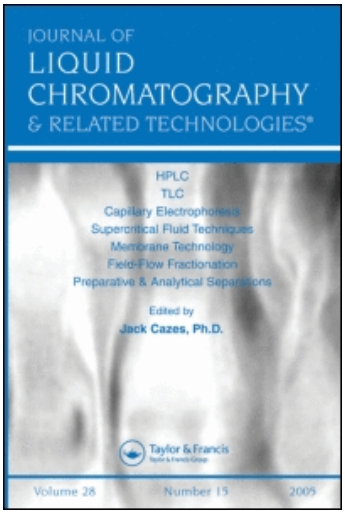

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COUNTERCURRENT CHROMATOGRAPHY FOR ISOLATION OF FLAVONOL GLYCOSIDES FROM GINKGO BILOBA LEAVES

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ABSTRACT

A procedure which combined countercurrent chromatography with gradient elution and preparative high-performance liquid chromatography was developed for the isolation and the purification of the seven predominant flavonol glycosides from *Ginkgo biloba* leaves .

INTRODUCTION

The therapeutical properties of *Ginkgo biloba* leaves have been lately reviewed; flavonoids as well as terpenic compounds were regarded as the active constituents (1). The flavonol glycosides composition of the plant has been recently revised and completed (2-4); however, the described procedures used for flavonol isolation from plant material are tedious and require multiple chromatographic steps on silica gel, polyamide and Sephadex columns (2-4).

The present paper reports a simplified and rapid method for flavonoids isolation which combined countercurrent

chromatography (CCC) and preparative high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Apparatus.

CCC was performed using an horizontal flow-through planet centrifuge (5,6) with an Ito Multilayer coil (7)(P.C. Inc.,Potomac,MD,USA)equipped with a 2.6mm I.D. column (length:66m ;capacity:350ml). The solvents were pumped through the column with an apparatus from Millipore-Waters (Bedford,Mass.,USA) equipped with two pumps (Model 6000A) and a solvent programmer (Model 660). The rotational speed was 800 rpm. A manual sample injection valve (Lobar column accessories,Merck,Darmstadt,West-Germany) equipped with a 10ml loop was used to introduce the samples into the column.

The continuous monitoring of the eluent was achieved with a Shimadzu spectrophotometer UV 120-02 (Kyoto,Japan)operating at 340nm in a 1mm flow-through cell, whereas the fractions (17ml) were collected with a LKB Ultrorac 7000 fraction collector (Stockolm, Sweden).

The HPLC system was equipped with a pump (Model 6000A from Millipore-Waters), a sample loop (Model U6K from Millipore -Waters), a Hibar Pre-packed column RT 250-10 (Lichrosorb RP-18,7µ,250mm x 10mm I.D. stainless steel column,Merck) and a high-speed spectrophotometric detector (Model 1040 M from Hewlett-Packard,Avondale,PA,USA). A 6ml/min flow-rate was applied; sample loading corresponded to 150µl injections of a 5% solution of the pre-purified flavonoids dissolved in the mobile phases (mixtures of water-acetonitrile-acetic acid in various ratio).

Reagents.

All chemicals were analytical grade.

CCC Separation Procedures.

The solvents systems were prepared by equilibrating, in a separating funnel, equal volumes of water (Phase A) and ethyl acetate (Phase B), and , equal volumes of water and of an ethyl acetate - 2-butanol (6:4 v/v) mixture (Phase C). After equilibration and decantation, the phases were degassed in an ultrasonic bath.

Phase A (350ml) was pumped into the column at 7ml/min. The sample solution was introduced through the injection port. As rotation began, phases B and C (total volume:2liters) were pumped into the column at 4.5ml/min following a gradient curve (fig.1). The separations were performed at room temperature. The stationary phase retention was 61%.

Preparation of Samples for CCC.

A commercialized extract of *Ginkgo biloba* leaves (500mg) was dissolved in 11ml of an (1:1 v/v) mixture of Phases A and B; after filtration, 10ml of this solution were loaded on the column.

Fractionation Monitoring.

The composition of the fractions was determined by TLC on RP-18F_{254}s plates (Merck) using a mixture of water - acetonitrile -acetic acid (70:30:1 v/v) as mobile phase (two developments).

RESULTS AND DISCUSSION

Figure 1 shows the separation of the major flavonol glycosides from a commercialized extract of *Ginkgo biloba* leaves; the use of such an extract allowed to avoid the preliminary purifications steps necessary with a crude plant material.

The solvents were chosen on the basis of partition coefficients of the flavonoids in the liquid phases. Partition

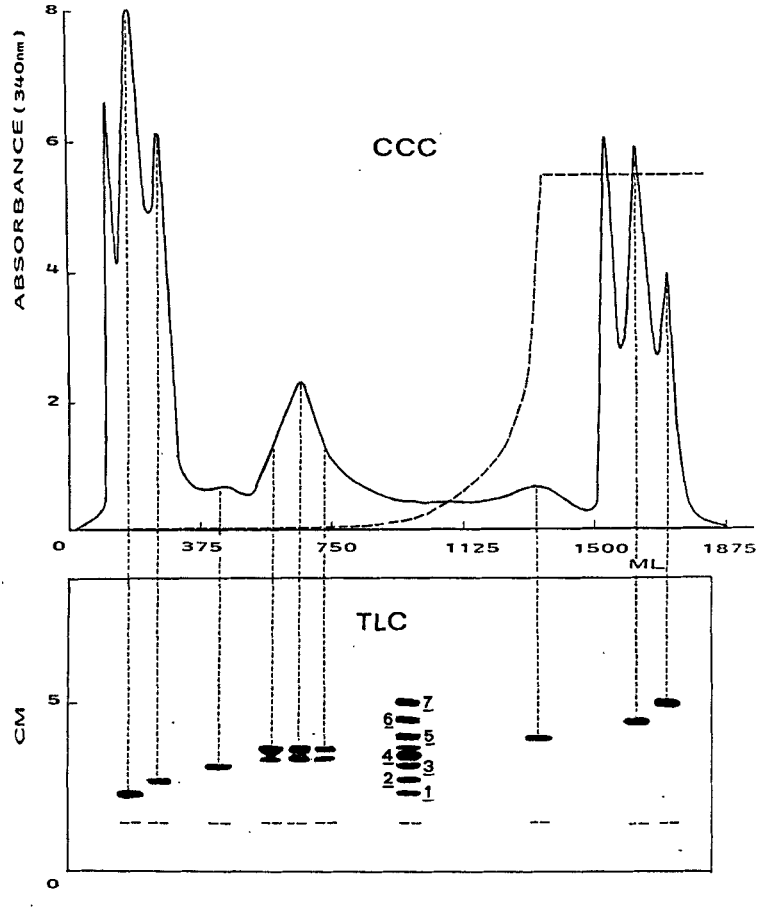

Figure 1. Preparative CCC of a commercialized extract of *Ginkgo biloba* leaves.

Sample: 500mg dissolved in 10ml of an 1:1 v/v mixture of Phases A and B.
Separation conditions: Stationary phase: H_2O (Phase A); starting solvent system: EtOAc; ending solvents system: EtOAc - 2-butanol (6:4 v/v): gradient curve: -----; flow rate :4.5ml/min. Column: 2.6mm I.D.; 350ml capacity. Detection at 340nm.
Monitoring of the fractions: TLC on silica gel RP-18 $F_{254}s$ with H_2O:MeCN:HOAc (70:30:1 v/v;2 developments). UV detection at 254nm
E: total extract; Compounds 1 to 7: see Results and Discussion.

coefficients were determined using TLC on RP-18 silica gel. Owing to the large differences of polarities showed by the flavonoids (a very complex mixture of mono-, di- and tri-glycosides), the use of only one solvents system was not adequate to achieve the separation of all compounds. Therefore, a procedure using a gradient elution of the column was developed. It involves water as stationary phase and two solvents systems: ethyl acetate for the less polar flavonoids (partition coefficients between 0.75 and 1.00) and ethyl acetate containing increasing amounts of 2-butanol for the more polar compounds (partition coefficients between 0.80 and 1.00). Such a gradient elution procedure was only once reported for dipeptides separation (7). The selected conditions allowed the separation of seven major flavonoids within 7h. However, compounds 3 and 4 were not completely resolved. Other less hydrophilic constituents from the extract of *Ginkgo biloba* such as biologically active terpenic constituents (1) were eluted in the first fractions obtained by CCC. A restriction connected to the outlet of the column was essential to avoid bubbling in the cell of the UV detector, an excessive elution of the stationary phase and the consecutive rebalancing of the system during the run.

The direct monitoring of the eluent afforded only indicative results and a manual spectrophotometric lecture of each fraction performed after complete separation of the phases was essential to obtain interpretable elution profiles.

The flavonoids fractions isolated by CCC were further submitted to HPLC using a preparative RP-18 column and mixtures of water, acetonitrile and acetic acid as solvents systems; their structures elucidation was achieved without any other purification step by comparison of the spectroscopic data (2).

Four of the purified flavonol glycosides, kaempferol 3-O-(6'''-p-coumaroylglucosyl-β-1,4-rhamnoside), 1 (yield:2.5%),

quercetin 3-O-(6'''-p-coumaroylglucosyl-β-1,4-rhamnoside),2 (yield:2.1%), isorhamnetol 3-O-rutinoside,4 (yield:2,0%), quercetin 3-O-rutinoside,5 (yield:3.0%) were previously identified in *Ginkgo biloba* leaves (2-4); the three others, kaempferol 3-O-β-glucoside,3 (yield:1.8%), kaempferol 3-O-[α-rhamnosyl-(1→2)-α-rhamnosyl-(1→6)]-β-glucoside,6 (yield: 1.2%), quercetin 3-O-[α-rhamnosyl-(1→2)-α-rhamnosyl-(1→6)]-β-glucoside,7 (yield:0.8%) were newly identified in the plant (8,9) (yields calcd from 500mg of the commercialized extract)

The above results clearly indicate the advantages of the proposed method in the case of complex mixtures of flavonol glycosides: high recovery of the constituents, especially in the case of p-coumaroyl esters which are readily hydrolysable, high resolution and purification obtained within two short steps when compared to more traditional chromatographic methods.

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REFERENCES

1. Special issue on "Extract of *Ginkgo biloba* (EGb761)", La Presse Médicale, 15, n°31,1441-1604, 1986.
2. Nasr,C.G. "Eude chimique de substances originales du *Ginkgo biloba* L.(Ginkgoacées); dérivés flavoniques (hétérosides - esters) et quinoléines carboxyliques; essais pharmacologiques préliminaires.", Thèse de Doctorat, Laboratoire de Pharmacognosie de l'Université Louis Pasteur, Strasbourg, 1987.
3. Nasr,C., Haag-Berrurier,M. ,Lobstein-Guth,A. and Anton R. "Kaempferol coumaroyl glucorhamnoside from *Ginkgo biloba*." Phytochem., 25, 770, 1986.
4. Nasr,C., Lobstein-Guth,A. ,Haag-Berrurier,M. and Anton R. "Quercetin coumaroyl glucorhamnoside from *Ginkgo biloba*." Phytochem., 26, 2869, 1987.

5. Ito,Y. "A new horizontal flow-through coil planet centrifuge for countercurrent chromatography. I.Principle of design and analysis of acceleration." J.Chromatogr., 188, 33, 1980.

6. Ito,Y. "A new horizontal flow-through coil planet centrifuge for countercurrent chromatography. II. The apparatus and its partition capabilities." J.Chromatogr., 188, 43, 1980.

7. Ito,Y. ,Sandlin,J. and Bowers,W.G. "High-speed preparative countercurrent chromatography (CCC) with a coil planet centrifuge." J.Chromatogr., 244, 247, 1982.

8. Vanhaelen,M. and Vanhaelen-Fastré,R. "Flavonol triglycosides from *Ginkgo biloba*." Pl.Med., in press.

9. Vanhaelen,M. and Vanhaelen-Fastré,R. "Kaempferol 3-O-β-glucoside (astragalin) from *Ginkgo biloba*." Fitoterapia , in press.