DETERMINATION OF GINKGOLIDES A AND B IN *GINKGO BILOBA* LEAVES BY HPLC USING ACTIVATED CHARCOAL

MASSOUD AMANLOU, BITA MESGARPOUR, HASSAN FARSAM

Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran

ABSTRACT

Ginkgo biloba (GB) preparations are now among the leading herbal medicines that exert a broad spectrum of possible clinical applications. Several methods have been reported for quantification of ginkgolides of GB and its pharmaceutical preparations and the HPLC techniques are now considered to be the method of choice. However, most reported HPLC methods are not simple and their work-up procedure are inadequate. The present paper describes a simple and non-expensive method for extraction and determination of ginkgolides A and B in GB leaves and their phytopharmaceuticals. The method is based upon extraction of ginkgolides from aqueous solution by activated charcoal, followed by extraction with Methanol and injection of the Methanolic solution into chromatographic system. Ginkgolides were separated on an ODS column with a mobile phase of water-methanol (67:33 v/v) at a flow rate of 1.0 ml/min and were detected at 220 nm. The mean recoveries of ginkgolide A and B were 97 and 98.4%, respectively. This method is simple and can be used for routine analysis of GB extracts and phytopharmaceuticals preparations.

Key words: *Ginkgo biloba*, Ginkgolides, HPLC, Activated Charcoal, Tanakan

INTRODUCTION

Ginkgo biloba ,a monotypic dioecious plant, is the only living representative of the Ginkgoaceae family and its order (Ginkgoales) (1, 2). The leaves of this "living fossil" (according to Darwin) have been medicinally used since the oldest Chinese materia medica, 2800 B. C. (3). *Ginkgo biloba* leaf extract (GBE) is widely used as a natural PAF (Platelet Activating Factor) antagonist all around the world for alleviation of complaints such as circulatory disorders, asthma, tinnitus, cerebrovascular and dementia syndromes particularly in old people (2, 3). Characteristic constituents of this plant are diterpene lactones (ginkgolides and bilobalides) and flavonoides (4).

Several analytical techniques such as TLC, GC, HPLC and MPLC have been reported for determination and isolation of ginkgolides in *G. biloba* leaves, GBE and its phytopharmaceutical preparations (5-11). The HPLC technique is considered the method of choice (9-11).These methods mostly suffer from having a simple preparative procedure for separation of ginkgolides and detailed work-up.

The present paper describes a simple and non-expensive method for extraction and determination of ginkgolides A and B in GBE and its phytopharmaceuticals and can be used in the routine laboratory analyses.

MATERIAL & METHODS

Reagents

HPLC grade methanol, acetone, benzyl alcohol and ethanol, reagent grade acetic acid, petroleum ether and pure activated charcoal were all from E. Merck (Darmestat, Germany). Double-distilled deionized water was used for the preparation of all aqueous solution. Standards of ginkgolide A (**GA**) and ginkgolide B (**GB**) were provided by Prof. Dr. K. Weinges (Universität Heidelberg, Germany) as a gift and also from Sigma (St. Louis, MA, USA). Pharmaceutical preparations were purchased from the market. The purity of the ginkgolides was checked by RP-HPLC and 400 MHZ ^{1}H-NMR spectroscopy.

Plant material

Ginkgo biloba leaves were collected from the campus of Tehran University of Medical Sciences. Green leaves were harvested in July 1999. A voucher specimen was deposited in the herbarium of the Faculty of Pharmacy. The leaves were air dried in the shade, powdered and kept in tightened light protected containers before use.

Chromatography

The HPLC system consisting of a 510 pump equipped with a Module 486 multiwavelength tunable UV detector and a 746 data module were all from Waters (Milford, MA, USA). The

Correspondance: Hassan Farsam, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran, E-mail: farsam@ams.ac.ir

Archive of SID

www.SID.ir

reversed-phase system consisted of a µ-Bondapack ODS (C18) column (10 µm, 300x3.9 mm USA). The mobile phase was a mixture of water-methanol (67:33 v/v) isocratically pumping at a flow rate of 1.0 ml/min (7). The detector was set as 220 nm.

Sample preparation

Ginkgo leaves extract was prepared according to the TLC method reported by Tallevi and Kurz (5). Dry powder of leaves (30g) was extracted with boiling water (100 ml) for 20 min, and the mixture was first passed through No. 1 Whatman filter paper and then celit. Then activated charcoal (10 g) was added to the filtrate and the mixture was stirred for 12 hrs at room temperature overnight. The mixture was centrifuged (700g, 15 min), the supernatant solution was discarded and the activated charcoal was re-suspended in 20 ml of acetone. After filtration through a filter paper, the solvent was evaporated to dryness and the residue was dissolved in 3 ml methanol and 5µl of the resulting solution was injected into the chro-matographic system.

Standard solution

Two milligrams of GA and GB were dissolved in 5ml of methanol. Aliquots of 25µl of GA and GB solutions and 5µl of benzyl alcohol (0.5 mg/ml in methanol) as internal standard were injected into the HPLC system.

Recovery and precision

The recovery study was performed by addition of the various amount of GA and GB to 20mg of GBE and 0.5ml Tanakan (Beaufour IPSEN Industry, France). Ginkgolides were determined according to the described method. The recovery was calculated by comparison of the found amounts with the added ones.

RESULTS AND DISCUSSION

Chromatograms of blank activated charcoal, ginkgolides A and B, GBE and Tanakan are shown in Fig 1. The retention times for GA and GB were 18.5 and 22.3 min, respectively. The mean recovery of GA and GB were 97% and 98.4% in GBE with a relative standard deviation of 7.15 % and 6.82 % respectively (n=5).

Various techniques have been presented for determination of ginkgolides (9-11). Among these methods the HPLC technique is considered more practical for determination of these relatively polar compounds (9-11). These methods mostly have difficulties in separation and purification procedures. Ginkgolides are present in very small amounts in Ginkgo leaves and have poor UV absorption coefficients (5). Furthermore, other compounds such as flavonoides which are present in higher concentrations and have stronger UV absorption can interfere with determination of ginkgolides (5). Therefore, determination of ginkgolides in GBE leaves is problematic. The use of different solid-phased extraction column has failed to give a reliable result. The polyamide column has given poor chromatogram (4) and the HPLC separation is too complicated that can not be used in routine laboratory analyses.

In the presented procedure the ginkgolides were extracted and purified using activated charcoal, which has previously been used in the TLC detection tests. As it is seen in the Fig. 1, the ginkgolides A and B are well separated in GBE and Tanakan. Since we did not have access to the standards of other ginkgolides, our discussion is limited to two more important ginkgolides A and B. Nevertheless, in the chromatogram f, two more peaks are seen. The peak with RT= 7.1 might be attributed to bilobalide (**BB**) and the smoother peak with RT= 9.2 min might be ginkgolide C (**GC**) as reported by other investigators (10, 11).

In conclusion, this method for extraction of ginkgolides is simple, not expensive and could be used as a routine technique for determination of ginkgolides A and B. It may be concluded that this approach improves both the sample preparation and chromatographic separation and represents a simple method for the routine assay of ginkgolides in GBE and its preparations.

REFERERECES

1 Braquet, P. (1987) Ginkgolides: potent platelet activating factor antagonists isolated from *Ginkgo biloba* L.: chemistry, pharmacology and clinical applications. Drugs Future.12: 643-699.

2 Braquet, P., Esanu, A., Buisine, E., Hosford, D., Broquet, C., Koltai, M. (1991) Recent progress in ginkgolide research. Med. Res. Rev. 11: 295-355.

3 The Review of Natural Products; The most complete source of natural product information. (2001) 1st ed, Facts and Comparisons, Lippincott Williams & Wilkins, USA, pp: 247-251.

4 Blumental, M., Goldberg, A., Brinckman, J. (2000) Herbal Medicine Expanded Commission E monographs. American Botanical Council with Integrative Medicine Communications. 1st

Correspondance: Hassan Farsam, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran, E-mail: farsam@ams.ac.ir

Archive of SID

www.SID.ir

ed. Austin, TX, pp: 160- 169.
5 Van Beek, T. A., Scheeren, R. T., Runtio, T., Melger, W. C., Lelyveld, G. P. (1991) Determination of ginkgolides and bilobalide in *Ginkgo biloba* leaves and phytopharma-ceuticals. J. Chromatogr. 543: 376-387.
6 Tallevi, S. G., Kurz, W. G. W. (1991) Detection of ginkgolides by thin layer chromatography. J. Nat. Prod. 54: 624-625.
7 Van Beek, T. A., Lelyveld, G. P. (1993) Thin layer chromatography of bilobalide and ginkgolides A, B, C and J on sodium acetate impregnated silicagel. Phytochem. Anal. 4: 109-114.
8 Hasler, A., Meier, B. (1992) Determination of terpens in *Ginkgo biloba* L. by capillary gas chromatography. Pharm. Pharmacol. Lett. 2: 187-190.
9 Lobstein-Guth, A., Briancon-Scheid, F., Anton, R. (1983) Analysis of terpenes from *Ginkgo biloba* L. by high-performance liquid chromatography. J. Chromatogr. 263: 431- 438.
10 Van Beek, T. A., Lelyveld, G. P. (1997) Preparative isolation and separation procedure for ginkgolides A, B, C and J and bilobalide. J. Nat. Prod. 60: 735-738.
11 Chen, P., Su, X. L., Nie, L. H., Yao, S. Z., Zeng J. G. (1998) Analysis of Ginkgolides and Bilobalides in *Ginkgo biloba* L. Extract for Its Production Process Control by High- Performance Liquid Chromatography. J. Chrom. Sci. 36(4): 197-200.

Archive of SID

Correspondance: Hassan Farsam, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran, E-mail: farsam@ams.ac.ir

www.SID.ir

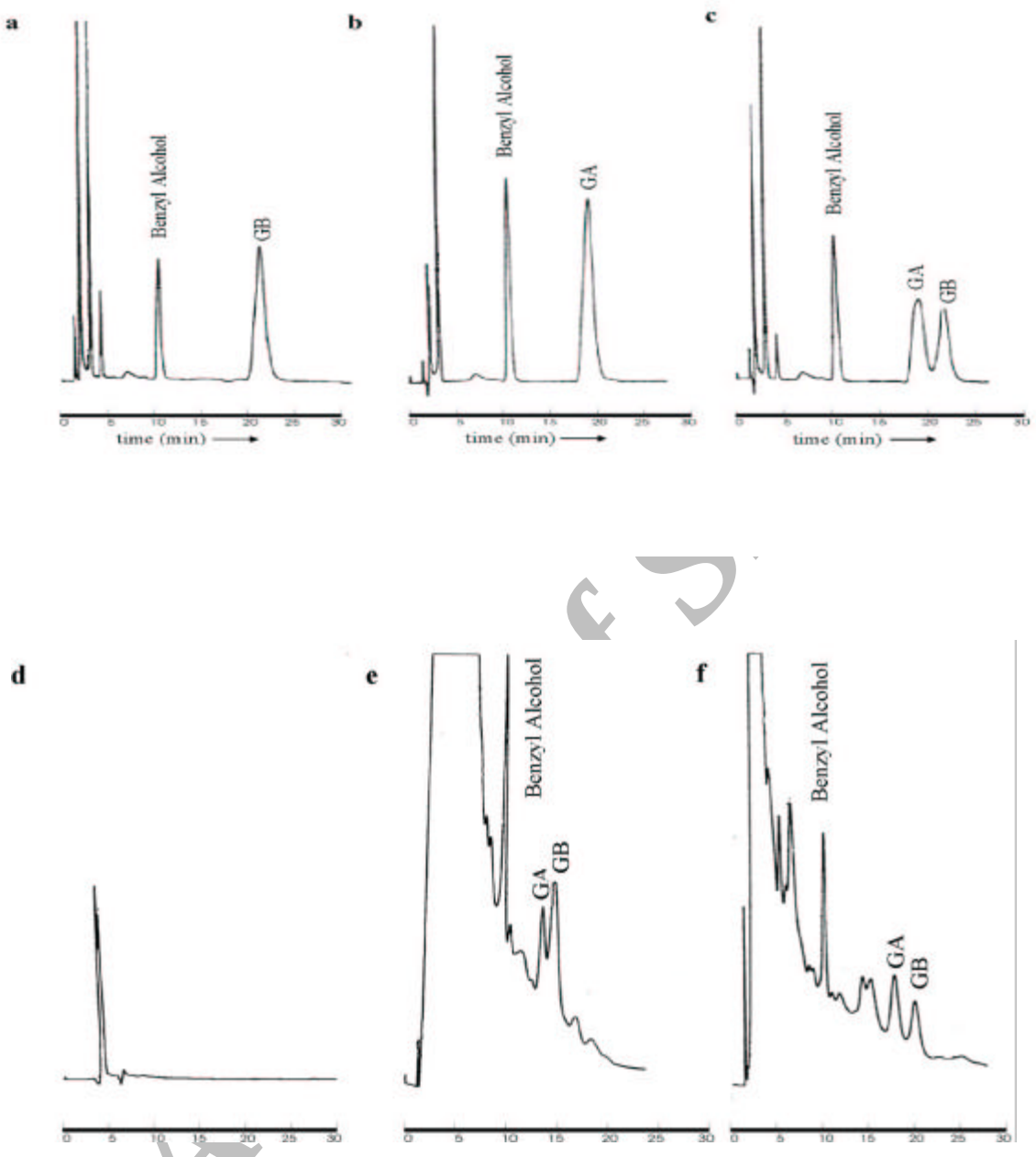

Figure 1. (a) Chromatogram of standard preparation of Ginkgolide B (GB) and benzyl alcohol, (b) Chromatogram of standard preparation of Ginkgolide A (GA) and benzyl alcohol, (c) Chromatogram of standard preparation of GA, GB and benzyl alcohol, (d) Chromatogram of the activated charcoal, (e) Chromatogram of Tanakan preparation in addition to GA, GB and benzyl alcohol, (f) Chromatogram of Iranian *Ginkgo biloba* leaves extract purified with activated charcoal in addition to GA, GB and benzyl alcohol.

Correspondance: Hassan Farsam, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran, E-mail: farsam@ams.ac.ir

www.SID.ir