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Pharmaceutical quality of different *Ginkgo biloba* brands

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Abstract

Ginkgo biloba-containing brands are one of the top sellers within the growing market for herbal remedies in many European countries as well as in the USA. In the consumers' interest, these brands should feature a certain quality and should be transparent in quality claims. In this investigation, a variety of products on the USA market was studied with respect to pharmaceutical quality, such as quantity of constituents and in-vitro dissolution. In terms of the content of active substances, flavone glycosides ranged from 24 % to 36 % and terpene lactones from 4 % to 11 %. With ginkgolic acids, there was a very large range, from < 500 ppm to about 90 000 ppm. Comparing the dissolution rates of terpene lactones and flavone glycosides within the single products, most were approximately the same. Thus, terpene lactones and flavone glycosides were released from these products and dissolved at the same rate in most cases. Furthermore, most of the products investigated released more than the required 75 % of the content of both components within 30 min. However, several products showed clear and relevant differences in dissolution rates to the rest (e.g. < 75 % within 30 min or even less than 25 % after 60 min in one case, indicating much poorer pharmaceutical quality). Beside the comparability respectively standardisation of the extracts used, the in-vitro dissolution of the relevant constituents should be similar to other drugs to guarantee comparable in-vivo performance of herbal products. An important step in standardising pharmaceutical quality is the pharmacopoeial monograph for *Ginkgo biloba* extract in Germany, standardising the content of pharmacologically relevant substances (flavone glycosides 22–27 % and terpenlactones 5–7 %, 2.8–3.4 % ginkgolides A, B, C and 2.6–3.2 % bilobalide thereof). Many of the investigated products, which refer to the German Commission E (of the Federal Institute for Drugs and Medicinal Devices) monograph, are not in accordance with this specification. Thus, they can not be considered to be pharmaceutically equivalent.

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Introduction

Ginkgo biloba has become a widely used herbal remedy for increasing cognitive functions in elderly people, improving symptoms and progression of vascular and neurodegenerative dementia, improving blood flow and treating tinnitus mainly of circulatory origin (Le Bars et al 1997; DeFeudis 1998).

The actual size of the market for herbal medicinal products in the USA is difficult to assess, since these products are sold mostly in health-food stores, by mail order or by multi-level marketing organisations, for which accurate statistics are not available. Nevertheless, the size was estimated at about $1.6 billion in 1994 (Brevoort 1999), with projections up to $3.24 billion in 1997 (Johnston 1997). Top sellers are *Ginkgo*, ginseng, garlic, echinaceae, St John's wort and saw palmetto.

Most herbal remedies are sold in the USA as dietary supplements under the Dietary Supplement Health and Education Act of 1994 (DSHEA). As a consequence, there is no approval by the FDA (Food and Drug Administration), which means that for dietary supplements, activity and efficacy has not been documented by adequate experimental and clinical studies.

In contrast, herbal medicinal products (e.g. *Ginkgo biloba*) are marketed in Germany as ethical medicines. They are approved by the Federal Institute for Drugs and Medicinal Devices (BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte). This approval is based, in most cases, on monographs summarising relevant material on pharmacology, toxicology, safety and clinical efficacy of a specific plant. These monographs are composed after extensive review of published and unpublished data by an independent expert committee (Commission E) set up by the German Federal Institute of Health (BGA (Bundesgesundheitsamt), now BfArM).

For *Ginkgo biloba*, the evaluation of the German Commission E was positive (Monographie: Trockenextrakt (35-67:1) aus Ginkgo-biloba-Blättern, Bundes-Anzeiger Nr 133, 1994) and referred to the following specifications: 22–27% flavone glycosides, 5–7% terpene lactones (2.8–3.4% ginkgolides A, B, C and 2.6–3.2% bilobalide thereof) and not more than 5 ppm ginkgolic acids, constituents with known allergic and cytotoxic potency (Koch & Jaggy 1997; Siegers 1999; Koch et al 2000; Baron-Ruppert & Luepke 2001). The monographs of the Commission E are now available in an English translation as well (Blumenthal 1998), as the interest in plant-derived medicinal products has grown substantially in other countries (e.g. in the USA). Besides the Commission E, the WHO (World Health Organisation) also published a positive monograph on *Ginkgo* leaf extracts, which is in principle comparable (Folium Ginkgo, WHO monographs Vol. I, 1999).

It must be remembered, however, that other components and constituents may also contribute to clinical efficacy (e.g. organic acids, proanthocyanidins or other unknown compounds). The concentration of these as yet unidentified compounds can only be kept constant when the substances with known relevant pharmacological properties are also constant in a tight range. For this reason, the Commission E monograph specifies a range (5–7% terpene lactones) and not a limit (e.g. more than 5% terpene lactones). The *Ginkgo biloba* extract used in most clinical trials (EGb761) has been standardised to the specifications just mentioned.

Because dietary supplements in the USA are exempt from rigorous FDA regulation, substantial qualitative and quantitative deviations from the product label have been documented for ginseng (Cui et al 1994), feverfew (Heptinstall et al 1992), ephedra (Gurley et al 2000), echinaceae (Bergeron et al 2000) and also for *Ginkgo* (ConsumerLab.com 2000).

Therefore, it was of interest to compare the pharmaceutical quality of herbal remedies containing *Ginkgo biloba* available on the US market. The products were sold in health-food stores and in supermarkets and were analysed following the methods described in official pharmacopoeias (DAB 2000, USP 1998). Furthermore, a selection of samples were investigated for their in-vitro dissolution profile, another important criterion for pharmaceutical quality.

During the course of our investigations a few comparable results of another laboratory were published (ConsumerLab.com 2000).

Materials and Methods

Materials

The *Ginkgo biloba*-containing brands were purchased in health-food stores and supermarkets. A description is given in Table 1.

Chemicals

Quercetin, kaempferol, isorhamnetin, ginkgolic acids, ginkgolides A, B and C, bilobalide and EGb 761 extract were gifts from Dr Willmar Schwabe GmbH & Co., Karlsruhe. The purity of all substances was $>98\%$.

HPLC analysis

The HPLC analyses were performed using a Jasco Pump PU-980, a Jasco autosampler AS-950, a Jasco gradient former LG-980-02 and Jasco detector (UV-975 (UV detector) and RI-930 (refractive index detector)). The calculations were done by Borwin-Software V 1.21.

To obtain information on the spectrum of constituents in the extract, a gradient HPLC analysis (mobile phase A: acetonitrile–water acidified with phosphoric acid (99:1:0.3 v/v/v) and mobile phase B: acetonitrile–phosphoric acid (100:0.3 v/v)) was done on an RP-column (RP18 LiChrospher 100, 5 μm, 125 × 4 mm; Merck, Darmstadt). The spectrum of constituents was eluted by pure phase A for 5 min followed by mixtures of phase A and B, 60/40% for 50 min and 1/99% for 45 min. To recondition the column, pure phase A was

used for 15 min. UV detection was performed at 211 and 360 nm. In these runs a quantitative determination of ginkgolic acids was possible, using authentic ginkgolic acids as standard.

The flavone glycosides were measured by HPLC as described in the literature (Folium Ginkgo, WHO monographs Vol. I, 1999; Ginkgo Dry Extract, standardised, Pharmeuropa 11, 1999). Accordingly, an amount corresponding to about 40 mg extract was dissolved in methanol–7% HCl and heated in a boiling water bath for 25 min to hydrolyse the glycosides. The resulting aglycones, quercetin, kaempferol and isorhamnetin, were separated on an RP-column (RP18 LiChrospher 100, 5 μm, 125×4 mm; Merck, Darmstadt) using isopropanol–acetonitrile–water (5:47:100 v/v/v) with citric acid (6 g L^{-1}) as mobile phase. Detection was performed at 370 nm using quercetin as reference. Quantitation of the aglycones quercetin, kaempferol and isorhamnetin in total were based on the response of quercetin, assuming equal detector responses for all aglycones. Because of the calculative conversion to flavone glycosides with an average molecular weight of M_R = 756.7 results were multiplied by 2.514.

For the determination of the terpene lactones (Ginkgo Dry Extract, standardised, Pharmeuropa 11, 1999; Folium Ginkgo, WHO monographs Vol. I, 1999), the extract was dissolved in phosphate buffer, pH 5.8, and extracted with ethyl acetate. The organic phase was evaporated to dryness and the residue dissolved in mobile phase, which was tetrahydrofuran–methanol–water (10:20:75 v/v/v). The separation was carried out on an RP-column (RP 8 LiChrospher 100, 5 μm, 250×4 mm; Merck, Darmstadt) using benzyl alcohol as reference. Detection was performed by a refractive index detector. Quantitation of ginkgolides A, B and C and bilobalide was based on the response of benzylalcohol assuming equal detector responses for all substances. Because of calculative conversion to each individual substance with its own average molecular weight, results were multiplied by 1.22 for ginkgolide A, 1.19 for ginkgolide B, 1.27 for ginkgolide C and 1.20 for bilobalide.

The methods used were validated according to ICH 3 guidelines (International Conference of Harmonisation). For quality control purposes, standardised extract EGb 761 was measured in every run determining flavone glycosides and terpene lactones. The single values of 7 days with n = 6 for every day were analysed. Precision and accuracy were calculated by determination of the content of flavone glycosides and terpene lactones in percent of extract and the recovery (mean ± s.d., n = 7): respectively, flavone glycosides 25.09 ± 0.806 and 99.68 ± 3.21; ginkgolide A 1.30 ± 0.046 and 106.53 ± 3.77; ginkgolide B 0.73 ± 0.185 and 101.06 ± 25.61; ginkgolide C 1.24 ± 0.055 and 101.32 ± 4.50 and bilobalide 2.56 ± 0.106 and 99.85 ± 4.13. Precision for ginkgolic acids was determined at three concentrations, each three replicates, by use of one of the U.S. products as 1609 ± 105 ppm (RSD: 6.53%). Accuracy (percent recovery) for ginkgolic acids, as determined by adding known quantities of ginkgolic acids to a placebo mixture at three concentrations (three replicates each), was 105.09 ± 5.28%.

Dissolution test

Tests were performed in the paddle apparatus which had previously been checked for suitability of the system as described in USP 23. Sinkers were attached to the capsules, which would otherwise float. Investigations were conducted in 900 mL of 0.1 M HCl dissolution medium at 37°C ± 0.5°C and a rotation speed of 100 rev min^{-1}. Samples were taken at 15, 30 and 60 min. Samples withdrawn for analysis were to be replaced with equal volumes of fresh dissolution medium at 37°C. The samples were filtered (Whatman glass microfibre filters GF/D) and 10 mL was used for determination of the amount of flavone glycosides in solution at a wavelength of maximum absorbance at about 360 nm in comparison with a filtered solution of ground tablets or capsules. Ground tablets or capsules dissolved in 0.1 N HCl served as reference, respectively, 100% dissolution control. For determination of the terpene lactones the remaining solution (20 mL) was prepared as described above.

Statistical analysis

Contents of the investigated products in terms of flavone glycosides and terpene lactones were compared by *t*-test to the specified ranges set by Commission E: 22–27% flavone glycosides and 5–7% terpene lactones (2.8–3.4% ginkgolides A, B and C and 2.6–3.2% bilobalide thereof), with $P < 0.05$ being considered statistically significant outside the recommended range.

Results

In a first series of analyses, 27 *Ginkgo biloba*-containing formulations (Table 1) available on the USA market were studied (one batch each). Most of them claimed to

Table 1 Content (percent in extract) of flavone glycosides, total terpene lactones and ginkgolic acids in *Ginkgo biloba*-containing brands available on the US market.

Product	Batch	Dose (mg extract)	Standardisation as described on label (% flavone glycosides, % terpene lactones)	Recommended daily dose (no. of tablets or capsules)	Galenical formulation	Flavone glycosides (% in extract (n))	Terpene lactones (% in extract (n))	Ginkgolic acids (ppm, (n))
1	7E01703	40	24	3	Tablet	25.49±0.82 (6)	6.93±1.18 (18)	< 500 (2)
2	8E03057	60[1]		1–2	Tablet	24.92±0.66 (12)	6.53±0.25 (6)	< 500 (2)
3	010403A	60	24, 6	2	Capsule	25.11±0.81 (6)	10.42±0.30*** (6)	1609±105 (2)
4	457–569	60	24, 6	2	Capsule	29.41±1.89*** (18)	3.87±1.09 (6)	< 500 (2)
5	4801791	40	24	1–2	Tablet	26.12±0.67 (6)	7.85±0.96* (12)	< 500 (2)
6	802241P	40	24	3	Capsule	32.12±1.72*** (18)	10.57±0.30*** (12)	47 968±6574 (2)
7	71047	50	24	1	Tablet	27.69±0.33*** (12)	8.74±2.65* (12)	3082±148 (2)
8	4136812	30	24	3–4	Tablet	24.60±0.27 (6)	8.91±1.78** (12)	2991±36 (2)
9	IF13085	40	24	3	Capsule	26.80±0.82 (12)	7.82±0.83** (12)	7293±88 (2)
10	808169	60	24	2	Tablet	24.59±0.57 (6)	5.90±0.18 (18)	< 500 (2)
11	70564	60[2]	24, 6	1–2	Capsule	24.54±0.39 (6)	6.91±1.16 (12)	27 400±465 (4)
12	610004	60	24, 6	2	Tablet	26.71±0.62 (6)	6.61±0.12 (6)	1522±85 (2)
13	610040	60	27, 7	2	Tablet	29.26±0.47*** (10)	10.79±0.22*** (6)	2924±223 (2)
14	FD7276A	40		3	Tablet	23.88±0.21 (6)	5.88±0.30 (6)	< 500 (2)
15	8D03390	40	24	3	Capsule	28.94±0.66** (4)	8.53±0.16*** (6)	7895±814 (4)
16	8F5010	60	24, 6	2–4	Tablet	24.74±0.31 (6)	5.50±0.13 (6)	< 500 (2)
17	4802	60[3]	24	1–2	Capsule	27.00±0.92 (6)	7.84±0.20*** (6)	78 425±2040 (2)
18	8C04774	40	24	3	Capsule	25.77±0.58	6.61±0.53 (12)	2924±81 (2)
19	801051A	40	24	3	Tablet	25.45±0.84 (6)	5.30±0.23 (6)	6746±1029 (2)
20	6223	40	24, 6	3	Capsule	35.54±1.03*** (6)	11.31±0.17*** (6)	89 576±2297 (2)
21	6331	–[4]		3	Capsule	Not determinable	Not determinable	> 50 000 (2)
22	8D05413	40	24	3	Capsule	32.79±0.83*** (6)	7.65±1.40 (12)	1088±181 (2)
23	2794-J8	60	24, 6	2	Tablet	30.34±0.62*** (6)	7.74±0.43** (6)	2667±74 (2)
24	IF10313	40	24	3	Tablet	29.39±0.27*** (6)	9.60±0.49*** (6)	2560±1 (2)
25	T4676E04	60	24, 6	2–4	Capsule	27.13±0.57 (10)	9.27±0.49*** (6)	8175±114 (2)
26	7287	40	27, 7, 1.2% ginkgolide B	3	Capsule	28.98±0.71*** (12)	9.46±0.13*** (6)	1055±88 (2)
27	8E01516	60	24, 6	3	Caplet	29.82±0.84*** (12)	7.48±2.20 (12)	3192±63 (2)

Data represent means±s.d. [1]In addition to 60 mg choline, 1 mg vitamin B_6 and 3 μg vitamin B_{12}. [2]In addition to 300 mg ginkgo leaves. [3]In addition to 375 mg ginkgo leaves. [4]400 mg ginkgo leaves. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with specified ranges set by Commission E (22–27% flavone glycosides, 5–7% terpene lactones (2.8–3.4% ginkgolides A, B and C and 2.6–3.2% bilobalide thereof).

contain *Ginkgo biloba* extracts standardised to 24% flavone glycosides or 24% flavone glycosides and 6% terpene lactones with reference to the German Commission E monograph. The recommendations dosages of these products were in accordance with the proposals of Commission E (120–240 mg extract). Two other products (nos 13 and 26) were standardised to 27% flavone glycosides and 7% terpene lactones, which were at the upper limits of the Commission E monograph, and three products (nos 10, 14 and 16) contained the original extract EGb 761 as described in the Commission E monograph. In contrast, one product (no. 21) contained *Ginkgo biloba* leaves, two other products contained extract and *Ginkgo* leaves in one capsule (nos 11 and 17) and one formulation contained *Ginkgo* extract together with some vitamins (no. 2). In brief, the composition of the investigated products differed from each other as labelled.

Figures 1–3 show typical fingerprint chromatograms of different products. It is obvious that the products are not comparable. Lipophilic properties increase from the left to the right side in the chromatogram. Thus, hydrophilic components like organic acids and flavone glycosides (0–40 min) are eluted first, while biflavones (40–80 min) and ginkgolic acids (80–90 min) are detected later.

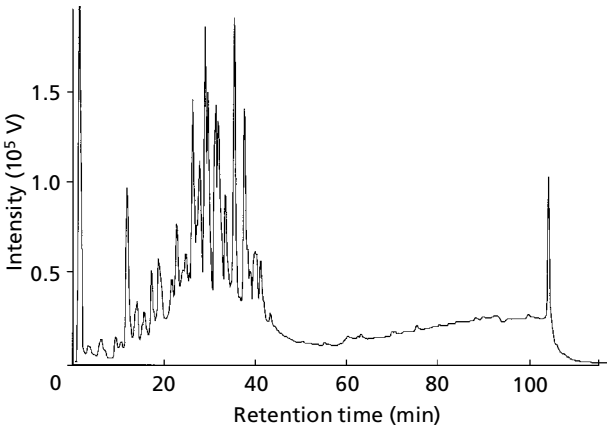

Figure 1 Fingerprint chromatogram of product no. 10 (see Table 1) containing EGb 761 extract. Gradient elution: A (H_2O–acetonitrile–H_3PO_4) and B (acetonitrile–H_3PO_4); flow: 1.2 mL min^{-1}; UV: 211 nm; column: RP-18.

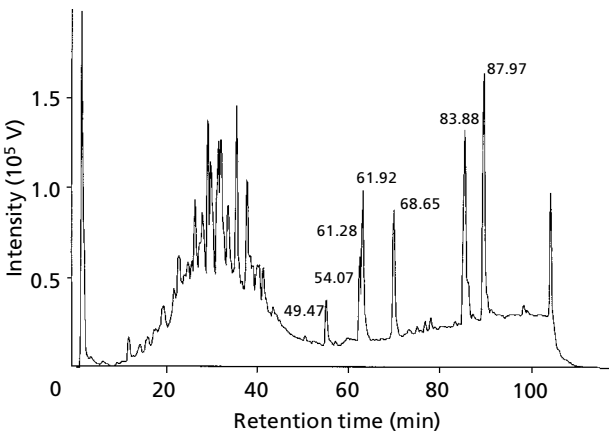

Figure 2 Fingerprint chromatogram of product no. 20 (see Table 1) containing extract. Gradient elution: A (H_2O–acetonitrile–H_3PO_4) and B (acetonitrile–H_3PO_4); flow: 1.2 mL min^{-1}; UV: 211 nm; column: RP-18.

Figure 1 shows a typical chromatogram of EGb 761 extract; its content of bioflavones and ginkgolic acids is very low. Moreover, it is conspicuous that in the hydrophilic area a relatively large peak ($\sim$ 12 min) is present, which is much smaller in the other extracts.

Figure 2 shows an unspecified extract of one US product. According to the different parameters of extraction, the amount of hydrophilic and lipophilic substances are very different in comparison with EGb 761 (Figure 1).

Figure 3 displays a special product containing *Ginkgo biloba* leaves exclusively. The ratio of hydrophilic-to-lipophilic substances is shifted more to the lipophilic components.

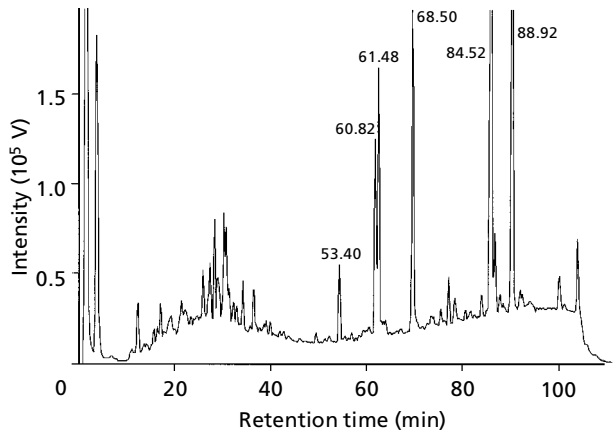

Figure 3 Fingerprint chromatogram of product no. 21 (see Table 1) containing leaves only. Gradient elution: A (H_2O–acetonitrile–H_3PO_4) and B (acetonitrile–H_3PO_4); flow: 1.2 mL min^{-1}; UV: 211 nm; column: RP-18.

In brief, as seen in the fingerprint chromatograms, the spectrum of lipophilic and hydrophilic substances in the extracts and the quantity of substances show great differences.

Results from the analysis of total flavone glycosides, total terpene lactones and ginkgolic acids are shown in Table 1. The content of terpene lactones specified as ginkgolides A, B and C and bilobalide is given in Table 2. The concentrations of the flavone glycosides and terpene lactones in the three products containing the original EGb 761 extract (nos 10, 14 and 16) are in accordance with the Commission E specification. Moreover, the content of ginkgolic acids was below the limit of quantification ($<$ 500 ppm). In contrast, findings for 17 other products with regard to flavone glycosides and terpene lactones were statistically above the specified range of 24–27% or 5–7%, respectively, as specified by the Commission E. The ginkgolide content was above the specified limit (2.8–3.4%) as well, whereas for bilobalide the concentration was below the limits of 2.6–3.2% in most products (Table 2).

The highest values were found in product no. 20 in comparison with the contents of the other investigated products. The concentrations of flavone glycosides and terpene lactones in this extract were 35.54% and 11.31%, respectively, both statistically outside of the recommended range. In this case, a very high concentration of ginkgolic acids (89576 ppm) was determined as well.

The content of ginkgolic acids was measured using a slightly modified Ph Eur (European Pharmacopoeia) method. The limit of quantitation (LLQ) was

Table 2 Content (percent in extract) of terpene lactones specified as ginkgolides A, B and C and bilobalide in *Ginkgo*-containing remedies.

Product	Ginkgolide A (%)	Ginkgolide B (%)	Ginkgolide C (%)	Total ginkgolides	Bilobalide (%)	n
1	2.63±0.46	1.07±0.25	1.40±0.23	5.10±0.88***	1.83±0.31***	18
2	1.79±0.09	0.63±0.04	1.54±0.05	3.97±0.13***	2.57±0.12	6
3	2.98±0.10	1.70±0.07	1.98±0.07	6.65±0.16***	3.77±0.16***	6
4	1.01±0.33	0.75±0.10	0.81±0.27	2.57±0.65	1.31±0.45***	6
5	2.62±0.10	1.65±0.63	1.55±0.21	5.81±0.85***	2.03±0.14***	12
6	2.95±0.15	1.20±0.31	1.85±0.08	6.00±0.26***	4.56±0.16***	12
7	2.39±0.65	1.51±0.81	1.64±0.44	5.54±1.76**	3.20±0.91	12
8	2.42±0.42	1.55±0.60	1.73±0.30	5.70±1.21***	3.20±0.59	12
9	2.65±0.17	1.50±0.55	1.44±0.10	5.59±0.72***	2.23±0.16***	12
10	1.37±0.04	0.49±0.11	1.27±0.04	3.13±0.12	2.78±0.09	18
11	2.12±0.37	0.79±0.15	1.31±0.22	4.22±0.69**	2.69±0.48	12
12	2.64±0.04	1.14±0.06	1.23±0.02	5.00±0.09***	1.61±0.04***	6
13	3.16±0.06	1.60±0.13	2.35±0.07	7.11±0.16***	3.67±0.08***	6
14	1.46±0.06	0.54±0.07	1.14±0.06	3.14±0.16	2.75±0.15	6
15	3.33±0.04	1.61±0.11	2.00±0.03	6.94±0.13***	1.59±0.04***	6
16	1.31±0.02	0.45±0.06	1.12±0.03	2.88±0.08	2.62±0.06	6
17	3.38±0.07	1.51±0.12	1.40±0.04	6.28±0.17***	1.55±0.05***	6
18	2.26±0.24	0.98±0.10	1.53±0.09	4.77±0.38***	1.84±0.15***	12
19	2.37±0.14	1.04±0.06	1.09±0.02	4.50±0.20***	0.80±0.02***	6
20	3.82±0.04	1.83±0.15	2.31±0.08	7.96±0.21***	3.35±0.04***	6
21	Not determinable[1]	Not determinable	Not determinable	Not determinable	Not determinable	–
22	3.66±0.79	2.00±0.37	0.82±0.13	6.48±1.17***	1.17±0.25***	12
23	3.50±0.18	1.33±0.22	1.51±0.05	6.35±0.42***	1.39±0.03***	6
24	3.09±0.18	1.39±0.08	1.12±0.03	5.60±0.28***	4.00±0.22***	6
25	3.62±0.17	1.75±0.13	2.08±0.12	7.45±0.39***	1.81±0.10***	6
26	3.28±0.04	1.87±0.04	1.16±0.02	6.31±0.08***	3.15±0.06	6
27	2.11±0.68	1.42±0.61	1.45±0.37	4.98±1.44**	2.50±0.81	12

Data represent means±s.d. [1]For preparation reasons. Levels of significance $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with specified ranges set by Commission E (22–27% flavone glycosides, 5–7% terpene lactones (2.8–3.4% ginkgolides A, B and C and 2.6–3.2% bilobalide thereof).

~ 500 ppm. Despite this limitation, a quantification of ginkgolic acids was possible in most products, since the content of those allergenic and cytotoxic compounds was in the range of 500–90 000 ppm and thus far above the LLQ.

In brief, regarding the specifications set from Commission E, the investigated products were not comparable.

The dissolution rates of terpene lactones and flavone glycosides within the single products were approximately comparable (Table 3). Thus, terpene lactones and flavone glycosides were released from the different brands and dissolved at a similar rate in all cases. Furthermore, most of the brands released more than 75% of both components within 30 min (Figure 4). Only 3 of the 14 products investigated exhibited clear deviations from this dissolution behaviour (> 75% in 30 min) (Table 3). Accordingly, products no. 8, 17 and 4a (where a indicates the same product, but a different batch no.), dissolved only to about 70, 70 and 10%, respectively, within 30 min. Dissolution profiles of product no. 24a, which underwent a rapid dissolution in comparison with products no. 4a and 17, are presented in Figure 4.

In brief, the pharmaceutical quality of the investigated products is easily not comparable and in terms of in-vitro dissolution profiles there are serious differences in a few.

Discussion

The market for herbal remedies in the USA is growing continuously. This led the US government to create a new legal definition for herbs, vitamins and minerals in the Dietary Supplement Health and Education Act of 1994. Accordingly, the consumers' interest in dietary supplements and the growing sales rates should ideally

Table 3 Dissolution rates by means of terpene lactones specified as ginkgolides A, B and C and bilobalide in *Ginkgo biloba*-containing remedies available on the US market.

Product	Time (min)	Ginkgolide A (%)	Ginkgolide B (%)	Ginkgolide C (%)	Bilobalide (%)	n
2	15	90.62±4.19	83.73±8.55	95.45±3.53	98.27±1.42	6
	30	93.35±3.92	78.00±10.26	97.20±1.95	100.47±4.23	
	60	93.79±2.58	94.31±2.58	93.60±2.74	103.29±1.16	
3	15	83.40±2.22	79.10±4.76	81.67±7.61	86.85±1.95	6
	30	91.50±3.86	89.96±5.76	91.78±1.60	93.84±1.46	
	60	96.54±3.46	95.53±3.47	91.17±4.18	98.33±2.76	
5	15	85.68±8.29	69.38±7.58	87.99±7.55	89.40±8.49	12
	30	96.95±3.24	81.74±2.93	97.47±3.57	98.81±2.62	
	60	97.19±2.16	93.20±6.55	95.52±2.78	98.32±2.73	
7	15	103.56±3.70	93.15±5.24	100.25±2.02	101.35±3.51	6
	30	102.87±1.49	90.53±7.07	98.28±2.42	100.60±1.43	
	60	99.22±1.06	94.26±5.52	100.37±2.30	97.14±1.59	
8	15	23.14±2.82	17.01±4.12	23.40±1.38	33.26±3.09	12
	30	69.45±8.51	66.86±10.73	71.46±8.95	78.59±8.58	
	60	95.05±3.21	92.06±3.95	94.62±4.31	96.36±3.00	
19	15	88.88±2.03	90.71±6.79	83.19±3.10	88.03±6.60	6
	30	97.72±4.13	97.35±4.15	89.67±2.30	95.69±5.85	
	60	98.44±3.22	100.41±8.26	93.97±3.54	97.51±11.64	
11	15	87.86±5.97	81.14±5.38	97.13±5.75	95.95±4.35	6
	30	92.42±9.88	93.17±13.17	98.78±5.11	102.77±7.64	
	60	87.14±4.15	87.69±5.87	95.60±3.26	97.64±0.57	
14a	15	93.67±2.64	94.56±1.56	96.58±2.94	95.20±3.46	6
	30	99.85±1.75	98.25±1.10	97.34±1.23	100.94±1.98	
	60	99.14±2.10	98.37±1.96	98.54±1.54	99.65±2.65	
12	15	61.10±23.61	50.33±21.78	65.37±23.28	62.69±17.35	12
	30	81.52±12.32	72.07±10.99	83.60±12.78	86.26±10.72	
	60	95.83±2.31	89.04±5.07	96.76±5.77	98.57±3.53	
15a	15	90.64±4.88	90.82±6.25	94.33±6.21	96.20±5.65	12
	30	98.81±5.57	99.93±6.32	101.27±5.27	102.19±5.61	
	60	100.93±5.10	105.80±5.47	103.84±4.07	102.16±4.05	
20	15	89.71±2.22	81.67±2.68	95.82±2.33	90.92±5.00	6
	30	96.25±5.02	96.46±5.33	100.65±5.39	99.49±4.89	
	60	96.44±3.41	94.32±8.33	90.28±4.18	95.61±2.05	
17	15	31.01±2.02	28.28±1.82	33.45±3.09	34.36±2.59	6
	30	66.14±1.93	62.88±2.43	70.81±2.37	73.47±1.15	
	60	85.99±1.75	83.00±4.98	90.68±2.89	96.10±3.86	
24a	15	100.70±1.43	95.08±5.77	105.70±2.05	101.37±3.49	6
	30	105.44±4.44	100.98±3.87	104.56±2.75	104.48±3.99	
	60	101.15±2.54	101.29±6.81	103.38±4.71	98.80±3.24	
4a	15	1.82±1.01	< Limit of	4.03±2.03	5.00±1.49	6
	30	7.09±1.32	quantification	7.81±1.41	10.08±1.42	
	60	10.12±1.83		14.48±1.64	19.26±1.95	

Data represent means±s.d.

be reflected by adequate quality standards by the manufacturers. This was our background to investigate the pharmaceutical quality of several US *Ginkgo biloba* brands, which are among the top sellers within this segment of the dietary supplement market.

Our investigation of *Ginkgo biloba* preparations obtained in health-food stores or supermarkets in the USA showed that many of the products were similar in terms of content of the active ingredients, flavone glycosides and terpene lactones. There were, however, relevant differences between a few products particularly with regard to the content of ginkgolic acids.

Most manufacturers of *Ginkgo* preparations refer directly or indirectly to the standards published by the German Commission E, partly in line with FTC (Federal Trade Commission) regulations. The use of *Ginkgo* for

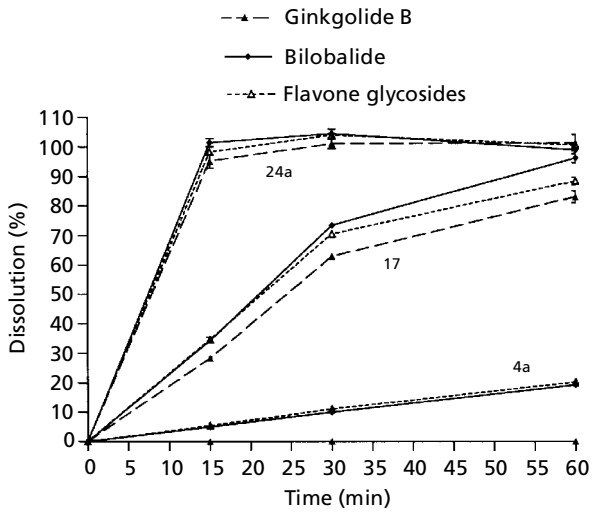

Figure 4 Comparison of dissolution profiles of three different products by mean curves of ginkgolide B, bilobalide and flavone glycosides. The lower array of curves belongs to product no. 4a, the middle to product no. 17 and the upper to no. 24a (see Table 3). Tests were conducted in 900 mL of 0.1 M HCl dissolution medium at 37°C ± 0.5°C with a rotation speed of 100 rev min^{-1}.

the treatment of peripheral and cerebral circulatory disorders is restricted to a clear specification of the extract. Accordingly, the extract should contain 22–27% flavone glycosides, 5–7% terpene lactones (2.8–3.4% ginkgolides A, B and C and 2.6–3.2% bilobalide thereof) and not more than 5 ppm ginkgolic acids.

The majority of the products were not in accordance with the above-mentioned specifications. The concentrations of flavone glycosides, terpene lactones and ginkgolic acids were above the specification whereas the contents of bilobalide were too low. The considerably high amounts of ginkgolic acids found should not be tolerated for safety reasons. The values determined for terpene lactones in *Ginkgo biloba*-containing herbal products on the US market are in accordance with a previously published study (Lang & Wai 1999).

Medicinal products are considered to be pharmaceutically equivalent if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards (Committee for Proprietary Medicinal Products: CPMP/EWP/QWP/1401/98). The concept of pharmaceutical equivalence is the object of USP 24 (United States Pharmacopeia) too and defined by the CPMP.

Most of the investigated products show clear differences in contents of active ingredients and for this reason they did not fulfill this criterion.

Pharmaceutical equivalence does not necessarily imply bioequivalence, as differences in the excipients or the manufacturing process (or both) can lead to faster or slower dissolution or absorption. This aspect is not considered by the standards published by the Commission E of the German authorities.

When comparing the dissolution rates of the *Ginkgo biloba*-containing remedies, most showed a rapid dissolution. However, a few of the products differed from the rest considerably. Differences in dissolution rates influence most likely the bioavailability of the active ingredients and thus efficacy in humans.

Therefore, the characterisation of the pharmaceutical quality of a medicinal product should further include the biopharmaceutical quality. Solid oral dosage forms intended for systemic action should be studied in suitable in-vitro and in-vivo investigations. Such information is usually not available for herbal medicinal products, due to their complex composition. The question as to whether in-vivo studies are necessary with respect to biopharmaceutical characterisation of herbal medicinal products is still under discussion (Schulz et al 1995; Blume & Schug 2000).

The question for clinical relevance of biopharmaceutical characteristics with respect to the question of interchangeability of different products in the case of synthetic drugs is the object of the Note for Guidance on the investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/98). This states that a medicinal product is essentially similar to an original product where it satisfies the criteria of having the same qualitative and quantitative composition in terms of active substances, of having the same pharmaceutical form, and of being bioequivalent unless it is apparent in the light of scientific knowledge that it differs from the original products as regards safety and efficacy. An essentially similar product can be used instead of its innovator product. Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same. By extension, it is generally considered that for immediate-release products the concept of essential similarity also applies to different oral forms (tablets and capsules) with the same active substance. In terms of interchangeability of different products the principles of essential similarity should be transferred from chemically defined drugs to herbal medicinal products as far as possible.

Our investigation of some *Ginkgo biloba* brands shows relevant differences in the content of active ingredients and dissolution rates. This would be expected to result

in different bioavailabilities of active ingredients in-vivo and thus different efficacy in humans. Therefore, an in-vivo bioequivalence study of some of the herbal medicinal products will be the subject of a further investigation.

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