation.

Quantitation: Determination of the ginkgolides (A, B, C, and J) and bilobalide (BB) present in the standardized Ginkgo biloba supplement was as follows: Average area counts (Å) for these constituents were obtained from the Brand A (capsule) over three days (n = 18, each injected in triplicate) for the reproducibility study. The terpene lactone content was also evaluated in brands B (tablet) and C (capsule) (n = 6, each injected in triplicate). The standardized liquid extract (Brand D) was injected six times to obtain Å. A solution of standards was injected seven times to obtain Å for the terpene lactones.

1. The average concentration (micrograms per capsule or tablet) of each terpene lactone (for example, BB) is:

\[
\text{μg (BB in STD)} \times \frac{\bar{A}}{\text{Å (BB in sample)}} \times 10 \text{ mL (BB in STD)} \times 10 \text{ mL (capsule or tablet)}
\]

where STD = standard

Note: The last conversion factor (10 mL/capsule or tablet) is not used for the liquid extract because it is unaltered.

2. Converting into milligrams per gram:

\[
\frac{g (BB in STD)}{\text{mg/capsule or tablet (avg. conc. from eq. 1)}}
\]

Table I: Terpene lactones in standard solution (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Bilobalide</th>
<th>Ginkgolide J</th>
<th>Ginkgolide C</th>
<th>Ginkgolide A</th>
<th>Ginkgolide B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area count</td>
<td>55.843</td>
<td>55.933</td>
<td>55.722</td>
<td>97.761</td>
<td>78.198</td>
</tr>
<tr>
<td>Concentration (μg/mL)</td>
<td>101.7</td>
<td>101.1</td>
<td>102.5</td>
<td>103.1</td>
<td>100.4</td>
</tr>
<tr>
<td>RSD</td>
<td>4.22%</td>
<td>5.08%</td>
<td>6.66%</td>
<td>2.86%</td>
<td>6.73%</td>
</tr>
</tbody>
</table>
Reproducibility and sensitivity: The precision of the method was assessed by examining sample-to-sample and day-to-day reproducibility. Run-to-run reproducibility also was evaluated for the standards. The limit of detection for the evaporative light-scattering detector was measured for this HPLC method by diluting the standard sample until an S/N ratio of 5 was reached.

Results and Discussion
Evaporative light-scattering detection (ELSD) was chosen over other methods of detection for this analysis for several reasons. Independent of functional group properties, it produces a response that gives a closer estimate of the true sample mass compared with UV detection, which depends upon the optical characteristics (extinction coefficients) of the analytes. The terpene lactones in *Ginkgo biloba* are poor chromophores with weak absorption in the 200–220 nm range. Refractive-index detection was considered and rejected because a stable baseline is more easily attained with ELSD, resulting in faster equilibration. In addition, ELSD is gradient compatible, whereas the refractive-index detection is not. Gas chromatography with flame ionization detection is a reliable and sensitive way to perform this analysis, but the process requires time-consuming sample preparation and derivatization, which potentially introduces more errors into the method (8).

The unique detection principle of ELSD involves nebulization, evaporation, and detection of the remaining nonvolatile solute particles. Inside the nebulizer, the column effluent passes through a needle and mixes with nitrogen gas to form an aerosol consisting of a uniform distribution of droplets. The size of each droplet is dependent upon the gas flow rate used in the analysis. The optimum gas flow rate will produce the highest S/N. In this study, the gas flow rate of 3.1 L/min gave the highest S/N.

Then the mobile phase evaporates in a heated stainless steel drift tube, leaving a fine aerosol of solute particles that are detected by a sensitive, noninterfering mass measurement detector. The elution times of the peaks are dependent upon the retention characteristics of the analytes and the properties of the mobile and stationary phases used in the analysis.

### Table II: Terpene lactones in standard solution of brand A

<table>
<thead>
<tr>
<th></th>
<th>Bilobalide</th>
<th>Ginkgolide J</th>
<th>Ginkgolide C</th>
<th>Ginkgolide A</th>
<th>Ginkgolide B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1 (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>27.31 ± 1.32</td>
<td>3.47 ± 0.32</td>
<td>21.92 ± 1.39</td>
<td>124.88 ± 7.22</td>
<td>77.17 ± 4.67</td>
</tr>
<tr>
<td></td>
<td>4.84%</td>
<td>9.22%</td>
<td>6.35%</td>
<td>5.78%</td>
<td>6.05%</td>
</tr>
<tr>
<td><strong>Day 2 (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>30.32 ± 1.25</td>
<td>3.02 ± 0.20</td>
<td>21.78 ± 0.61</td>
<td>126.80 ± 5.25</td>
<td>75.34 ± 2.34</td>
</tr>
<tr>
<td></td>
<td>4.12%</td>
<td>6.55%</td>
<td>2.81%</td>
<td>4.14%</td>
<td>3.10%</td>
</tr>
<tr>
<td><strong>Day 3 (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>29.79 ± 0.69</td>
<td>2.79 ± 0.15</td>
<td>19.67 ± 0.55</td>
<td>123.55 ± 4.00</td>
<td>65.80 ± 1.73</td>
</tr>
<tr>
<td></td>
<td>2.31%</td>
<td>5.53%</td>
<td>2.77%</td>
<td>3.24%</td>
<td>2.63%</td>
</tr>
<tr>
<td><strong>Overall (n = 3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>29.14 ± 1.61</td>
<td>3.10 ± 0.34</td>
<td>21.12 ± 1.26</td>
<td>125.08 ± 1.63</td>
<td>72.77 ± 6.10</td>
</tr>
<tr>
<td></td>
<td>5.52%</td>
<td>11.09%</td>
<td>5.95%</td>
<td>1.31%</td>
<td>8.39%</td>
</tr>
</tbody>
</table>
mist of dried sample particles in the solvent vapor. The optimum tube temperature will produce the most stable baseline without compromising sensitivity (S/N), which in this application was 110.0 °C. Detection occurs inside an optical cell, where nonvolatile sample particles interrupt a laser light beam. The scattered light is detected by a silicon photodiode, producing a signal that is sent to the analog output for data collection (9).

The HPLC method was applied to *Ginkgo biloba* standardized supplements in capsule, tablet, and liquid form. The terpene lactone peaks were identified by direct comparison of the retention times of the peaks in the standard chromatogram with the peaks resolved in the sample chromatogram. The order of elution is as follows: bilobalide, ginkgolide J, ginkgolide C, ginkgolide A, and ginkgolide B. Figure 2 shows the chromatograms of each of the samples. Figure 3 illustrates the separation of the terpene lactone standards. The methanol extraction was exhaustive, which was verified by analyzing the third extraction in which no measurable amounts of the terpene lactones were found.

Table I lists the average area counts with the corresponding concentrations and the run-to-run relative standard deviations ($\overline{SD}$) for each of the terpene lactones in the standard solution. For the reproducibility study, Table II reports the Brand A sample area counts along with the standard deviations ($\overline{SD}$) and relative standard deviations. The relative standard deviations for intraday samples ($n = 6$, each injected in triplicate) ranged from 2.31% (bilobalide, day 3) to 9.22% (ginkgolide J, day 1). Day-to-day relative standard deviations ranged from 1.31% (ginkgolide A) to 11.09% (ginkgolide J). As depicted in Figure 4, the limit of detection (S/N > 5) was less than 125 ng on-column for each of the five analytes. Quantitation of the terpene lactones was straightforward using equation 1, and the results reported in Table III. Table IV summarizes the amount of the total terpene lactones per serving size for the *Ginkgo biloba* herbal supplements.

A direct comparison illustrating the absolute and relative amounts of terpene lactones present in each of the four *Ginkgo biloba* supplements is shown in Figure 5.

Both the amounts of the *Ginkgo biloba* standardized extract and leaf content vary widely from brand to brand, as evidenced in Figure 4. The extract portion is standardized to > 6% total terpene lactones. However, the amounts of the terpene lactones are unregulated in the leaf material. Moreover, the terpene lactone content in the leaf can vary considerably depending upon the season and the geographic location of cultivation (10). The total terpene lactones, the ginkgolides A, B, C, and J, along with bilobalide, in the leaf are not taken into account in the quantitation listed on the product label, often leading to an underestimation of the amount of the total terpene lactones in the *Ginkgo biloba* supplement when the leaf is present, as Brands A and C indicate. Brand B, which contains no leaf, contains approximately 69.1% of the total terpene lactones it is purported to have according to the label, while Brand D, the liquid extract, contains 103.6% compared to its label claim, which is within experimental error. The low amount of total terpene lactones in Brand B relative to the label claim could be attributed to label inaccuracies by the man-

![Figure 4: Limit of detection of terpene lactones. See the text for conditions. Peaks: 1 = bilobalide, 2 = ginkgolide J, 3 = ginkgolide C, 4 = ginkgolide A, 5 = ginkgolide B.](image1)

![Figure 5: Comparison of terpene lactones in commercial *Ginkgo biloba* standardized supplements.](image2)
pared to other published methods. Li and colleagues (7) used a buffer, an acid, two polymer application, while Ganzera and his method. A ternary gradient is used in the flavoacetic acid, is much simpler in this phase, comprising methanol, water, and tri- pene lactones were assessed in the polymer within 25 min. In addition, only three ter- leagues (7) managed to resolve the lactones Massachussetts) (11) and Ganzera and col- leagues (7) attempts to determine both the terpene lactones and flavonol glycosides in Ginkgo biloba simultaneously. Their sample preparation involved a liquid–liquid extrac- tion, subsequent evaporation, and resuspension. In addition, the flavonol glycosides were not acid hydrolyzed into their respective aglycones. Without hydrolyzation, determination of the flavonol glycosides is impossible due to the different sizes of sugars that are otherwise bound to each of the flavonol glycosides, resulting in numerous peaks with varying retention times for each flavonol glycoside (12).

The limit of detection for the terpene lac- tones using ELSD is 125 ng on-column for the terpene lactones. This is much lower than both Polymer Laboratories' (11) and Ganzera and colleagues' (7) limits of detection, which were 250 and 203 ng on-column, respectively.

**Conclusion**
The total terpene lactones present in the Ginkgo biloba supplements analyzed ranged from 0.79% (w/w) for Brand B to 1.57% (w/w) for Brand C. The concentrations of the individual terpene lactones varied from 0.005% (w/w) for ginkgolide J in Brand B to 0.70% (w/w) for ginkgolide A in Brand C.

The HPLC method described here allows analysis of Ginkgo biloba much more rapidly than has been reported previously without compromising sensitivity and reproducibility. As a result, routine quality control in high-throughput laboratories within the nutraceutical industry can be performed much more efficiently.

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**References**
(12) D. McCrery, personal communication (2002).