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# Determination of ginkgolides and bilobalide in Ginkgo *biloba* leaves and phytopharmaceuticals

T. A. VAN BEEK*, H. A. SCHEEREN, T. RANTIO, W. Ch. MELGER and G. P. LELYVELD
***Phytochemical Section, Laboratory of Organic Chemistry, Agricultural University, Dreijenplein 8.6703 HB Wageningen (The Netherlands)***

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## ABSTRACT

A method has been developed for the determination of the pharmacologically active terpenoids ginkgolide A, B and C and bilobalide in ***Ginkgo biloba*** leaves and phytopharmaceutical preparations containing ginkgo extracts. The leaves (400-800 mg) are selectively extracted with methanol-water (10:90) and the resulting extract is purified by means of a polyamide and a $C_{18}$ solid-phase extraction column. After concentration, the terpenoids are determined by high-performance liquid chromatography on a $C_{18}$ column with methanol-water (33:67) as eluent and refractive index detection. Benzyl alcohol is used as an internal standard. The recovery of the method is 95 ± 5%. The reproducibility is dependent on the concentration and varies from 2 to 15%: The minimum concentration that can be determined in leaves is 10 $\mu$g of terpenoid/g of dry leaves. With a small modification the method can be used equally well for phytopharmaceuticals. Several ginkgo medicines were investigated and the total concentration of terpenoids was found to vary by a factor 18. The concentration in leaves varied by a factor 40.

## INTRODUCTION

Ginkgolides, which occur in the leaves of the tree ***Ginkgo biloba,*** possess unique pharmacological properties and are part of many phytopharmaceutical preparations. Reviews have been published by Braquet [1], Schilcher [2] and Hosford ***et al.*** [3] and in a special issue of ***La Presse Medicale*** [4] and a multi-author book on ginkgolides [5].

Their analysis and that of the closely related sesquiterpenoid bilobalide is difficult because of the poor UV characteristics of these compounds and the highly complex matrix. Guth and co-workers [6,7] were the first to publish two different reversed-phase high-performance liquid chromatographic (RP-HPLC) methods with UV detection. Both methods have in common time-consuming purification steps prior to HPLC analysis, a high detection limit of 30 $\mu$g and unsatisfactory results.

In 1988 Teng [8] published a good chromatogram of a ***Ginkgo biloba*** extract with a baseline separation of bilobalide and ginkgolide A, B, C and J and no other visible peaks. Separation was achieved by RP-HPLC with refractive index detection. Unfortunately, no details of the work-up procedure or recovery were given so the work cannot be reproduced. Komoda ***et al.*** [9] also reported an RP-HPLC method with refractive index detection. Their purification procedure involved two partitions, among other steps. No recovery data or extraction efficiencies were given.

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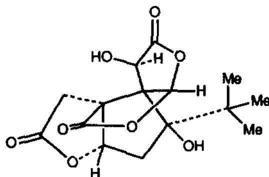


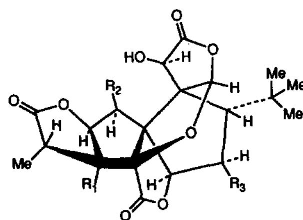


| | | |
|---|---|---|
| Bilobalide | Ginkgolide A | $R_1$ =OH, $R_2$=$R_3$=H |
| | Ginkgolide B | $R_1$=$R_2$=OH, $R_3$=H |
| | Ginkgolide C | $R_1$ =$R_2$=$R_3$=OH |
| | Ginkgolide J | $R_1$ =$R_3$=OH, $R_2$=H |

Recently, Wagner et al. [10] published a method for the determination of flavonoids and ginkgolides in leaves and phytopharmaceuticals by means of HPLC and thin-layer chromatography (TLC) followed by detection with a chromogenic reagent. Prior to detection they used a lengthy purification procedure including column chromatography. After investigating several phytopharmaceuticals they concluded that ginkgolides were either absent or present in extremely low concentrations. This statement prompted a polemic in the same journal by one of the producers of the extracts [11], who stated that the extracts do contain at least 3% of ginkgolides and that these can be determined with sufficient accuracy if the proper analytical techniques are employed. The method used by Wagner *et al.*, especially the TLC part, was considered to be inadequate.

Finally, Pietta *et al.* [12] recently published a simple purification step for ginkgolides followed by RP-HPLC with UV detection. Unfortunately, using this type of detection, several trace impurities co-eluting with ginkgolide C and A are determined at the same time, which gives rise to too high values for these two compounds. This follows from the on-line UV spectra which show too much absorption in the 200-220 nm range, and the baseline of the chromatogram, which returns to zero only after elution of ginkgolide B. No recoveries were given.

In summary, there are no reliable methods that will allow smaller companies and pharmacies to compare different batches of leaves, to control extracts or to standardize phytopharmaceuticals. Phytopharmaceuticals could thus be lacking in the important ginkgolides. Nothing is so far known about differences in the content of ginkgolides as a function of harvest time, age, sex or geographic origin of the tree or weather conditions.

In this paper we report a simple method for the determination of ginkgolides and bilobalide in ginkgo leaves and phytopharmaceuticals containing ginkgo extracts.

## EXPERIMENTAL

### *Plant materials and phytopharmaceuticals*

Dutch ginkgo leaves were collected in the botanical garden of the Agricultural University, Wageningen, from a 55-year-old male tree. The year and month of harvest are mentioned with the individual samples. German ginkgo leaves were collected in mid-September, 1989, from two trees near the Organic Chemistry building of the University of Heidelberg. For the Chinese and French leaves no details are known.

Phytopharmaceutical preparations were provided by the producers or bought in a pharmacy.

### *Drying*

Dutch and German leaves were dried as soon as possible after collection for 24 h at 70°C in an oven with forced ventilation.

### *Standards*

Ginkgolide A, B and C and bilobalide used for some of the recovery experiments and the determination of the response factors were supplied by Prof. K. Weinges. The purity was checked by means of RP-HPLC, UV spectroscopy and 200-MHz $^{1}H$ NMR spectroscopy.

### *Solvents*

Methanol (M4058), acetonitrile and tetrahydrofuran (THF), all from Fisons (Loughborough, U.K.), were of HPLC quality. Methyl acetate was of synthetic quality (Merck, Darmstadt, Germany) and was redistilled prior to use. Hexane was of analytical-reagent grade (Merck). Water was doubly distilled in an all-glass apparatus. All solvents used for HPLC were filtered (0.45 μm) and ultrasonically degassed before use.

### *Instrumentation*

All separations were carried out isocratically at room temperature (20°C) with a Kratos (Manchester, U.K.) Spectroflow 400 HPLC pump, equipped with a MUST (Spark, Emmen, The Netherlands) injector with a 50-μm (analytical) or 250-μm (preparative) loop. Detection was carried out with a Gilson (Villiers le Bel, France) Model 131 refractive index (RI) detector ($3 \cdot 10^{-5}$ refractive index units full-scale). In some initial experiments detection was carried out with a Kratos Spectroflow 773 UV detector at 219 nm. Integration was performed with a Shimadzu (Kyoto, Japan) C-R3A Chromatopac integrator. The analytical column (231 × 4.6 mm I.D.) packed with Spherisorb (Clwyd, U.K.) 5-μm $C_{18}$-modified silica gel was prepared in our laboratory. The preparative column (250 × 10 mm I.D.) packed with Microsorb 5-μm $C_{18}$-modified silica gel was prepared by Rainin (Woburn, MA, U.S.A.).

### *Chromatographic conditions*

Three different eluents were used: (1) water-methanol-THF (7:2:1), flow-rate 1 .O ml/min; (2) water-acetonitrile-THF (7:2:1), flow-rate 1 .O ml/min; (3) water-methanol (67:33), flow-rate 1 .O ml/min (analytical) or 4.0 ml/min (preparative).

*Identification and purity of peaks*

Ginkgolide A, B and C and bilobalide were identified by comparing the retention times of the various peaks with those of authentic reference samples. Ginkgolide J was identified by comparing its chromatographic behaviour in the different eluents with literature data [7,8].

The purity of the peaks was checked by working up a l-g amount of French leaves according to the standard procedure (see below) and, after evaporation of the ethyl acetate-hexane extract, injecting the ginkgolides onto the preparative HPLC column. Each peak was collected separately and after several injections the solvent was removed. The five residues were investigated by means of analytical HPLC with solvents 1 and 2 with refractive index detection. When only one peak was observed the ginkgolide or bilobalide peak in solvent 3 was considered to be pure.

***Extraction and purification procedure***

***(a) Phytopharmaceuticals.*** A Bond Elut (Analytichem International, Harbor City, CA, U.S.A.) 500-mg $C_{18}$ solid-phase extraction column (Art. No. 607 303) is placed below a similar extraction tube packed with 500 mg of MN-polyamid SC 6 for column chromatography (Macherey, Nagel & Co., Düren, Germany). The combination of the two purification columns is first washed with 10 ml of methanol and then conditioned with 10 ml of 2% aqueous methanol. Unless stated otherwise, the column is never allowed to run dry. A 1 .OO-ml volume of the medicine is diluted with 4 ml of water in a 20-ml conical flask and boiled for 5 min in an oil-bath of 150°C without a reflux condenser. After cooling, the solution is transferred to another flask. The first flask is washed three times with 1.5 ml of 2% aqueous methanol.

The diluted ginkgo extract and the washings (*ca.* 8 ml) are transferred to the polyamide column and sucked through the two columns at a rate of 1 drop/s. The flask is washed three times with a total of 5 ml of 2% aqueous methanol and the washings are transferred to the column as well. The two columns are then washed with 5 ml of 5% aqueous methanol and dried by sucking air through the columns for 2 min with a water pump at maximum suction. The polyamide column is discarded, the $C_{18}$ column is washed with 1 ml of water and again dried for 2 min.

The dried $C_{18}$ column is first washed with 6 ml hexane (washings discarded) and then eluted with 7 ml of hexane-methyl acetate (60:40) into a 20-ml conical flask. After evaporation of the solvent, the residue is dissolved in 70-350 μl of methanol. Subsequently 1.00 μl of benzyl alcohol is added with a Hamilton (Bonaduz, Switzerland) zero-dead-volume Model 7001 injection needle, followed by 130-650 μl of water. The flask is stoppered and placed in a water bath at 50°C. After 5 min, the solution is ready for injection in the HPLC system.

***(b) Leaves.*** The dried leaves are pulverised either mechanically or with a pestil and mortar. Leaves (400-800 mg) are carefully weighed and placed in a 20-ml conical flask, 5 ml of 10% aqueous methanol and a boiling stone are added and the solution is refluxed for 15 min. During the last minute the condenser is removed. The hot solvent is filtered under vacuum over a small Büchner funnel (3.3 cm I.D.) with a filter-paper (Schut, Heelsum, The Netherlands). The flask and the leaves are washed with 2 ml of 2% aqueous methanol. After 1 min of suction the leaves are replaced in the flask and extracted a second time in the same way with 4 ml of 10% aqueous methanol. After filtration the flask and the residue of leaves are washed twice with 1.5 ml of 2% aqueous methanol.

The combined filtrates (cu. 12 ml) are transferred to the polyamide column (see above) and drawn through the two columns at a rate of 1 drop/s. The flask is washed three times with a total of 3 ml of 2% aqueous methanol and the washings are transferred to the column.

The remainder of the procedure for the leaves is the same as that for the phytopharmaceuticals from "The two columns are then washed with 5 ml ...".

### *Determination of response factor*

Benzyl alcohol (Aldrich, Milwaukee, WI, U.S.A.) was used as an internal standard. Response factors were calculated for ginkgolide A, B and C and bilobalide by plotting for each of the ginkgo compounds the ratio of the area of the ginkgo peak to that of the internal standard peak (benzyl alcohol) against the ratio of the weight of the ginkgo compound to that of the internal standard. The slope of each line gives the relative response factor. For each ginkgolide seven different weight ratios, varying from 0.00 to 2.1, were used.

### *Reproducibility of integration*

The reproducibility of the integration of the RI signals was determined for each ginkgolide at six different concentrations. Each solution was injected five times and relative standard deviations were calculated.

### *Recovery experiments*

Known amounts of the ginkgolides and bilobalide in aqueous methanol were submitted to the extraction and purification procedure and the recovery was determined. Three different amounts of starting material were investigated. For each amount the purification process was repeated three times. Each extract obtained was injected three times for HPLC analysis.

### *Quantification*

The ginkgo leaves and phytopharmaceuticals to be investigated were analysed two to five times according to the above purification procedure. The concentrations of ginkgolide A, B and C and bilobalide were calculated with the following equation:

$$\text{concentration (ppm)} = \frac{\text{area (G)}}{\text{area (I.S.)}} \times \frac{10^6}{R} \times \frac{1.045}{M}$$

where area (G) is the peak area of the ginkgo compound of interest, area (I.S.) is the peak area of the internal standard (benzyl alcohol), $R$ is the relative response factor of the ginkgo compound (see Table I) and $M$ is either the sample volume of the phytopharmaceutical preparation ($\mu$l) or the weight of the ginkgo leaves (mg).

Owing to the poor separation of bilobalide and ginkgolide J in some chromatograms the concentration given for bilobalide includes ginkgolide J, whereas in other chromatograms it was possible to give a separate value for ginkgolide J. As no pure ginkgolide J was available to determine the response factor relative to benzyl alcohol, an estimated value of 1.21 was chosen (average of $R_{GB}$ and $R_{GC}$).

## RESULTS AND DISCUSSION

Although ginkgolides can be determined by gas chromatography (GC) [1] as their trimethylsilyl derivatives, HPLC seems a more logical choice for these relatively polar compounds. All the earlier investigations used RP-HPLC, which gives good separations of the ginkgo compounds [7]. Initially we choose UV detection because of the ready availability of UV detectors, high stability and good sensitivity. The ginkgolides and bilobalide all have poor UV characteristics with $\lambda_{max}$ = 219 nm and $\varepsilon \approx$ 300 l/mol · cm [13]. Nevertheless, the detection limit was 30 ng, which was more than sufficient for our purposes. Water-methanol (67:33) was used as eluent, which gives a good separation of, in order of increasing retention time, bilobalide and ginkgolide J, C, A and B in 20 min. Only bilobalide and ginkgolide J nearly coelute.

With pure reference compounds several purification procedures were developed using different combinations of solid-phase extraction columns and extraction procedures. Combinations showing adequate recovery were then tested on leaves and sometimes phytopharmaceuticals. Interfering compounds present in ginkgo leaves include many phenolics, e.g., flavonoids and tannins, and more apolar compounds, e.g., chlorophyl, anacardic acids and lipids. Although ginkgolides and especially ginkgolide B are not very soluble in cold water, they are soluble in boiling water and this was found to be a selective primary extraction solvent.

However, many flavonoids are co-extracted and have to be removed because of their high concentration and their much stronger W absorption, but no single purification column or combination of two or even three solid-phase extraction columns gave an extract that could be analysed by RP-HPLC with UV detection. Purification columns used include $C_{18}$ silica, Diol, Extrelut, polyamide and phenylboronic types. Precipitation agents for phenolics such as $Pb^{2+}$ ions and polyvinylpolypyrollidone (PVPP) did not improve the chromatograms.

The best results were eventually obtained with a combination of a polyamide and a $C_{18}$ column. The polyamide column removes most of the phenolics. The non-retained ginkgolides are then retained by the $C_{18}$ purification column if the methanol content is low enough. By increasing the elution strength the ginkgolides can easily be removed from the $C_{18}$ column. However, even leaves with a high content of ginkgolides gave poor chromatograms with this procedure and UV detection. Fig. la shows the chromatogram of an already partially purified phytopharmaceutical with a high content of terpenoids after the polyamide–$C_{18}$ column work-up. Only at the end of the chromatogram is the baseline back to zero. Leaves could not be analysed at all.

We therefore tried this clean-up procedure in combination with RI detection. This immediately gave much better results and, after some modifications, this method was also usable for leaves and phytopharmaceuticals with a low yield of ginkgolides. Fig. lb shows the same purified extract as in Fig. la analysed under identical conditions except that RI detection instead of UV detection was used. The noise level is naturally much higher but the baseline immediately returns to zero after the solvent and essentially no peaks other than those of the terpenoids of interest are observed. Apparently the unwanted compounds still present in the purified extract occur in low concentrations relative to the ginkgolides but possess $\varepsilon$ values at 219 nm which are ca. 100 times higher than those for the ginkgolides. Hence the RI detector, which shows much less variation in response factors, is better suited even though the sensitivity and

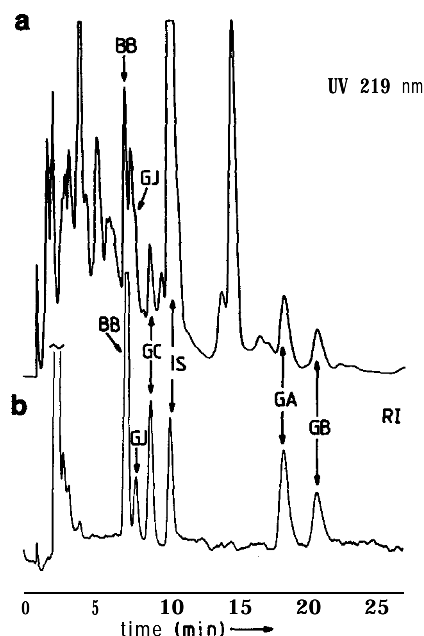


Fig. 1. HPLC traces of 1 .OO ml of Tanakan preparation purified according to extraction and purification procedure (a). Solvent 3; flow-rate, 1 .O ml/min. BB = Bilobalide; IS = internal standard (benzyl alcohol); GJ, GC, GA, GB = ginkgolide J, C, A and B, respectively. (a) W detection (220 nm); (b) RI detection.

baseline stability are much poorer. The detection limit was 0.5 $\mu$g for bilobalide and ginkgolide C and 1 $\mu$g for ginkgolide A and B. For quantitative purposes ten times more is needed, which means that the purified extract obtained after the last purification column is usually too dilute for direct analysis by HPLC. The final extract has to be evaporated and the residue dissolved in a small volume (0.2-1.0 ml) of the HPLC eluent before injection. Because water is difficult to evaporate quickly we tried to elute the ginkgolides with a purely organic solvent. Hexane–ethyl acetate (6:4) or hexane-methyl acetate (6:4) gave good results. As ethyl acetate is more difficult to evaporate and appeared as an additional peak in the chromatogram near ginkgolide J and C, methyl acetate, which lacks these disadvantages, was chosen. A wash step with hexane just prior to the hexane–methyl acetate elution was necessary to remove the water still present on the $C_{18}$ column after drying. Hexane does not remove ginkgolides because they are totally insoluble in this solvent.

Another modification was the addition of a few percent of methanol to the primary extraction solvent, which increased the extraction efficiency. Removal of the methanol before transferring the extract onto the polyamide by boiling briefly without a reflux condenser was necessary to retain as much impurities as possible on the polyamide column. A small percentage of methanol was left in the various conditioning, washing and elution solvents to increase the retention of the $C_{18}$ solid-phase extraction column. This method could also be used for phytopharmaceutical preparations if their initial high alcohol content (50–60%) was lowered almost to zero by boiling for 5 min in an open flask.

TABLE I

RESPONSE FACTORS OF GINKGOLIDE A, B AND C AND BILOBALIDE

| Compound | Response factor | Correlation coefficient |
|---|---|---|
| Ginkgolide A | 1.12 | 0.9988 |
| Ginkgolide B | 1.16 | 0.9972 |
| Ginkgolide C | 1.26 | 0.9989 |
| Bilobalide | 1.11 | 0.9984 |

To avoid fixed end volumes and to make the method usable by laboratories which do not possess pure ginkgolides, benzyl alcohol as an internal standard was added just prior to the HPLC analysis. Relative response factors for ginkgolide A, B and C and bilobalide were determined and are reported in Table I. The relative standard deviation in the integration process is dependent on the concentration and becomes considerable at concentrations of 100 ppm or lower (see Table II).

The recovery of the whole filtration and purification process was determined for various initial concentrations of the ginkgo constituents. The results are presented in Table III. The recovery was 95 ± 5% for all concentrations except for very high concentrations of ginkgolide B. The reason for this is the very poor solubility of this compound in water (0.01%). Concentrated solutions of ginkgolide B in water have a tendency to form crystals which do not redissolve easily. Most of the ginkgolide B in the most concentrated test solutions remained as tiny crystals on the polyamide column. With leaves or phytopharmaceutical preparations containing higher concentrations than the reference solutions there was never any evidence of crystallization of ginkgolide B. Probably other substances present in ginkgo leaves keep ginkgolide B in solution. Only in the final solution for injection into the HPLC system, which sometimes contains high concentrations of the ginkgolides (> 0.1%), could crystallization again be observed. This could be prevented by warming the solution in a closed flask at 50°C.

The efficiency of the extraction of the leaves was determined by extracting the leaves for 30 min a third time after the first two times. It was found that an additional 1.8% of ginkgolide C and bilobalide, 3.5% of ginkgolide A and 4.5% of ginkgolide B

TABLE II

RELATIVE STANDARD DEVIATIONS OF INTEGRATION RESULTS FOR GINKGOLIDE (G) A, B AND C AND BILOBALIDE (BB) ($n$ = 3)

| Concentration (μg/ml) | Relative standard deviation (%) | | | |
|---|---|---|---|---|
| | G-A | G-B | G-C | BB |
| 100 | 3.8 | 14.3 | 5.6 | 6.0 |
| 200 | 7.5 | 4.8 | 3.4 | 3.8 |
| 300 | 5.2 | 3.4 | 5.2 | 2.4 |
| 435 | 3.7 | 2.4 | 3.6 | 1.6 |
| 625 | 2.2 | 3.4 | 1.5 | 0.8 |
| 800 | 2.0 | 1.5 | 1.3 | 1.4 |

TABLE III

RESULTS OF RECOVERY EXPERIMENTS

For conditions, see Experimental ($n = 3$)

| Compound | Initial amount (μg) | Recovery (%) | Relative standard deviation (%) |
|---|---|---|---|
| Bilobalide | 2140 | 98.8 | 2.2 |
| | 719 | 98.5 | 2.9 |
| Ginkgolide C | 1160 | 102.5 | 9.2 |
| | 615 | 90.1 | 3.1 |
| | 290 | 97.3 | 5.8 |
| Ginkgolide A | 1300 | 96.6 | 0.6 |
| | 738 | 94.8 | 2.6 |
| | 320 | 97.0 | 8.9 |
| Ginkgolide B | 708 | 86.9 | 2.5 |
| | 590 | 92.3 | 2.5 |
| | 150 | 99.5 | 7.9 |

could be extracted. Thus in the normal procedure 95–98% of the ginkgolides and bilobalide are extracted.

As there is always a possibility that an impurity which is not removed during the purification process coelutes with one of the compounds of interest, the purity of the peaks was assessed. A larger amount of leaves with a high content of ginkgolides was purified in the usual manner and then separated on a preparative column. The peaks corresponding with ginkgolide A, B, C and J and bilobalide were collected, evaporated and then reinjected onto an analytical column with a tetrahydrofuran-containing solvent. Such solvents have a large influence on the capacity factors of ginkgolide A, B and C and bilobalide [7]. The chance that a possible interfering compound undergoes exactly the same shift in retention can be considered small. The appearance of one or more peaks in addition to the ginkgolide peak is then evidence for an impurity co-eluting in the standard water-methanol (67:33) solvent. No peaks larger than the noise level were observed, so no major impurities co-elute.

Finally, the reproducibility of the method when applied to leaves with a high ginkgolide content was determined. For this purpose four samples of 500 mg of leaves were extracted and purified according to the standard procedure. Each of the four extracts was analysed three times by HPLC. The results are given in Table IV. For leaves and phytopharmaceuticals with a low content of ginkgolides the relative errors become larger owing to larger integration errors (see also Table II). If “electronic noise” and not “chemical noise” is the main problem, decreasing the volume of the final solution from 1.0 to 0.3 ml gives more reliable data.

The method was applied on some commercial ginkgo preparations. The results are given in Table V. Two corresponding chromatograms are shown in Fig. 2. Tebonin and Rökan look similar to Tanakan in Fig. lb. It is immediately clear that controlled partially purified ginkgo medicines (Rökan, Tanakan, Tebonin) contain much higher concentrations of ginkgolides and bilobalide than Dutch phytopharma-

TABLE IV

REPRODUCIBILITY OF EXTRACTION AND PURIFICATION PROCESS FOR 500 mg OF GINKGO LEAVES ($n$ = 4)

| Compound | Weight (μg) | | | | | Standard deviation (μg) | Relative standard deviation (%) |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Average | | |
| Bilobalide | 531 | 507 | 517 | 520 | 519 | 10 | 1.9 |
| Ginkgolide C | 3 16 | 313 | 321 | 327 | 319 | 6 | 1.9 |
| Ginkgolide A | 226 | 224 | 218 | 232 | 225 | 6 | 2.6 |
| Ginkgolide B | 197 | 196 | 205 | 196 | 198 | 4 | 2.2 |

ceuticals (see Fig. 2a), which are prepared according to the homeopathic pharmacopoeia. This is not surprising as, according to this pharmacopoeia, 10 kg of fresh leaves, which correspond to ca. 2.5 kg of dry leaves, give cu. 20 1 of ginkgo tincture. The partially purified French and German preparations contain far more "leaves"/ml. The ginkgogink preparation is entirely different from all the others. It contains high concentrations of all the ginkgolides but bilobalide could not be detected. As bilobalide is the major terpenoid in all the leaves and other phytopharmaceuticals investigated, this suggests that the bilobalide has been selectively removed during the manufacturing process. Some extra peaks not present in any other sort of leaves or phytopharmaceuticals can be observed at the beginning of the chromatogram (see Fig. 2b).

According to Stumpf [11], Tebonin extract contains at least 0.12% of "ginkgolides". We found a total of 0.115% (excluding bilobalide) or 0.21% (including bilobalide), which is in agreement with his claim. Hence the HPLC-TLC procedure put forward by Wagner et al. [10] is indeed not usable. According to Drieu [14], Tanakan contains 0.24% of ginkgolides and bilobalide. We found 0.22%. As we do not know how they determined these compounds, it is difficult to say whether their determination gives systematically slightly higher results than ours or whether the

TABLE V

GINKGOLIDE (G) A, B, C AND J AND BILOBALIDE (BB) CONCENTRATIONS IN GINKGO PHYTOPHARMACEUTICALS

For Dutch preparations only a combined value for bilobalide and ginkgolide J is given.

| Phytopharmaceutical | Concentration in μg/ml fluid | | | | | Total (%) |
|---|---|---|---|---|---|---|
| | BB | G-J | G-C | G-A | G-B | |
| Geriaforce A (Dutch) | 98 | | 20 | 26 | 24 | 0.017 |
| Geriaforce B (Dutch) | 80 | | 22 | 23 | 21 | 0.015 |
| Ginkgoplant (Dutch) | 72 | | 18 | 27 | 16 | 0.013 |
| Naphyto DØ (Dutch) | 58 | | 22 | 24 | 19 | 0.012 |
| Ginkgogink (French) | <20 | 120 | 363 | 372 | 256 | 0.111 |
| Tanakan (French) | 943 | 184 | 336 | 460 | 278 | 0.220 |
| Rökan (German) | 921 | 174 | 280 | 360 | 180 | 0.192 |
| Tebonin (German) | 988 | 167 | 302 | 398 | 279 | 0.213 |

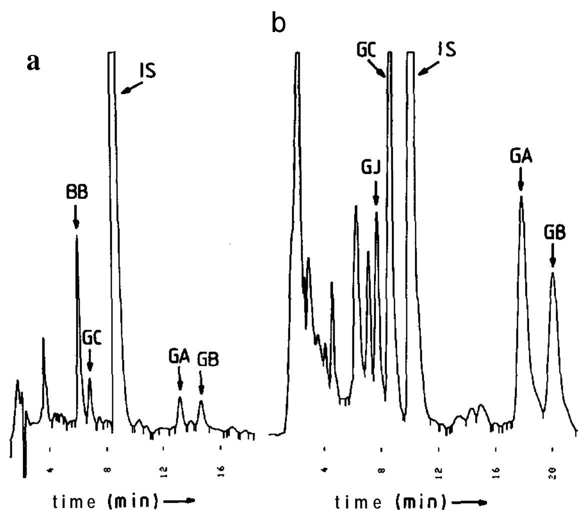


Fig. 2. HPLC traces of 1.00 ml of commercial fluid ginkgo phytopharmaceuticals after purification according to extraction and purification procedure (a). Solvent 3; flow-rate, 1 .0 ml/min; RI detection. Abbreviations as in Fig. 1. Sample: (a) Geriaforce; (b) Ginkgogink.

batch is below their specification. If we make a 3–4% correction for the usual losses (see recovery experiments) the difference is hardly significant.

Some different leaves were compared and large variations were found (see Table VI). French leaves contained the highest amount of ginkgolides. Dutch leaves from the good summer of 1989 contained almost as much bilobalide but ca. 40% less ginkgolides, whereas Chinese leaves, German leaves (1989) and Dutch leaves (1987) contained much less. Dutch leaves from May 1990 contained a small amount of bilobalide and no detectable ginkgolides. Apparently the sun and the time of harvest play an important role in the yield of these terpenoids. In all instances the bilobalide

TABLE VI

GINKGOLIDE (G) A, B, C AND J AND BILOBALIDE (BB) CONCENTRATION IN GINKGO LEAVES

For Chinese leaves only a combined value for bilobalide and ginkgolide J is given.

| Origin of leaves | Date of collection | Concentration in $\mu g/g$ dry leaf | | | | | Total (%) |
|---|---|---|---|---|---|---|---|
| | | BB | G-J | G-C | G-A | G-B | |
| Chinese | – | 758 | | 162 | 265 | 155 | 0.134 |
| Dutch | Oct. 1987 | 318 | 7 | 9 | 17 | 21 | 0.037 |
| Dutch | Sept. 1989 | 1100 | 42 | 260 | 380 | 220 | 0.196 |
| Dutch | May 1990 | 65 | <2 | <2 | <3 | <3 | 0.006 |
| French | – | 1250 | 190 | 424 | 646 | 348 | 0.266 |
| French | Aug. 1982 | 964 | 74 | 638 | 450 | 396 | 0.252 |
| German | Sept. 1989 | 290 | <4 | <4 | <4 | 29 | 0.032 |

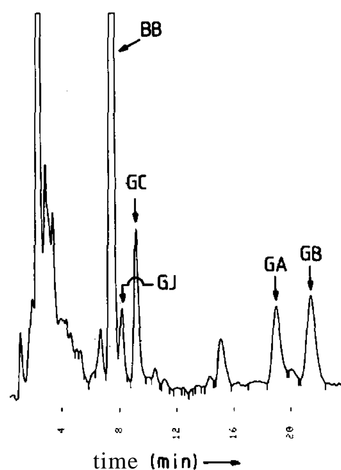


Fig. 3. HPLC trace of 1.000 g of Dutch ***Ginkgo biloba*** leaves (September 1989) after purification according to extraction and purification procedure (b). Solvent 3; flow-rate, 1.0 ml/min; RI detection. Abbreviations as in Fig. 1.

content was equal to or higher than that of the total ginkgolides. A typical chromatogram is presented in Fig. 3.

## CONCLUSION

Selective extraction of ginkgo leaves with methanol-water (10:90) followed by a sample clean-up with a polyamide and a $C_{18}$ solid-phase extraction column in series gives an extract that can be readily analysed by RP-HPLC with RI detection. All the leaves and phytopharmaceuticals investigated were found to contain bilobalide and ginkgolides, although large differences between different leaf batches or ginkgo preparations from different manufacturers were observed.

Owing the intrinsic poor stability and sensitivity of the RI detector, the minimum concentration that can be determined with acceptable accuracy is fairly high (*ca.* 10 ppm). Improvements to the proposed analytical procedure should in the first place concentrate on this aspect.

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