Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 1077–1079, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00
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Short Communication

Rapid liquid chromatography of terpenes in *Ginkgo biloba* L. extracts and products*

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Keywords: Ginkgo biloba *L.; terpenes; reversed-phase HPLC.*

Introduction

The diterpenes ginkgolide A, ginkgolide B, ginkgolide C and the sesquiterpene bilobalide (Fig. 1) are known to be present in *Ginkgo biloba* L. extracts [1, 2]. High-performance liquid chromatography on reversed-phase columns (RP-HPLC) is the method of choice for the analysis of these extracts. However, the presence of several other components such as flavonoid glycosides [3] and biflavonoids [4] makes the assay of the terpenes difficult and purification is a crucial step for a successful analysis. Recently, the authors described [5] a procedure for the preparation of samples based on elution of the terpene fraction on Extrelut

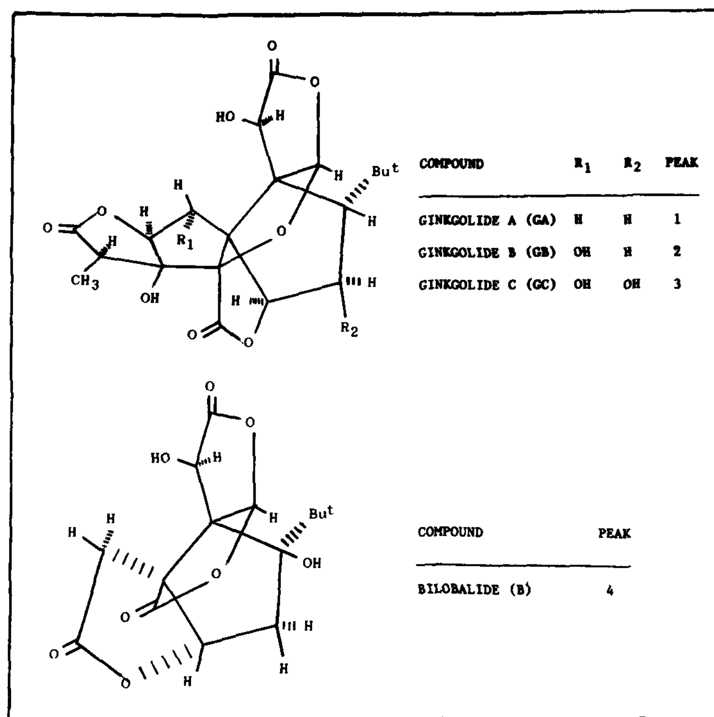

Figure 1
Structures of *Ginkgo biloba* L. terpenes.

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.
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with ether followed by further clean-up by means of alumina cartridges. In the present paper a single-step purification is described together with improved chromatographic conditions; this allows the rapid analysis of terpenes in *Ginkgo biloba* L. extracts and pharmaceutical preparations.

Experimental

Materials

Ginkgolide A (GA), ginkgolide B (GB), ginkgolide C (GC) and bilobalide (B) were kindly provided by Ipsen (France). *Ginkgo biloba* L. extracts (EGB) and formulations (EGB tablets and syrup) were purchased from different sources. Methanol, 1-propanol, tetrahydrofuran and water were of HPLC grade. Bakerbond Alumina columns were from J.T. Baker Chemicals B.V. (Deventer, Holland).

Chromatographic conditions

HPLC was performed on a Waters (Milford, MA, USA) liquid chromatograph equipped with a Model 510 pump, a Model U6K universal injector, a Model Lambda Max 480 UV detector connected to a CR3A integrator (Shimadzu, Kjoto, Japan). The 250 × 4 mm i.d. column was packed with 5-μm C_8 Nucleosil 300 (Macherey-Nagel). The eluent was 1-propanol–tetrahydrofuran–water (1:13:86, v/v/v) at a flow rate of 1 ml min^{-1} at room temperature. The peaks were monitored at 220 nm (0.04 a.u.f.s.).

Sample preparation

Ginkgo biloba *L. extracts (EGB).* Eighty milligrams of dry extract was dissolved in 2 ml of methanol and 0.5 ml of this solution was applied to a Bakerbond alumina cartridge pre-washed with 10 ml of methanol. The sample was then eluted with methanol and the first 2 ml of the eluate was collected. The solution was filtered through a 0.45-μm membrane (Spartan 13, Schleicher and Schuell, Dassel, Germany), and 5-μl aliquots were injected into the chromatograph.

EGB tablets. An accurately weighed amount from tablets corresponding to 80 mg of *Ginkgo biloba* L. extract was placed in a centrifuge tube and 2 ml of methanol was added; the mixture was mixed in a vortex mixer for 10 min and centrifuged at 5000*g* for 2 min; 0.5 ml of the supernatant was treated as described for the extracts.

EGB syrup. Two millilitres of syrup, corresponding to 80 mg of *Ginkgo biloba* L. extract was evaporated to dryness *in vacuo*, and the residue was dissolved in 2 ml of methanol; 0.5 ml of this solution was treated as described for the extracts.

Standard solution. B, GA, GB and GC (2 mg of each) were dissolved in 5 ml of methanol. Different aliquots (0.04–0.5 ml) of this stock solution were then treated as described for the extracts. Aliquots of 5 μl were injected ($n = 8$) and the peak areas were integrated against the corresponding masses of B, GA, GB and GC.

Results and Discussion

In a previous paper [5] the authors reported that extraction with ether using Extrelut columns followed by clean-up through an alumina cartridge was necessary for the purification of *Ginkgo biloba* L. samples. This procedure overcomes difficulties due to background interference; nevertheless, the method is lengthy and troublesome. In contrast, the method described in the present work is very simple and allows a rapid and clean determination of ginkgolides and bilobalide in

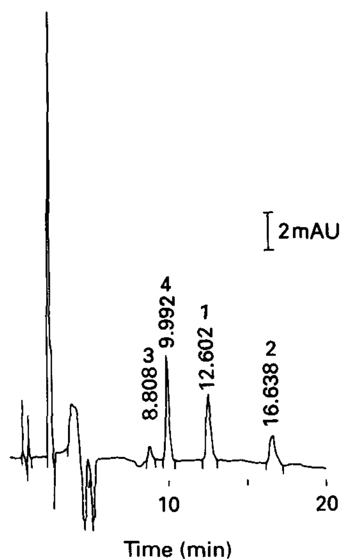

Figure 2
HPLC of GC, B, GA and GB standards. Eluent: 1-propanol–tetrahydrofuran–water (1:13:86, v/v/v). Flow rate, 1 ml min^{-1}; UV detection, 220 nm.

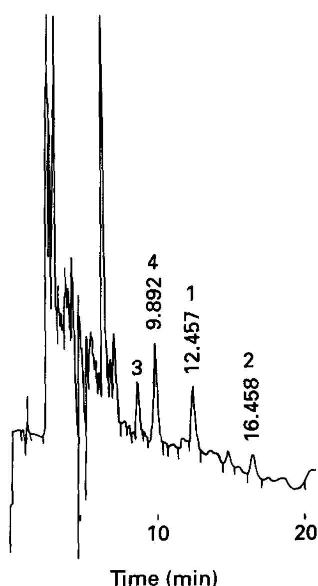

Figure 3
Typical chromatogram of EGB. Chromatographic conditions as in Fig. 2.

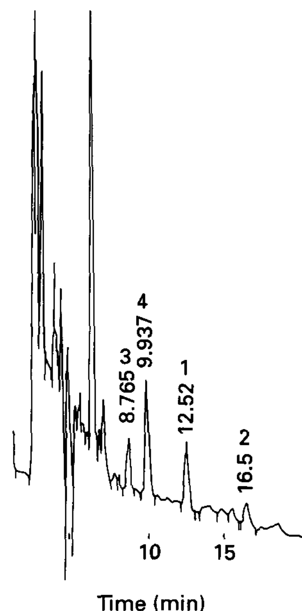

Figure 4
Typical chromatogram from EGB tablets. The syrup gives a practically identical chromatogram. Chromatographic conditions as in Fig. 2.

extracts and their pharmaceutical preparations. Moreover, use of the C_8 Nucleosil 300 column combined with an eluent based mainly on tetrahydrofuran as organic modifier results in a sharp baseline separation of GC, B, GA and GB (Fig. 2). Typical chromatograms obtained from EGB (Fig. 3) and from EGB tablets or syrup (Fig. 4) show well-resolved peaks, the retention times varying by $<3\%$ during 30 days; in addition, effects of the solvent front are much reduced and the impurities that normally overlap GC and B are absent. The quantitative determination of B and ginkgolides in EGB and formulations was achieved using external standardization (SD = 1.2%, $n = 8$). The calibration curves were linear in the range investigated (0.04–0.5 mg ml^{-1}). The correlation coefficients and relative standard deviations ($n = 8$) were: 0.997 and 1.3% for GA; 0.988 and 1.1% for GB; 0.989 and 1.2% for GC; and 0.992 and 1.4% for B. The minimum detectable amount was 100 ng.

It may be concluded that this new approach improves both the sample preparation and chromatographic separation and represents a valuable method for the routine assay of GA, GB, GC and B in *Ginkgo biloba* L. extracts and formulated preparations.

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[*Received for review 5 May 1992; revised manuscript received 26 June 1992*]