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Determination of the Flavonoids from Ginkgo Biloba Extract by High Performance Liquid Chromatography

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Abstract: HPLC method for analysis of the flavonoids from ginkgo biloba extract (GBE) was studied. By suitable selection of columns, symmetrical chromatographic peaks were obtained without using acidic modifier in the mobile phase, which can eliminate the time for cleaning the chromatographic system and simplify the analystic method for GBE. Experimental conditions: column: Hypersil BDS C_{18}, 5μm×4×250 mm; column temperature: 35°C; mobile phase: 46% methanol-54% water; flow rate: 0.7 mL/min; detection wavelength: 360nm.

Keywords: Ginkgo biloba extract, flavone glycoside, high performance liquid chromatography.

Today Ginkgo biloba extract (GBE) is one of the most widely used food supplements and herbal medicines. The amounts of flavone glycosides, one of the key active components in GBE, vary according to the source of the ginkgo leaves and the extraction and enrichment procedures used to prepare the extract. A typical GBE contains from 22% to 27% of flavone glycosides.

Ginkgo flavone glycosides are a group of small complex molecules that can be hydrolyzed to give kaempferol, quercetin and isorhamnetin. It is difficult to analyze flavone glycosides in GBE directly. HPLC method for indirect determination of total flavone glycosides has been widely used, namely after hydrolyzing GBE, analyzing the hydrolyzed products kaempferol, quercetin and isorhamnetin, then converting the result to total flavone glycosides. Spectrometry can also be employed in inexact assay of total flavonoids in GBE when rutin is used as standard[1]. Many efforts have been made to analyze flavonoid glycosides in ginkgo biloba by HPLC methods[1,2]. Reversed phase columns were employed in all these works; a mobile phase containing phosphoric acid was used in most cases, acetic acid was used in only one experiment with poor resolution. Because flavonoid glycosides and their hydrolyzed products are polar and slightly acidic polyphenol compounds, will cause tailing peaks on a traditional C_{18} column, adding acidic modifier in a mobile phase in such RP-HPLC system can often improve peak shapes. But acidic eluent may shorten the column life, and the chromatographic system needs cleaning after finishing the analysis. In this study we modified the HPLC method for analysis of ginkgo flavonglycosides by special selection of certain columns, and obtained symmetrical chromatographic peaks and good resolution without using acidic modifier in the mobile phase.

Reagents and Samples

Methanol，hydrochloric acid and phosphoric acid, AR, (Guangzhou Chemicals Factory); Ginkgo Biloba extract, (Hunan Hongshengtang Natural Product Factory); quercetin, kaempferol and isorhamnetin standards (China National Institute for the Control of Pharmaceutical and Biological Products). Pure water, distilled water prepared through a Millipore ultra clear system (Millipore, USA).

Sample preparation

Sample solution: weigh exactly 0.1 g of ginkgo biloba extract was weighed exactly in a boiling flask, it was dissolve in 20 mL methanol, added 20 mL of 1.5 mol/L HCl solution, the mixture was refluxed for 120 min, then cool down. Transfer the solution into a 50 mL volumetric flask and fill methanol was added till the mark. Filter the solution through a 0.45 μm membrane filter for chromatographic analysis. Injection volume: 20 μL. Standard solution: weigh exactly about 10 mg of quercetin, kaempferol and isorhamnetin standards were weighed exactly in a volumetric flask, and dissolve in 10 mL methanol. Injection volume: 20 μL.

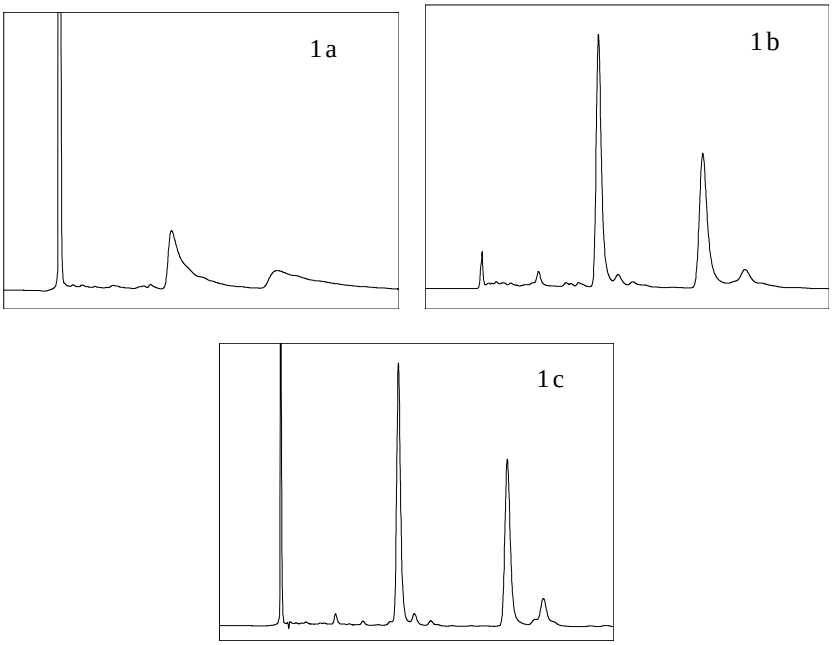
Chromatographic conditions

Instrument: HP1100 high performance liquid chromatograph (Hewlett Packard, USA) with a diode array detector; detection wavelength =360 nm, slit width =16 nm, reference wavelength = 600 nm，slit width =100 nm；columns: 1) Hypersil BDS C_{18}, Φ 4.0×250 mm, 5 μm; 2) YWG C_{18}, Φ4.0 × 300 mm, 10 μm; Hypersil ODS2 C_{18}, Φ 4.0 × 250 mm, 5 μm, (Dalian Elite Analytical Instruments Co., Ltd, China); 3) Shim-pack C_{18}, Φ6×150 mm, Shimazhu; 4) Zorbax 300 SB-C_{18}, Φ4.6×150 mm, 5 μ m, Hewlett-Packard；column temperature ：35°C；Mobile phase as illustrated in **Figure 1**; flow rate: 0.7 mL/min.

Results and Discussion

In Zhuang's report[2] a Zorbax ODS column and a mobile phase with acetic acid were employed, and a distinct tailing peak of quercetin was found in its chromatogram. Other studies showed good resolutions and symmetrical peaks when C_{18} column, for example Spheri-5 rp-18[1], and mobile phase with phosphoric acid were used. In our work, it was found that the peaks were severely tailing and could not be integrated when a YWG C_{18} column and a mobile phase without acid were employed (see **Figure 1a**); when phosphoric acid was added the peaks appeared normal (**Figure 1b**). The same result was found when a Shim-pack C_{18} column was used. It was changed when some specially selected C_{18} columns (so called base deactivated, endcapped or ultra pure silica) were used. Without adding of phosphoric acid in the eluent we obtained symmetrical peaks on Hypersil BDS C_{18}（**Figure 1c**）, Zorbax 300 SB-C_{18}, and ODS2 C_{18}. If the analysis time was within 25 minutes, the best column efficiencies were gotten on a BDS C_{18} and a Zorbax 300 SB-C_{18} columns. Good resolution was achieved in about 25 minutes on a Hypersil ODS2 C_{18} column, but the column efficiency reduced if the elution time was reduced by increasing methanol concentration in the eluent.

Figure 1 Chromatograms of the flavonoids from Ginkgo Biloba



t_R (quercetin) = 10.5-12.3 min, t_R (kaempferol) = 17.1-18.3 min, t_R (isorhamnetin) =19.7-20.5min); stationary and mobile phases: (a) YWG C_{18}, 36% methanol-64% water; (b)YWG C_{18}, 36% methanol-64% water-0.5% H_3PO_4; (c) Hypersil BDS C_{18}, 36% methanol-64 % water.

Although Zorbax 300 SB-C_{18} is a short column (15 cm), on it the same resolution as on BDS C_{18} could be obtained only when the analysis time was as long as on a 25 cm column. This means the short column does not show the merit of short analysis time for assaying such compounds. Hypersil BDS C_{18} was chosen for later work because of its lower price, its nearly same analysis time and resolution compared with Zorbax 300 SB-C_{18}. In **Figure 1c** some peaks were overlapped with some small peaks. By reducing the intensity of the eluent and increasing separation time till 45 minutes we could completely separate these overlapped peaks. In a GBE sample these impurity peaks are about 3% of those large peaks. If it is tolerant to neglect these peaks, condition illustrated in **Figure 1** can be applied and the analysis may be completed in 25 minutes.

References

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