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Non-biased investigation of standardized *Ginkgo biloba* preparations by ^{1}H NMR-Based Metabolomics and HPLC-PDA-MS-SPE-NMR

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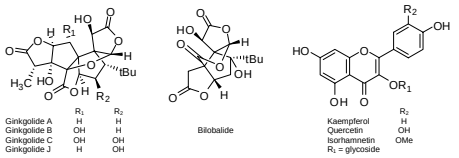
BACKGROUND

Extracts of *Ginkgo biloba* leaves are among the best selling herbal preparations worldwide and are used for improvement of the peripheral and central blood circulation, improvement of memory, symptomatic treatment of dementia, Alzheimer's disease as well as for their antioxidant and neuro-protective activities.

Commercial preparations are very complex mixtures prepared from raw leaf extract by a series of extraction and prepurification steps. During this process the extracts are enriched in some compounds while other compounds are removed.

The final *Ginkgo biloba* extract (EGb 761) are standardized to contain 24% flavonoids and 6% terpene trilactones (TTL's; 3.1% ginkgolides and 2.9% bilobalide) which are considered the active constituents. However the current quality control does not assure the same distribution of individual TTL's and flavonoid glycosides from batch-to-batch or brand-to-brand and other constituents that may possess pharmacological activity are not included in the standardization.



ACTIVE CONSTITUENTS OF GINKGO BILOBA

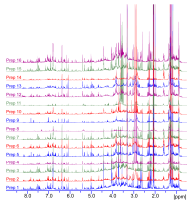
In the current work, ^{1}H NMR-based metabolomics was used for non-biased investigation of the global composition of 16 commercially-available *Ginkgo biloba* preparations. In addition, hyphenation of HPLC, PDA, MS, and NMR with an automated solid-phase extraction (SPE) device, i.e., HPLC-PDA-MS-SPE-NMR, was used for identification of eight major flavonoid glycosides.

METHODOLOGY

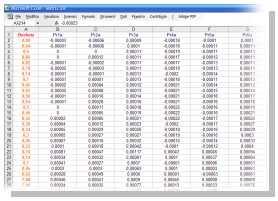
Samples of all tablets and capsules were prepared in triplicate by extraction of pulverized material with 70% methanol. The dry residue was dissolved in acetone-d_6, and ^{1}H NMR spectra of all extracts were acquired at a resonance frequency of 600 MHz. All ^{1}H-NMR spectra were reduced to 850 sequencially integrated spectral regions (buckets) of 0.01 ppm width from 0.5 to 9 ppm using Bruker AMIX software. PCA analysis were performed using SIMCA-P software on centered data.

SAMPLE PREPARATION

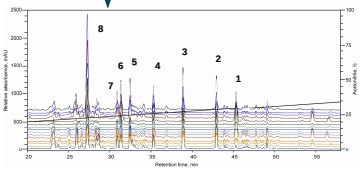
NMR ANALYSIS



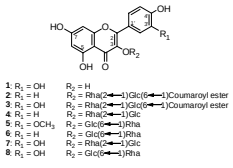
DATA PREPROCESSING



DATASET FOR PCA ANALYSIS



HPLC ANALYSIS



STRUCTURE IDENTIFICATION

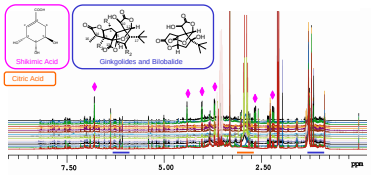
HPLC-PDA-MS-SPE-NMR was used for the isolation of 8 selected flavonoids. Peaks were trapped 8 times on a SPE cartridges using threshold-based absorbance at 254 nm for selection of analytes.

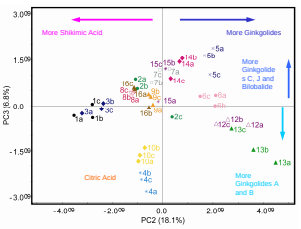
Lack of commercially available reference substances for flavonol glycosides is a well know problem in the analysis of flavonoids.

HPLC-PDA-MS-SPE-NMR has constituted a fast and reliable method for the identification of the major flavonoids in *Ginkgo biloba* extracts.

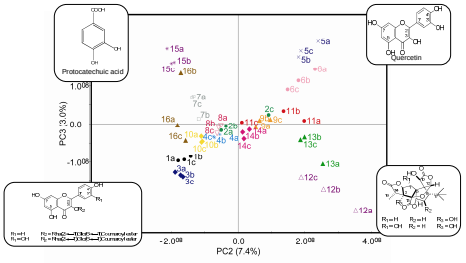
RESULTS AND DISCUSSION

PCA analysis was performed on the entire spectrum. The preparations results separated based on their relative content of shikimic acid and ginkgolides. Discrimination between relative content of single TTL's was possible. Sample 4 and 10 cluster together for the presence of citric acid as excipient.

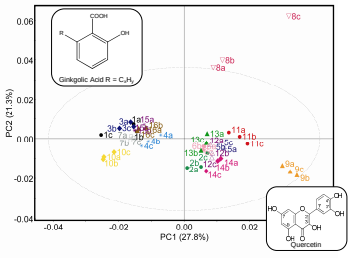




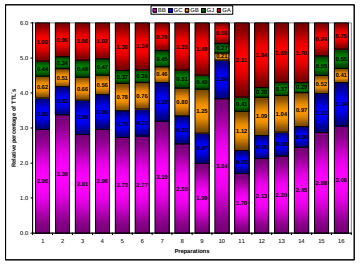
Citric acid constitute a frequently used excipient in pharmaceutical preparations. However, as demonstrated in a recent publication (Larsen et al., 2009), citric acid can form potentially harmful reaction products with constituents of pharmaceutical preparations and its addition in complex extract like *Ginkgo biloba* extracts should therefore preferably be avoided.

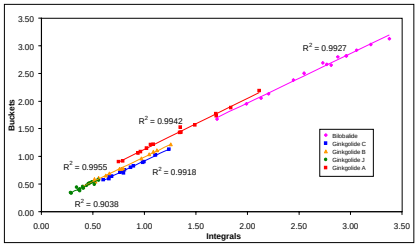


A separated model was performed in the aromatic region of the spectra and further characterization of the samples based on the relative amount of TTLs and flavonoids was possible.



The same dataset but scaled on the total intensity, enabled the identification of ginkgolic acid in sample 8 and observation of quercetin as almost the only flavonoid in sample 9, clear symptom of fortification.





A measure of the relative amount of the single TTLs (normalized to sum of 6%) can be easily obtained from the buckets and results equivalent to the manual integration of the peaks. The relative amount of TTL's confirm the results of PCA analysis.

CONCLUSIONS

^{1}H-NMR based metabolomics is able to discriminate between the various preparations according to their global composition. The analysis disclosed differences in the content of flavonol glycosides and terpene trilactones, as well differences in other constituents normally not considered in the standardization procedure. ^{1}H-NMR based metabolomics constitute an attractive method for global characterization of standardized *Ginkgo biloba* preparations.