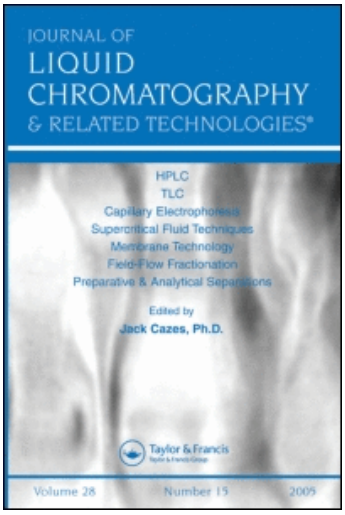

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Adsorption Separation for the Extracts from *Ginkgo biloba* Leaves Using Intermediate Polarity Resins

Shih-Ming Lai[a]; Rui-Long Chen[a]; Shing-Yi Suen[b]
[a] Department of Chemical Engineering, National Yunlin University of Science and Technology, Touliu, Yunlin, Taiwan, R.O.C. [b] Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan, R.O.C.

Online publication date: 29 September 2003

To cite this Article Lai, Shih-Ming , Chen, Rui-Long and Suen, Shing-Yi(2003) 'Adsorption Separation for the Extracts from *Ginkgo biloba* Leaves Using Intermediate Polarity Resins', Journal of Liquid Chromatography & Related Technologies, 26: 17, 2941 — 2960
To link to this Article: DOI: 10.1081/JLC-120025055
URL: http://dx.doi.org/10.1081/JLC-120025055

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 17, pp. 2941–2960, 2003

Adsorption Separation for the Extracts from *Ginkgo biloba* Leaves Using Intermediate Polarity Resins

Shih-Ming Lai,[1,*] Rui-Long Chen,[1] and Shing-Yi Suen[2]

[1]Department of Chemical Engineering, National Yunlin University of Science and Technology, Touliu, Yunlin, Taiwan, R.O.C.
[2]Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan, R.O.C.

ABSTRACT

An adsorption separation method using intermediate polar resins (Amberlite XAD-7HP) was applied for concentrating the active ingredients from *Ginkgo biloba* leaves. First, the crude *G. biloba* L. extracts were prepared using a Soxhlet extractor operated under an optimal extraction condition (using 160 mL of 70% ethanol aqueous solution for 3 h per 10 g dry leaves). The extraction yield was 25–30% and the purity was 2.5–3.0 wt.% for flavonoid glycosides and 0.5–1.0 wt.% for terpene lactones. Before the column operation, the extracts were dissolved in 10% ethanol and used as the feed solution, which led to a 1.5–2.0 fold concentration and a 10–20% loss for both active ingredients. Then, the

*Correspondence: Shih-Ming Lai, Department of Chemical Engineering, National Yunlin University of Science and Technology, Touliu, Yunlin 640, Taiwan, R.O.C.; E-mail: laism@yuntech.edu.tw.

DOI: 10.1081/JLC-120025055
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

column was operated under four steps, i.e., adsorption, washing, elution, and regeneration, in sequence. The results show that using the eluent with a high ethanol concentration ($\geq 70\%$) in the isocratic elution mode gave good column performance. Both flavonoid glycosides and terpene lactones were nearly completely eluted, their purities all met the standardized contents of *G. biloba* L. extracts (24 wt.% for flavonoid glycosides and 6 wt.% for terpene lactones), and their yields were nearly 100%. Finally, for a simulated two-step elution mode (first step: 30% and second step: 70% ethanol), the product purity can be further improved, but at the expense of the product yield.

Key Words: *Ginkgo biloba* L. extracts; Flavonoid glycosides; Terpene lactones; Adsorption separation; Intermediate polar resins; Column performance.

INTRODUCTION

Extracts from *Ginkgo biloba* leaves (GBE) have been widely utilized as a medicine or a nutrient food for the treatment of some diseases, especially related to cerebrovascular and peripheral circulatory insufficiency of the elderly. The most important active ingredients of the extracts are flavonoid glycosides and terpene lactones.[1–7] Flavonoid glycosides are thought to have many useful biological activities such as dilating coronary vessels, improving peripheral and brain blood circulation, and preventing intravascular thrombogenesis.[4] On the other hand, terpene lactones are potent and selective platelet-activating-factor antagonists and are considered to be responsible for the medicinal properties of GBE.[4,6,7]

Ginkgo biloba L. extracts are usually standardized in terms of flavonoid glycoside (24 wt.%) and terpene lactone (6 wt.%) contents.[2] Current procedures for isolation and purification of GBE include liquid–liquid extraction, precipitation by alkalinization or adding ammonium solution, and adsorption separation.[4,6,8–10] A few limitations and problems for these methods, however, have been observed and discussed in the literature.[6,9–11] For example, in the liquid–liquid extraction method, large quantities of variable organic solvents are consumed, phase separation is required, and a trace of undesirable solvents may be retained in the final products. In the precipitation method by means of alkalinization using metal hydroxides, the remaining metals would cause troubles. Compared to the above methods, the adsorption separation method may be advantageous because of its simple process and low cost. The adsorbents adopted or mentioned in the literature include polyamide,[4] synthetic MA(methacrylate)-DVB(divinylbenzene) copolymer beads,[9] and macroporous resins such as Duolite S-761 and Diaion HP-20,[12,13]with either

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

polar or hydrophobic adsorption characteristics. In spite of the numerous patents and publications concerning isolation and purification for GBE by adsorption separation processes, there still exists a need for processes which are efficient, relatively inexpensive, and easy to scale-up into industrialized usage.

Regarding the functional structures of the active ingredients, flavonoid glycosides belong to natural phenolic derivatives and terpene lactones are cage-like molecules full of oxygen.[9] Intermediate polar resins are believed to have a good adsorption selectivity for both of them. In this study, Amberlite XAD-7HP, possessing intermediate polarity, was employed for the adsorption separation process for GBE because of their high performance and durability in several similar applications reported by the manufacturer.[14] Prior to the adsorption process, the crude *G. biloba* L. extracts were prepared using ethanol aqueous solutions. The column operation included the adsorption, washing, elution, and regeneration steps in sequence. In the adsorption step, 10% ethanol was used as the feed solvent. In the elution step, different eluent compositions were attempted in the isocratic elution mode and their effects on the column performance (in terms of purity, concentration ratio, and yield for flavonoid glycosides and terpene lactones in the purified extracts) were investigated. Finally, the possibility of further improvement of the column performance by a two-step elution mode was also investigated.

EXPERIMENTAL

Materials

Dry green *G. biloba* L. leaves were obtained from Mainland China. Flavonoid aglycone and terpene lactones standards were purchased from commercial sources: quercetin dihydrate from ACROS (Geel, Belgium), isorhamnetin (90% purity) from Extrasynthese (France), kaempferol (90% purity), Ginkgolide A and B (GA and GB, 90% purity), and bilobalide (BB, 95% purity) from Sigma (St. Louis, MO).

Purified water was obtained using a Milli-Q purifier (Millipore, USA). Hydrochloric acid (HCl), methanol, acetonitrile, and tetra-hydrofuran (THF) were bought from TEDIA (Fairfield, OH). Ethanol was from SCI (Kyungki-do, Japan) and ortho-phosphoric acid (H_3PO_4, 85% purity) was from Riedel-de haen (Seelze, Germany). All the organic solvents used were of HPLC grade and were ultrasonically degassed before use.

Amberlite XAD-7HP polymeric adsorbent was from Rohm and Haas Company (Philadelphia, PA), with the particle size of 0.3–1.2 mm (90% within). Due to its non-ionic aliphatic acrylic structure, this adsorbent possesses

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

intermediate polarity, which induces the adsorptivity of non-polar compounds from aqueous systems and polar compounds from non-polar solvents.[14]

Preparation of the Crude *Ginkgo biloba* L. Extracts

Prior to use, dry *G. biloba* L. leaves were further dried in an oven at 60°C for 5 h and then pulverized. A batch of 10 g amount of dried and pulverized *G. biloba* L. leaves (put in a fiber cup) was refluxed with a certain amount and concentration of ethanol aqueous solution (put in a 250 mL round-bottomed flask) using a Soxhlet extractor (Cole-Parmer, USA) for a certain time. The effects of different extraction conditions, e.g., the amount and concentration of ethanol aqueous solution, and extraction time, have been investigated using the response surface methodology and reported in a previous study by the authors.[15] The optimal extraction conditions were using 160 mL of 70% ethanol aqueous solution for 3 h, which were adopted in this study. After cooling, the extract solution was filtrated through a 0.45 μm nylon membrane (Critical Inc., USA) and the filtrate (Sample A) was concentrated using a rotary evaporator (Eyela, Japan), and the concentrated extracts were weighed.

The concentrated extracts were then dissolved in a certain amount of 10% ethanol aqueous solution. Some water-insoluble lipophilic components were precipitated. The resulting solution was centrifuged at 12,000 rpm for 15 min and then filtrated using a 0.45 μm disposable syringe PVDF filter (Millipore Miller-HV, Ireland). The filtrate (Sample B) will be used as the feed solution for adsorption separation using column chromatography.

The amount and weight fraction of flavonoid glycosides and terpene lactones in the concentrates of Sample A and Sample B were analyzed by HPLC in the next section. The loss and concentration ratio of flavonoid glycosides and terpene lactones of the extracts from Sample A (70% ethanol aqueous solution) to Sample B (10% ethanol aqueous solution) were calculated.

Quantitative Analysis of the Active Ingredients

HPLC System

The high performance liquid chromatography (HPLC) system included a Jasco Model PU-980 solvent metering pump, a Jasco Model UV-970 UV detector (Tokyo, Japan), a Showa Denko Model Shodex RI-71 RI detector (Tokyo, Japan), a Rheodyne Model 7125 6-way syringe loading valve fitted with a 20 μL sample loop (Cotati, CA), and a Sunway Model 940-CO column oven (Taipei, Taiwan). The millivolt signal from the detector was converted to a digital form with the aid of an analog-to-digital interface card (Scientific

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Information Service Corp., Taipei, Taiwan) interfaced with a microcomputer for data storage and processing. The HPLC column was Sphereclone ODS (2), 250 L × 4.6 mm I.D., 5 μm (Phenomenex, Torrance, CA). All analyses were carried out at 30°C.

Quantitative Analysis of Flavonoid Glycosides

Although the total amount of flavonoid glycosides is not easy to estimate, due to their great variety and the difficulty to get the pure standards, they can be reduced to three major aglycones, i.e., isohamnetin, kaempferol, and quercetin, by hydrolysis.[1,16] According to the analysis conducted by Hasler et al.,[1] the aglycone content could be correlated to the flavonoid glycoside content. In this study, hydrolysis of flavonoid glycosides in *G. biloba* L. extracts was carried out using 25% (v/v) HCl. The volume ratio of *G. biloba* L. extract solution (dissolved in methanol) to 25% HCl was 3 : 1. A complete hydrolysis is required for an accurate evaluation of the aglycone content and, hence, a precise determination of the flavonoid glycoside content. The effects of different hydrolysis conditions (e.g., hydrolysis temperature and time) have been investigated in a previous study by the authors[17] and the optimal conditions were found to be 70°C and 1 h, which were adopted in this study.

The hydrolyzed solution was then filtrated using a 0.45 μm disposable syringe PVDF filter (Millipore Miller-HV, Ireland) and the filtrate was analyzed by the reversed-phase HPLC. The isocratic mobile phase was THF–methanol–0.5% H_3PO_4 (5 : 5 : 12, v/v/v). The flow rate was 1.0 mL/min and the peaks were monitored with a UV detector under 370 nm. Figure 1(a) shows the corresponding chromatogram of one particular example, where the peaks for three aglycones were clearly separated. The whole chromatogram is similar to that obtained by Hasler et al.,[1] i.e., kaempferol and quercetin are the main peaks and the concentration of isohamnetin is approximately five times lower. This isocratic mobile phase was proved workable and accordingly adopted in the rest of this work.

When each aglycone content in the chromatogram of the hydrolyzed extract solution was determined using the calibration curves for aglycone standards, the total flavonoid glycoside content could be evaluated using the following formula:[1]

$$\text{Total amount of flavonoid glycosides} = \sum(\text{amount of each aglycone}) \times 2.51 \quad (1)$$

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

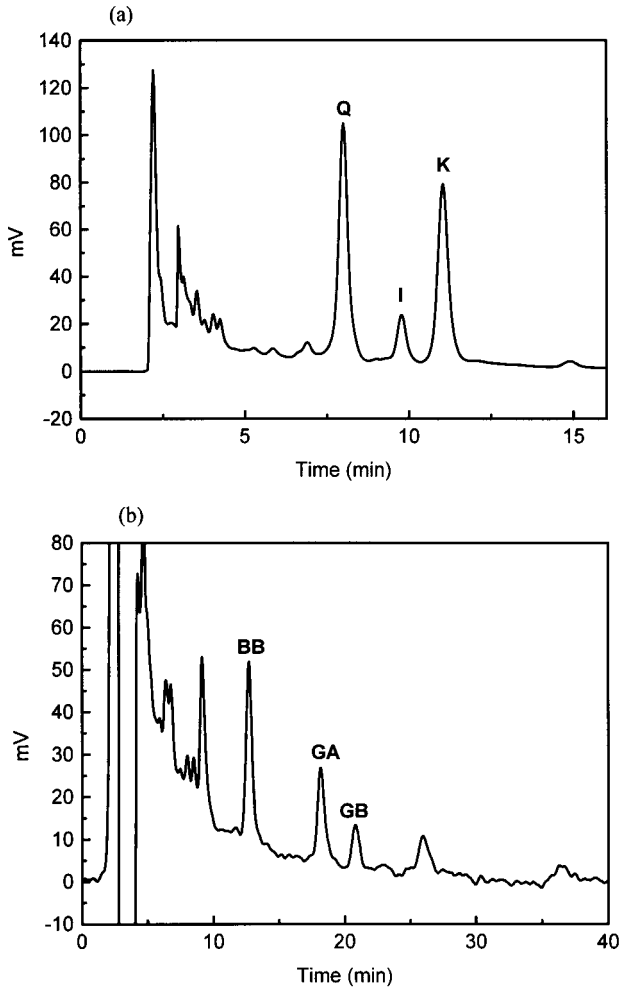

Figure 1. Typical HPLC analysis results of the crude *G. biloba* L. extracts. (a) Flavonoid glycosides (after hydrolysis: injection volume 20 μL, injection concentration 1.28 mg/mL); Q, quercetin, I, isohamnetin, K, kaempferol. (b) Terpene lactones (direct analysis: injection volume 20 μL, injection concentration 250 mg/mL); BB, bilobalide; GA, Ginkgolide A; GB, Ginkgolide B.

The averaged conversion factor for the three major aglycones, i.e., 2.51, is determined with the molecular weight of flavonol coumaroyl ester glycosides (MW = 756.7).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Quantitative Analysis of Terpene Lactones

The pharmacologically active components of terpene lactones present in *G. biloba* L. include the diterpenes, Ginkgolide A, B, C, and J (GA, GB, GC, and GJ), and the sesquiterpene, bilobalide (BB). In this study, because there are no commercial GC and GJ standards available for calibration, only the contents of GA, GB, and BB were used as the quantitative indices of terpene lactones.

To analyze the terpene lactones in the crude *G. biloba* L. extracts, the extracted solution (dissolved in methanol) was directly analyzed by the reversed-phase HPLC. The isocratic mobile phase was H_2O–acetonitrile–THF (10 : 2 : 1, v/v/v). The flow rate was 1.0 mL/min and the peaks were monitored with the RI detector. Figure 1(b) shows the corresponding chromatogram of one particular example, where a successful separation of BB, GA, and GB was obtained. The result is similar to that obtained with the RP-C_{18} column by van Beek et al.[7] This isocratic mobile phase was also proved workable and, accordingly, adopted in the rest of this work.

Ginkgolide A, GB, and BB were then identified and quantified by direct comparison with reference standards. Multiplying BB, GA, and GB concentrations by the total extracted solution volume, the contents of BB, GA, and GB and the total terpene lactone content (the value was under-estimated since only the contents of BB, GA, and GB were evaluated) in the crude extract solution were determined.

Adsorption Separation Using Column Chromatography

Medium Pressure Liquid Chromatograph System

The medium pressure liquid chromatograph (MPLC) system included a Lab Alliance Model Series II medium pressure pump (Lemont, PA), a Jasco Model UV-970 UV detector (Tokyo, Japan), a ISCO Model 2360 gradient programmer, and a ISCO Model Retriever® 500 fraction collector. The millivolt signal from the detector was also converted to a digital form. A 7.5 mL of the XAD-7HP resin beads were dry packed in a glass column, sizing 1.5 cm I.D. and 7.1 cm length. The column was located in a water bath, where the temperature was controlled at 30°C.

As intermediate polar adsorbents, Amberlite XAD-7HP resins can adsorb flavonoid glycosides, moderately polar molecules,[9] and terpene lactones, less polar molecules,[9] from aqueous solutions. Based on the "like attracts like" principle,[18] a feed solution at a low ethanol concentration (e.g., $\leq 10\%$) in the adsorption step is preferred; on the contrary, an eluent at a high ethanol concentration (e.g., $\geq 50\%$) in the desorption step is favorable.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Adsorption Separation Process

A whole adsorption separation process included four steps in sequence: adsorption, washing, elution, and regeneration steps in sequence. First, the column was conditioned with 10% ethanol solution and the feed solution was loaded onto the column. At the end of adsorption, the column was washed with de-ionized water in order to remove the impurities remaining in the pore of the adsorbents and the packing spacing. Then, the packed column was washed with de-ionized water and eluted isocratically with the ethanol aqueous solution of a certain composition, e.g., 30%, 50%, 70%, or 100% ethanol solution. Finally, the column was regenerated with 10 bed-volumes of the ethanol aqueous solution varying step-gradiently from 30% to 100%.

Evaluation of the Column Performance

The effluent for each step in the adsorption separation process was collected for compositional analysis. Each effluent was concentrated and each concentrate was weighed. The column performance indices, including purity, concentration ratio, and yield of flavonoid glycosides and terpene lactones, are defined as follows:

$$\text{Purity} = \frac{\text{weight fraction of the active ingredient content in the concentrated elution effluent fraction}}{\text{purity in the concentrated elution effluent fraction}} \tag{2}$$

$$\text{Concentration ratio} = \frac{\text{purity in the concentrated elution effluent fraction}}{\text{purity in the concentrated feed solution, Sample B}} \tag{3}$$

$$\text{Yield} = \frac{\text{weight of the active ingredient content in the concentrated elution effluent fraction}}{\text{sum of the weights of the active ingredient content in the all effluent fractions of the adsorption separation process}} \tag{4}$$

The column performance was evaluated for each different elution composition, e.g., 30%, 50%, 70%, and 100% ethanol solution.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

RESULTS AND DISCUSSION

Preparation of the Crude *Ginkgo biloba* L. Extracts

Averaging the results (Sample A) of five batches of 10 g of *G. biloba* leaves, the weight of the concentrated extracts ranged from 2.5 to 3.0 g and the weight of flavonoid glycosides and terpene lactones ranged from 70 to 80 mg and 20 to 25 mg, respectively. The yield of the crude extracts (weight of the concentrated extracts/weight of dry leaves used) ranged from 25% to 30% (w/w). The weight fraction (or purity) of flavonoid glycosides and terpene lactones in the concentrated extracts ranged from 2.5% to 3.0% (w/w) and 0.5% to 1.0% (w/w), respectively. These results are close to the values reported in the literature.[4] It should be noted that the same extraction conditions may not lead to identical results in each batch. The difference may be attributed to many factors such as non-uniform flavonoid glycoside and terpene lactone contents distributed in different leaves, or probable loss at each stage (extraction, filtration, concentration, or hydrolysis) during the whole process.

The feed solution (Sample B) for adsorption separation process by column chromatography was prepared by collecting the crude extracts from six batches of 10 g dry leaves, concentrating, and then dissolving in 100 mL of 10% ethanol solution. The concentrates of Sample A and Sample B were analyzed and the results are shown in Table 1. The weight fractions (or purities) in the concentrates of Sample A and Sample B were found to be 2.5% and 4.9%, respectively, for flavonoid glycosides and 0.77% and 1.30%, respectively, for terpene lactones. The concentration ratio (purity in the concentrate of Sample B/purity in the concentrate of Sample A) was found to be 1.96 for flavonoid glycosides and 1.69 for terpene lactones. The loss of the extracts from Sample A (70% ethanol aqueous solution) to Sample B (10% ethanol aqueous solution) was found to be 9.5% for flavonoid glycosides and 22.3% for terpene lactones. Finally, the concentrations of flavonoid glycosides and terpene lactones of the feed solution (Sample B) were calculated to be 4.55 and 1.20 mg/mL, respectively.

Adsorption Separation Using Column Chromatography

Dynamic Adsorption Performance

A dynamic adsorption experiment was carried out on the packed glass column (1.5 cm I.D. and 7.1 cm L) at a flow rate 1.0 mL/min. Sample B was used as the feed solution. The effluent fractions of the adsorption step were

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Quantitative analysis results for the crude extracted solution (Sample A) and the feed solution (Sample B).

	Sample A		Sample B[a]
Concentrate			
Averaged weight (g/batch)	2.91		1.34
Flavonoid glycosides			
Averaged weight (mg/batch)	72.7		65.8
Loss% from Sample A to Sample B		9.5	
Wt.% (Purity)	2.50		4.91
Concentration ratio from Sample A to Sample B		1.96	
Terpene lactones			
Averaged weight (mg/batch)	22.4		17.4
Loss% from Sample A to Sample B		22.3	
Wt.% (Purity)	0.77		1.30
Concentration ratio from Sample A to Sample B		1.69	

[a]The feed solution was prepared by dissolving six batches of the crude extracts of 10 g leaves in 100 mL of 10% ethanol. Final volume of the feed solution was about 87 mL (14.5 mL/batch).

collected in several intervals, e.g., 5–10 mL for each interval, for compositional analysis. The dynamic adsorption curves of flavonoid glycosides and terpene lactones are shown in Fig. 2. It can be seen from the comparison of these two curves, that the break point of flavonoid glycosides (~25 mL) took place earlier than that of terpene lactones (~75 mL). To avoid the loss of flavonoid glycosides and terpene lactones from the adsorption stage, 25 mL of the feed solution was taken as the loading amount for the following adsorption processes.

It is worth noting, that the equilibrium points of flavonoid glycosides and terpene lactones were about 200 mL, the mass transfer zones (from the break point to the equilibrium point) were very wide, and the shape of dynamic adsorption curves became an elongated S-form. According to the theory of fixed-bed adsorption,[19] the wide mass transfer zones were formed due to the significant mass transfer resistances from the XAD-7HP resins (0.3–1.2 mm diameter). To achieve a narrower mass transfer zone, a smaller particle size should be used. Therefore, the shape of the dynamic adsorption curves can be steeper, and, hence, a larger loading amount can be allowed. However, a higher-pressure drop will be needed for a column packed with particles of smaller sizes.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

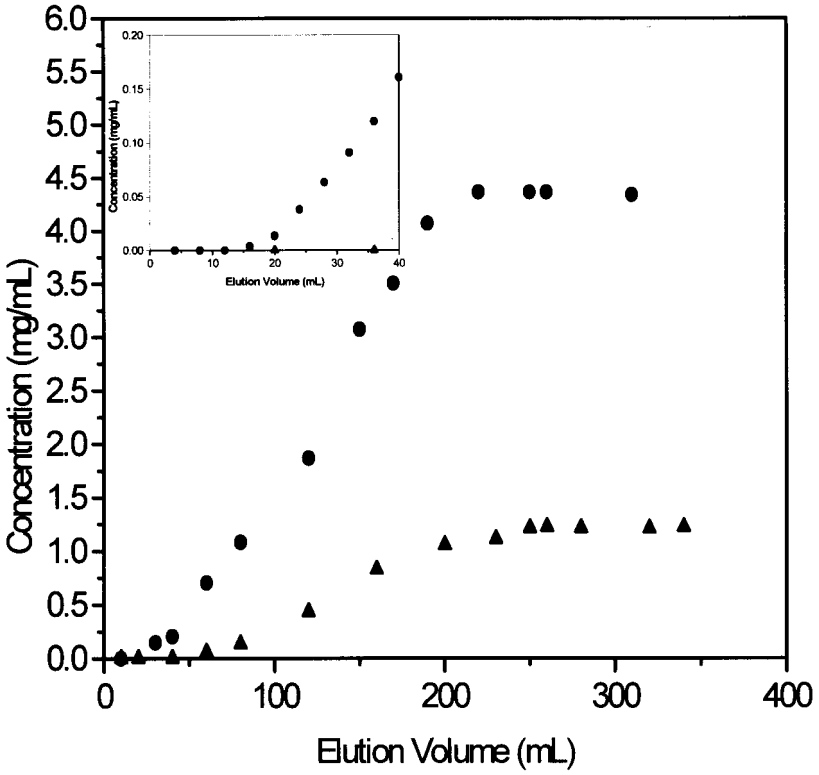

Figure 2. The dynamic adsorption curves for (●) flavonoid glycosides and (▲) terpene lactones. Column (XAD-7HP): I.D. = 1.5 cm, L = 7.1 cm; Feed solvent: 10% ethanol; Feed concentrations of flavonoid glycosides and terpene lactones: 4.55 and 1.20 mg/mL, respectively; Flow rate: 1 mL/min. Insert: the enlarged figure for the first 40 mL elution.

Dynamic Elution Performance

A dynamic elution experiment was carried out isocratically with a higher percentage ethanol aqueous solution on the same column at a flow rate 1.0 mL/min, following the adsorption step of 25 mL feed solution and the washing step of 50 mL de-ionized water. Four different elution systems (eluent compositions for systems A, B, C, and D = 30%, 50%, 70%, and 100% ethanol, respectively) were tested. The effluent fractions of the elution step were collected in several intervals, e.g., 30 mL for each interval, for compositional analysis. Their dynamic elution curves of flavonoid glycosides and

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

terpene lactones are shown in Fig. 3. The results show that, for both flavonoid glycosides and terpene lactones, their elution peaks became narrower as the content of ethanol in the eluent increased, i.e., they could be efficiently eluted with a smaller amount of eluent with high percentage ethanol. The

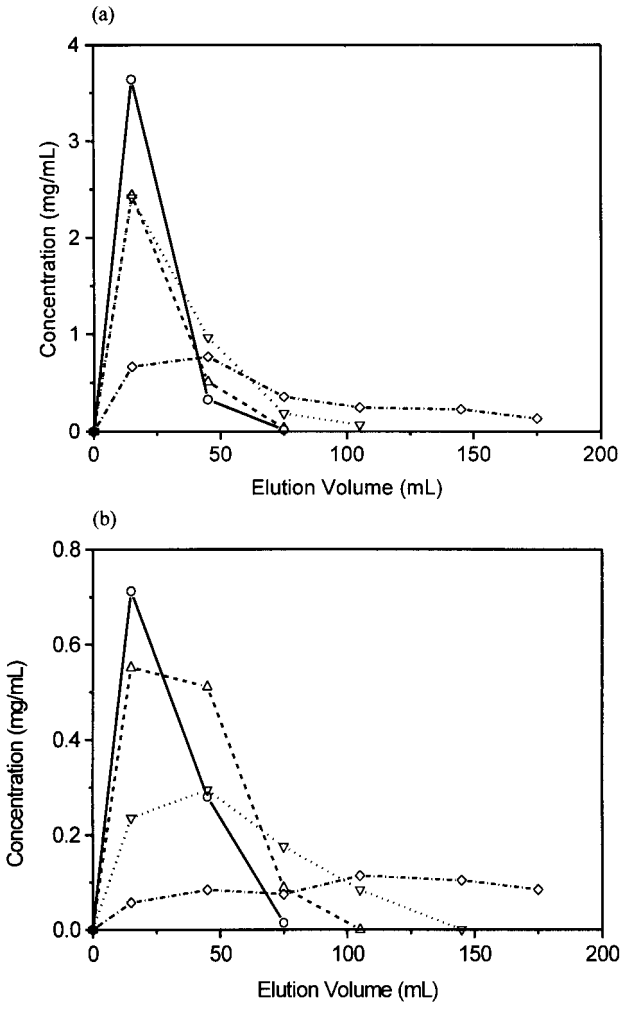

Figure 3. The dynamic elution curves for (a) flavonoid glycosides and (b) terpene lactones. Column (XAD-7HP): same as Fig. 2; Feed solution: 25 mL 10% ethanol; Elution solution: 30% ethanol (---◇---), 50% ethanol (····∇····), 70% ethanol (----Δ----), 100% ethanol (—○—); Flow rate: 1 mL/min.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

elution volumes for 30%, 50%, 70%, and 100% ethanol were about 250 mL (System A), 150 mL (System B), 100 mL (System C), and 75 mL (System D), respectively.

Effect of the Eluent Composition on the Column Performance

The column performances among the four different elution systems (eluent compositions for systems A, B, C, and D = 30%, 50%, 70%, and 100% ethanol, respectively) were compared, as presented in Tables 2 and 3. Each experiment was repeated twice. The difference between these two trials was the way of fractional collection in the elution step. The effluent fractions of the elution step were collected in several intervals for the first trial (Trial 1), but collected in the entire interval for the second trial (Trial 2).

Table 2 shows the mass weight of concentrate, the mass weight and weight fraction of flavonoid glycosides and terpene lactones in each of the effluent fractions of three stages (adsorption together with washing, elution, and regeneration) of these two trials. The column performance in terms of purity, concentration ratio, and yield of flavonoid glycosides and terpene lactones, evaluated by Eqs. (2–4), for each different elution system, is presented and compared in Table 3. The results from these two different trials were close, although some data points were not perfectly matched. Since the tendencies were similar in both trials, the column separation results were considered reproducible.

It can be seen from Table 2 that, in the adsorption-washing step, both flavonoid glycosides and terpene lactones were fully adsorbed when 25 mL feed solution was loaded. In the elution step, both flavonoid glycosides and terpene lactones were nearly completely eluted for elution systems C and D, but only partially eluted for elution systems A and B. The retained species in the elution step were then recovered in the regeneration step. For the three effluent fractions collected, the sum of concentrates weighed around 2400 mg, the sum of flavonoid glycosides weighed around 110 mg, and the sum of terpene lactones weighed around 30 mg. The collected amounts of the concentrate, flavonoid glycosides, and terpene lactones were very close to their loading amounts in the 25 mL feed solution. The regeneration step was proven efficient.

In Table 3, for elution systems C and D, the purity of each active ingredient content could all meet the standardized contents of *G. biloba* L. extracts, i.e., 24 wt.% of flavonoid glycosides and 6 wt.% of terpene lactones.[2,9] In addition, since nearly 80% of the total weight of concentrates was removed without any loss of flavonoid glycosides and terpene lactones in the adsorption-washing and regeneration steps, the active ingredient contents were then efficiently concentrated more than 5.0 times (concentration ratio >5.0) in the elution step. In comparison, for elution systems A and B,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 2. Quantitative analysis results for the effluent fractions collected from the column operation under different elution conditions.

Elution system	Trial number	Step of column operation	Weight of concentrate (mg)	Weight of flavonoid glycosides (mg)	Weight of terpene lactones (mg)
A (30% ethanol solution)	1	Adsorption (25 mL)-washing (50 mL)	1,981	0	0
		Elution			
		0–30 mL	148	19.88	1.71
		30–60 mL	112	22.91	2.52
		60–90 mL	59	10.59	2.24
		90–120 mL	43	7.31	3.41
		120–150 mL	29	6.73	3.12
		150–250 mL	23	3.92	2.54
		Total	414 (Product)	71.34	15.54
		Regeneration (180 mL)	103	47.32	13.00
	2	Adsorption (25 mL)-washing (50 mL)	1,850	0	0
		Elution (250 mL)	376	73.55	18.92
		Regeneration (180 mL)	154	33.46	9.65
B (50% ethanol solution)	1	Adsorption (25 mL)-washing (50 mL)	1,943	0	0
		Elution			
		0–30 mL	321	72.44	7.07
		30–60 mL	111	28.89	8.84
		60–90 mL	34	5.47	5.26
		90–150 mL	23	2.01	2.52
		Total	489 (Product)	108.81	23.69
		Regeneration (180 mL)	38	1.95	2.53

	2	Adsorption (25 mL)-washing (50 mL)	1,923	0	0
		Elution (150 mL)	439	108.80	24.68
		Regeneration (180 mL)	107	2.40	2.54
C (70% ethanol solution)	1	Adsorption (25 mL)-washing (50 mL)	1,929	0	0
		Elution			
		0–30 mL	381	73.16	16.56
		30–60 mL	61	25.21	15.35
		60–100 mL	8	10.07	2.65
		Total	450 (Product)	108.44	34.56
		Regeneration (180 mL)	0	0	0
	2	Adsorption (25 mL)-washing (50 mL)	1,956	0	0
		Elution (100 mL)	414	113.60	26.80
		Regeneration (180 mL)	0	0	0
D (100% ethanol solution)	1	Adsorption (25 mL)-washing (50 mL)	1,967	0	0
		Elution			
		0–30 mL	393	109.17	21.39
		30–60 mL	82	9.75	8.41
		60–75 mL	0	0.44	0.43
		Total	475 (Product)	119.36	30.23
		Regeneration (180 mL)	0	0	0
	2	Adsorption (25 mL)-washing (50 mL)	1,992	0	0
		Elution (75 mL)	440	113.80	32.20
		Regeneration (180 mL)	0	0	0

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. Column performances of the four different elution systems.

Elution system	Trial number	Flavonoid glycosides			Terpene lactones		
		Purity (%)	Concentration ratio[a]	Yield (%)	Purity (%)	Concentration ratio[a]	Yield (%)
A (30% ethanol solution)	1	17.23	3.51	60.12	3.75	2.88	54.45
	2	19.56	3.98	68.73	5.03	3.87	66.22
B (50% ethanol solution)	1	22.25	4.53	98.24	4.84	3.72	90.35
	2	24.78	5.05	97.84	5.62	4.32	90.67
C (70% ethanol solution)	1	24.10	4.91	~100	7.68	5.91	~100
	2	27.44	5.59	~100	6.47	4.98	~100
D (100% ethanol solution)	1	25.13	5.12	~100	6.36	4.89	~100
	2	25.86	5.27	~100	7.32	5.63	~100

[a]The purity of the concentrated feed solution (Sample B) from Table 1 was adopted.

the active ingredient contents were not completely eluted in the elution step. The retained species were then recovered in the regeneration step, which was considered as a loss for the column operation. Finally, the yields of flavonoid glycosides and terpene lactones were nearly 100% for elution systems C and D, but less than 70% and 98% for elution systems A and B, respectively.

Simulation for a Further Improvement of the Column Performance

In the previous operations, the column was eluted isocratically and the product was collected from the whole effluent in the elution step. It would be of great interest to investigate if using a two-step elution mode and collecting only part of the effluent as the product could further improve the column performance.

The idea of a simple two-step elution was to elute some more polar species by an eluent of lower content of ethanol (e.g., 30%) first, and then, to elute the active ingredient contents by an eluent of higher content of ethanol (e.g., 70%) and collect the product. The elution data of Trial 1 of System A was used to simulate the column performance using the two-step elution mode. Two elution systems were simulated (System E and System F) and their results are shown in Table 4. The elution volumes of the first elution step (30% ethanol) were 30 mL for System E and 60 mL for System F, respectively. The results show that a significant amount of flavonoid glycosides and a lesser amount of terpene lactones were also eluted with the more polar eluent at the first elution step. For System E and System F, the purity was increased 10% and 20% for flavonoid glycosides and 25% and 50% for terpene lactones, but the yield was decreased 20% and 40% for flavonoid glycoside and 5% and 15% for terpene lactones. The product purity was proven to be improved with an increase in the elution volume of the first elution step, but only at the expense of the product yield.

CONCLUSIONS

The adsorption separation method, using intermediate polar resins (Amberlite XAD-7HP), was verified to be an efficient way for concentrating the active ingredients from *G. biloba* leaves. The major findings from this study are as follows:

The preparation of the crude *G. biloba* L. extracts, using a Soxhlet extractor operated at 160 mL of 70% ethanol aqueous solution and 3 h for a batch of 10 g dry leaves gave a satisfactory result. The yield of the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

MARCEL DEKKER, INC. 270 Madison Avenue, New York, New York 10016

Table 4. Simulated column performances using a two-step elution condition.

Elution system	Step of column operation	Weight of concentrate (mg)	Flavonoid glycosides				Terpene lactones			
			Weight (mg)	Purity (%)	Concentration ratio[a]	Yield (%)	Weight (mg)	Purity (%)	Concentration ratio[a]	Yield (%)
E	Adsorption (25 mL)-washing (50 mL)	1,981	0	26.77	5.45	83.25	0	7.27	5.59	94.01
	Elution									
	0–30 mL (30% ethanol)	148	19.88				1.71			
	30–130 mL (70% ethanol)	369 (Product)	98.78				26.83			
	Regeneration (180 mL)	0	0				0			
F	Adsorption (25 mL)-washing (50 mL)	1,981	0	29.52	6.01	63.94	0	9.46	7.28	85.18
	Elution									
	0–60 mL (30% ethanol)	260	42.79				4.23			
	60–160 mL (70% ethanol)	257 (Product)	75.87				24.31			
	Regeneration (180 mL)	0	0				0			

[a]The purity of the concentrated feed solution (Sample B) from Table 1 was adopted.

crude extracts ranged from 25% to 30% (w/w) and the purity of flavonoid glycosides and terpene lactones in the concentrated extracts ranged from 2.5 to 3.0 wt.% and 0.5 to 1.0 wt.%, respectively.

In order to be effectively adsorbed in the adsorption process, the crude extracts were dissolved in 10% ethanol and used as the feed solution. This procedure led to a 1.5–2.0 fold concentration and a 10–20% loss for flavonoid glycosides and terpene lactones.

For a column packed with 7.5 mL resins (0.3–1.2 mm diameter) and sizing 1.5 cm I.D. and 7.1 cm L, 25 mL of the feed solution was taken as the loading amount to avoid the loss of flavonoid glycosides and terpene lactones. The loading amount would be increased if the particle size of XAD-7HP resins is reduced, but at the expense of higher pressure drop.

The effect of different eluent compositions on the column performance suggests that a high ethanol concentration (e.g., $\geq$ 70%) should be used as the eluent. Using the eluent in the isocratic elution mode, the following column performance was achieved: both flavonoid glycosides and terpene lactones were nearly completely eluted, their purities all met the standardized contents of *G. biloba* L. extracts, and their yields were nearly 100%.

Using a simulated two-step elution mode (first step: 30% and second step: 70% ethanol), the product purity can be improved (10–20% for flavonoid glycosides and 25–50% for terpene lactones), but at the expense of the product yield (20–40% for flavonoid glycoside and 5–15% for terpene lactones).

Finally, the above findings can also be applied in the isolation and separation of similar active ingredients from other natural products.

ACKNOWLEDGMENTS

The authors wish to thank Taiwan Sugar Co. and the National Science Council of the Republic of China for the financial support under grant no. NSC 89-TSC-7-224-001.

REFERENCES

1. Hasler, A.; Sticher, O.; Meier, B. J. Chromatogr. **1992**, *605*, 41.
2. Li, C.-L.; Wong, Y.-Y. Planta Med. **1997**, *63*, 563.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

3. Chen, P.; Su, X.-L.; Nie, L.-H.; Yao, S.-Z.; Zeng, J.-G. J. Chromatogr. Sci. **1998**, *36*, 197.
4. Zhang, D.-C.; Yu, Z.; Cooper, R.; Chang, M. US Patent 6,174531 B1, 2001.
5. Pietta, P.; Mauri, P.; Rava, A. J. Chromatogr. **1998**, *437*, 453.
6. Hwang, D.-Y.; Lin, S.-J. Formosan Sci. **1995**, *48*, 106.
7. Van Beek, T.A.; Scheeren, H.A.; Rantio, T.; Melger, W.Ch.; Lelyveld, G.P. J. Chromatogr. **1991**, *543*, 375.
8. Ayroles, G.; Rossard, R.-M.; Cadiou, M. US Patent 4,981,688, 1991.
9. Xu, M.-C.; Shi, Z.-Q.; Shi, R.; Liu, J.-X.; Lu, Y.; He, B. React. & Funct. Polym. **2000**, *43*, 297.
10. Zhang, C.; Hu, X.; Yue, H. Chem. Ind. Engin. Prog. **1999**, *18* (6), 27.
11. Lang, Q.; Wai, C.-M. Anal. Chem. **1999**, *71*, 2929.
12. Seikagaku, T. Japan Patent 3,275,629, 1991.
13. Umeda, S. Japan Patent 4,182,434, 1992.
14. Product Data Sheet, PDS 0529 A, Sept., Rohm and Haas Company, Philadelphia, 1995.
15. Lai, S.-M.; Shieh, M.-J.; Chen, R.-L. J. Technol. **2002**, *17* (1), 1289.
16. Hasler, A.; Sticher, O.; Meier, B. J. Chromatogr. **1990**, *508*, 236.
17. Yu, F.-C.; Lai, S.-M.; Suen, S.-Y. Sepn. Sci. & Technol. **2003**, *38*, (5), 1033.
18. Thurman, E.M.; Mills, M.S. *Solid-Phase Extraction: Principles and Practice*; John Wiley & Sons: New York, 1998; Chap. 2.
19. Ruthven, D.M. *Principles of Adsorption and Adsorption Processes*; John Wiley & Sons: New York, 1984; Chap. 8.

Received February 20, 2003
Accepted March 18, 2003
Manuscript 6096

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016