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Evaluation of Quantitative Analysis of Flavonoid Aglycones in *Ginkgo biloba* Extract and Its Products

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ABSTRACT

A simple and rapid HPLC method was developed to evaluate and validate the assay of quercetin, kaempferol and isorhamnetin in *Ginkgo biloba* extract and its products. The column used is Cosmosil 5C18-AR, and mobile phase is MeOH:0.3%H_3PO_4 (1:1). The correlation coefficients of linear calibration for quercetin, kaempferol and isorhamnetin are 0.9999, 0.9999, and 0.9989, respectively. The relative standard deviations of intra-day assays for quercetin, kaempferol, and isorhamnetin are 0.29%, 0.28%, and 1.90%, respectively; inter-day assays are 0.95%, 0.85%, and 1.67%, respectively. The recoveries of quercetin, kaempferol and, isorhamnetin are 87.4%, 94.0%, and 72.4%, respectively. The optimal condition for acid hydrolysis of flavonoid glycosides in Ginkgo biloba extract could be achieved by refluxing with 20.0% HCl at 85˚C for 15 min or with 5.5% HCl at the same temperature for 30 min.

Key words: *Ginkgo biloba* extract, HPLC, quercetin, kaempferol, isorhamnetin

INTRODUCTIOM

Ginkgo Semen is the seed of *Ginkgo biloba* L. and classified in Ginkgoaceae. Dried Ginkgo Semen was used as a traditional Chinese medicine with a common name “bai-guo”. The components in bai-guo include proteins, lipids, and carbohydrates. Apart from Chinese, the Westerners have also used *Ginkgo biloba* leaf extract as a medicine. Flavonoid glycosides and terpene lactones (including ginkgolides and bilobalides) are two main groups of active components in *Ginkgo biloba* leaf. Based on the literature[1-5] and the monograph reported by specialists in commission E of the Federal Institute for Drugs and Medical Devices of Germany, the medical functions of *Ginkgo biloba* include (1) improvement of hypoxic tolerance, particularly in cerebral tissue (2) inhibition of the development of traumatically or toxically induced cerebral edema and acceleration of its regression; (3) inactivation of toxic oxygen radicals (flavonoids); (4) antagonism of the platelet-activating factor (PAF) by ginkgolides; (5) protecting nerves by ginkgolides A and B and bilobalides.

Since *Ginkgo biloba* was proven to improve blood circulation of capillaries and cerebrum, recently it was manufactured into various products such as tablet, sugar-coated tablet, film-coated tablet, oral solution, drop, and injection. Therefore, the cases for registering *Ginkgo biloba* extract and its derived products are increasing in Taiwan. To date, flavonoids in these products are the only components that are requested to assay by the manufacturers. The analysis of flavonoids can be achieved by UV and HPLC methods. However, due to variations in rationale and detecting compound between these two methods, the calculation and quantitation are expressed in different ways.

The UV method used quercetin as the reference standard. The samples were hydrolyzed, reacted with $AlCl_3$ to form color, and then quantified with UV spectrophotometer. Since the factor used to calculate the total flavonoids was not a constant, the deviation of this method is always large. In addition, proanthocyanidines in samples could interfere with detection and reduce the reproducibility of the results. Furthermore, the UV method was not specific[4] and was gradually replaced by HPLC method. For this reason and the suggestion of the National Laboratory of Foods and Drugs, the Department of Health of Taiwan has stated in ordinance 1995.11.1.[7] that HPLC method is a recommended method to examine the components in raw material of *Ginkgo biloba* leaf extract and its derived products.

Using quercetin, kaempferol and isorhamnetin as the reference standards for HPLC method, the samples were hydrolyzed and then analyzed by HPLC. Although five kinds of C_{18} columns used to analyze flavonoids have been evaluated and reported[5], this currently developed method is, however, different from those reported and official methods in sample pre-treatment, column and mobile phase choices, detection wavelength, and flow rate of the mobile phase. Thus, the objective of this research is to re-evaluate HPLC method used to quantify quercetin, kaempferol, and isorhamnetin in *Ginkgo biloba* leaf extract and its derived products and to develop an optimum separation and quantification method. This research also studied the hydrolysis condition of flavonoid glycosides, selection of the HPLC column and mobile phase, repeatability of analyses, and recovery of each standard compound. This developed method was evaluated using commercial *Ginkgo biloba* products as the testing compounds in order to establish an official method to analyze flavonoids in these products.

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MATERIALS AND METHODS

I. *Instruments*

HITACI HPLC equipped with a L-7100 pump and a L-7400 detector.

II. *HPLC Columns*

1. Cosmosil $5C_{18}$-AR (4.6 × 150 mm, 5μm) (Waters, Milford, MA, USA)
2. LiChrospher 100RP-18 (4.0 × 125 mm, 5 μm) (Merck, Hohenbrunn, German)
3. μ-Bondapak C_{18} (3.9 × 300 mm, 10 μm) (Waters, Milford, MA, USA)
4. Inertsil ODS-2 (4.6 × 250 mm, 5 μm) (GL Science, Kyoto, Japan)
5. Cosmosil $5C_{18}$-MS (4.6 × 150 mm, 5 μm) (Waters, Milford, MA, USA)
6. SYMETRY (4.6 × 150 mm, 5 μm) (Waters, Milford, MA, USA)
7. Nucleosil $5C_{18}$ (4.6 × 150 mm, 5 μm) (Phenomenex, Torrance, CA, USA)
8. Hypersil $5C_{18}$ (4.6 × 150 mm, 5 μm) (Phenomenex, Torrance, CA, USA)
9. PRODIGY 5 ODS-2 (4.6 × 250 mm, 5 μm) (Phenomenex, Torrance, CA, USA)

III. *Standard Compounds and Reagents*

(I) *Standard Compounds*

Quercetin, kaempferol, and isorhamnetin were purchased from EXTRA SYNTHESE Co. (Genay, France). Morin was from NACALAI Co. (Kyoto, Japan).

(II) *Reagents*

$Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, and KH_2HPO_4 were from WAKO Co. (Osaka, Japan); sodium 1-octanesulfonate ($PICB_8$) was from SIGMA Co. (St. Louis, MO, USA); phosphoric acid and acetic acid were from Merck Co. (Hohenbrunn, Germany).

(III) *Solvents*

Methanol and acetonitrile were obtained from LABSCAN Co. (Dublin, Ireland); tertrahydrofuran (THF) was from Merck Co. All solvents were HPLC grade.

(IV) *Samples*

Ginkgo biloba extract was purchased from Tradshine Co. Ltd. (imported from Boral Quimica, S.A., Spain). Twelve commercial available *Ginkgo biloba* products includes 8 samples of film-coated tablets and one sample of sugar-coated tablet, tablet, drop, and oral solution.

IV. *Methods*

(I) *Study of the HPLC Conditions*

1. Selection of the Mobile Phase

Nine different mobile phases were evaluated to separate quercetin, kaempferol, and isorhamnetin, and Cosmosil $5C_{18}$-MS column was used as the HPLC column. The retention time (t_R), relative retention (α), and resolution (Rs) resulted from various mobile phases were compared, and the most appropriate mobile phase could be selected.

2. Selection of HPLC Column

Nine HPLC columns with methanol:0.3% H_3PO_4 (1:1) as the mobile phase were evaluated to analyze quercetin, kaempferol, and isorhamnetin. The retention time (t_R), asymmetry of peaks (As), resolution (Rs), and plate number (N) obtained from different columns were compared, and the best column could be chosen.

(II) *HPLC Analysis of Commercial Products*

1. Preparation of the Commercial Products for HPLC Analysis

Four to five sample tablets (or 5.0 mL for liquid sample) were ground and 20 mL 80% methanol solution was added to dissolve the sample. After shaking for 30 min, the mixture was placed aside until two phases were separated. Ten mL of the upper layer was removed and treated with 10 mL 5.5% HCl solution. The solution was refluxed in 85˚C water bath for 30 min. Finally the solution was filtered and ready for HPLC analysis.

2. HPLC Conditions

Column, Cosmosil $5C_{18}$-AR (150 mm); mobile phase, methanol:0.3% H_3PO_4 (1:1); flow rate, 1.0 mL/min; detection wavelength, 370 nm.

(III) *Standard Curves of Quercetin, Kaempferol, and Isorhamnetin*

Quercetin, kaempferol, isorhamnetin, and morin (internal standard) were measured precisely and were dissolved separately in 80% methanol solution. Quercetin solution was further diluted to 500, 110, 53, and 26 μg/mL; kaempferol solution to 470, 100, 50, and 25 μg/mL; isorhamnetin solution to 270, 54, 27, and 13 μg/mL. Internal standard (44 μg/mL) was spiked into each dilution, which was then analyzed by HPLC. The standard curves were obtained by plotting the peak area ratios (Y-axis) of standard compounds to the internal standard versus the concentrations of standard compound (X-axis). According to these standard curves, the linear regressions (Y = mX + b) and correlation coefficients

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were computed.

(IV) *Investigation of Hydrolysis Conditions*

1. Concentration of HCl

Ten mL of different concentrations of methanolic HCl solution (2.25%, 5.5%, 10%, 20%, and 25%) were added to five tubes (10 mL each) of diluted *Ginkgo biloba* extract (1.5 g sample dissolved in 200 mL 80% methanol solution) separately. Each mixture was refluxed in an 85˚C water bath for 30 min, after cooling, each mixture was spiked with internal standard. Finally, the volume was brought to 25 mL with 80% methanol solution and then was analyzed by HPLC. The HPLC conditions were the same as described above.

2. Hydrolysis Time

Ten mL of HCl solution (20%) was added into 4 tubes of diluted *Ginkgo biloba* extract (10 mL each), and each mixture was refluxed in a water bath (85˚C) for 15, 30, 60, and 120 min, respectively. After cooling, each mixture was spiked with I.S. and brought to 25 mL with 80% methanol solution. For another set of test, the same procedure was carried out except 20% HCl solution was replaced by 5.5% HCl.

(V) *Intra-Day and Inter-Day Assays of Standard Compounds*

The testing concentrations for quercetin, kaempferol, and isorhamnetin solutions were within the concentration ranges of their respective standard curves. Quercetin (0.11 mg/mL), kaempferol (0.10 mg/mL), and isorhamnetin (0.054 mg/mL) were analyzed by HPLC for 5 times/day and 1 time/day for 5 consecutive days. The resulting peak area ratios were used to calculate standard deviation and relative standard deviation.

(VI) *Recovery Test*

Standard solutions of quercetin (0.53, 1.06, 2.12, and 3.18 mg/mL), kaempferol (0.50, 1.0, 2.0, and 3.0 mg/mL), and isorhamnetin (0.27, 0.54, 1.08, and 1.62 mg/mL) were added separately into 4 tubes of diluted *Ginkgo biloba* extract (3 mL each). The control tube was without addition of standard solutions. Following addition of 10 mL of 5.5% HCl, each mixture was refluxed in an 85oC water bath for 30 min. After cooling, each tube was spiked with I.S. and diluted to 25 mL with 80% methanol solution. After HPLC analysis, the recovery of each standard compound could be calculated from the resulting data.

RESULTS AND DISCUSSION

I. *Study of HPLC Conditions*

(I) *Selection of Mobile Phase*

A total of 9 mobile phases from recent registered cases were applied to separate quercetin (Q), kaempferol (K), and isorhamnetin (I), and t_R, α, and Rs obtained from each mobile phase was calculated. Based on the literature, the criteria for a good separation are: α within 1.05-2.0 and Rs over 1.5. The t_R, α, and Rs of each mobile phase are listed in Table 1.

Since Q could separate well from the other two standard compounds in all 9 mobile phases, the mobile phase, which could best resolve K and I was chosen and used for this study. Therefore, α and Rs of K and I obtained from these 9 mobile phases were evaluated. As the result shown, with the exception of mobile phase M9, which could not resolve K and I, the other 8 mobile phases were able to separate peaks Q, K, and I, and their Rs were more than 1.5 and α between 1.05-2.0. Of all mobile phases, M2, M3, M6, M7, and M8 resulted in a better resolution, however, M2 containing phosphate salt could increase the difficulties in mobile phase preparation and HPLC column clean-up. Mobile phase M3 containing triethylamine could emit unpleasant odor. The retention times were too long for mobile phase M6 and M7. Therefore, M8 (methanol:0.3%H_3PO_4 = 1:1) was chosen as the mobile phase for later study. Figure 1 is the chromatogram for standard compounds using M8 as the mobile phase.

(II) *Selection of HPLC Column*

With M8 as the mobile phase, nine different HPLC columns were compared to resolve quercetin (Q), kaempferol (K), and isorhamnetin (I). The t_R, As, Rs, and N resulted from different columns were listed in Table 2. Based on the literature[5], As is the best between 1 and 1.2. Of all 9 columns, the As of column 1, 5, 6, 7, and 9 were within this range. Among these 5 columns, column 1 (Cosmosil 5C_{18}-AR), 5 (Cosmosil 5C_{18}-MS), and 7 (Nucleosil 5C_{18} 100A) were selected for better resolution (Rs > 4.5). However, column 7 was left out since its retention time was longer than

Table 1. Separation parameters with various mobile phases

Mobile[a] phase	t_R(min) Q	K	I	α(K-I)	Rs(K-I)
M1	19.2	43.1	46.1	1.07	5.05
M2	11.3	21.8	24.6	1.13	5.56
M3	12.0	23.1	25.8	1.12	5.36
M4	8.0	14.6	16.8	1.15	4.44
M5	12.3	17.2	20.1	1.17	4.90
M6	21.4	47.5	52.5	1.11	6.32
M7	15.9	31.3	34.6	1.11	5.50
M8	12.1	23.3	26.0	1.11	5.22
M9	10.1	19.4	–[b]	–	–

[a] M1. Phosphate buffer (containing $PICB_8$) : CH_3CN (77:23).
M2. 20 mM KH_2PO_4 : MeOH (51:49).
M3. Triethylamine : MeOH (53:47).
M4. 20% AcOH : MeOH (68:32).
M5. 1% AcOH : THF: MeOH (55:13:32).
M6. 0.3% H_3PO_4: CH_3CN (77:23).
M7. MeOH : 0.3% H_3PO_4 : CH_3CN (30:58:12).
M8. MeOH : 0.3% H_3PO_4 (1:1).
M9. H_2O : CH_3CN : Isopropanol (100:47:5) + 0.4% Citric acid.
[b] Isorhanetin could not be separated.

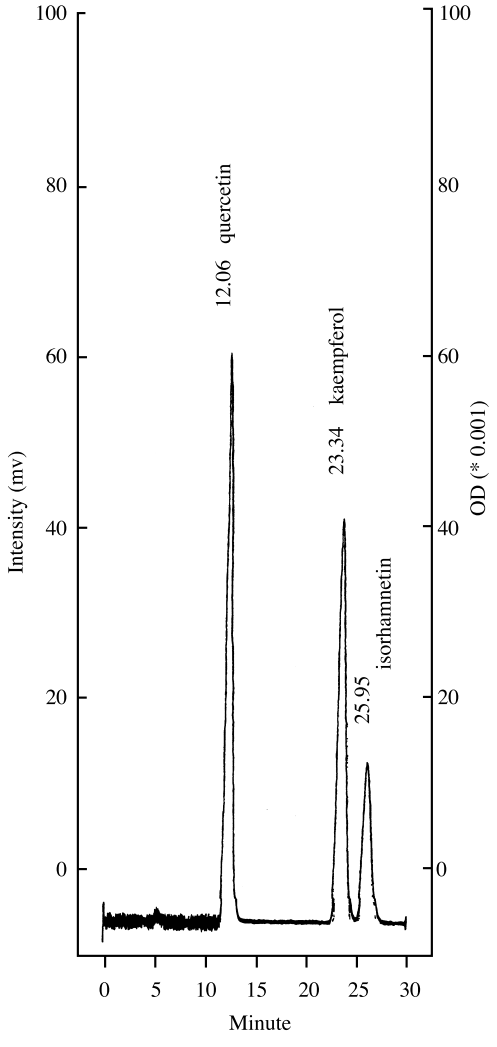

Figure 1. Chromatogram of quercetin, kaempferol and isorhamnetin, Column: Cosmosil $5C_{18}$-MS, Mobile phase: MeOH : 0.3% H_3PO_4 (1:1).

Table 2. Separation and efficiency parameters with various columns

Column[a]	t_R(min)						
	Q	K	I	As(K)	As(I)	Rs(K-I)	N
1	13.1	25.3	28.2	1.0	1.0	5.54	36556
2	7.8	14.4	16.2	1.5	1.5	3.29	18431
3	20.3	35.1	38.9	1.5	1.4	3.39	22458
4	18.4	37.5	41.1	1.3	1.3	4.29	44109
5	12.6	22.4	24.8	1.0	1.0	4.86	30830
6	10.8	19.6	21.7	1.0	1.0	4.20	24121
7	16.2	29.2	33.3	1.0	1.0	5.53	29466
8	9.8	16.9	18.9	1.5	1.1	2.83	12946
9	16.4	29.9	32.1	1.0	1.0	4.36	55033

[a] 1. Cosmosil $5C_{18}$-AR 4.6 × 150 mm (Waters).
2. Lichrospher 100 RP-184.0 × 125 mm (Merck).
3. μ-Bondapak C_{18} 3.9 × 30 mm (Waters).
4. Inertsil ODS-24.6 × 250 mm (GL Sciences Inc.).
5. Cosmosil $5C_{18}$-MS 4.6 × 150 mm (Waters).
6. SYMETRY 4.6 × 150 mm (Waters).
7. Nucleosil $5C_{18}$ 100A 4.6 × 150 mm (Phenomenex).
8. HYRERSIL $5C_{18}$ 4.6× 150 mm (Phenomenex).
9. PRODIGY5 ODS-2 4.6 × 250 mm (Phenomenex).

column 1 and 5, and its N value (29466) was smaller. Column 1 and 5 were similar but the former possessed a higher N value (36556) and better antiacidity than the later. In addition, column 1 was superior in column efficiency and Rs, therefore, it was the best choice for this study. Figure 2 is the chromatogram for reference standards separated by column 1.

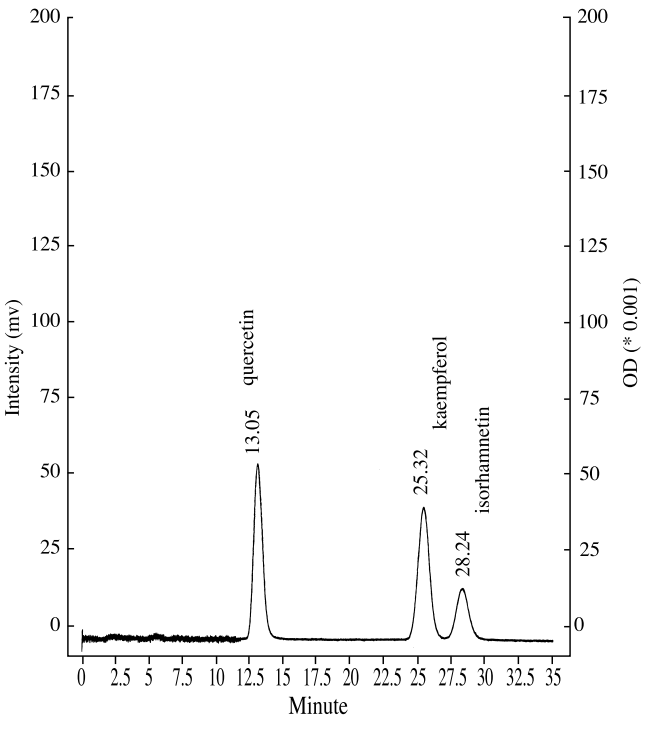

Figure 2. Chromatogram of quercetin, kaempferol and isorhamnetin, Column: Cosmosil $5C_{18}$-AR, Mobile phase: MeOH: 0.3%H_3PO_4 (1:1).

II. *Standard Curves of Quercetin, Kaempferol, and Isorhamnetin*

The chromatogram of quercetin, kaempferol, and isorhamnetin is shown in Figure 3. The linear regressions for the standard curves of quercetin, kaempferol, and isorhamnetin were as follows:

Quercetin: Y = 6.03X + 1.06E-03 r = 0.9999
Kaempferol: Y = 6.09X + 2.45E-03 r = 0.9999
Isorhamnetin: Y = 1.32X + 1.44E-02 r = 0.9989

III. *Investigation of Hydrolysis Conditions*

All the recent registered cases for the examination of *Ginkgo biloba* extract and other relevant products in Taiwan were hydrolyzed under acid conditions. Hasler[11] and Pietta[12] also hydrolyzed samples with HCl and under heated environments. Therefore, HCl was applied in this study for sample hydrolysis. The testing samples were treated with 5 levels of HCl solution (2.5%, 5.5%, 10%, 20%, and 25%) as described in Materals and Methods. The highest hydrolysis rate in these 5 treatments was assigned as 100%. As shown in Table 3, 20% HCl solution resulted in the highest hydrolysis rate. However, most registered cases used 5.5% HCl solution for sample hydrolysis. For this reason, both

5.5% and 20% were chosen to investigate the hydrolysis time. After reflux at a water bath for 15, 30, 60, and 120 min, the results were obtained and shown in Table 4 and Table 5. The treatments of 20% HCl for 15 min and 5.5% HCl for 30 min achieved the similar outcome (Table 6).

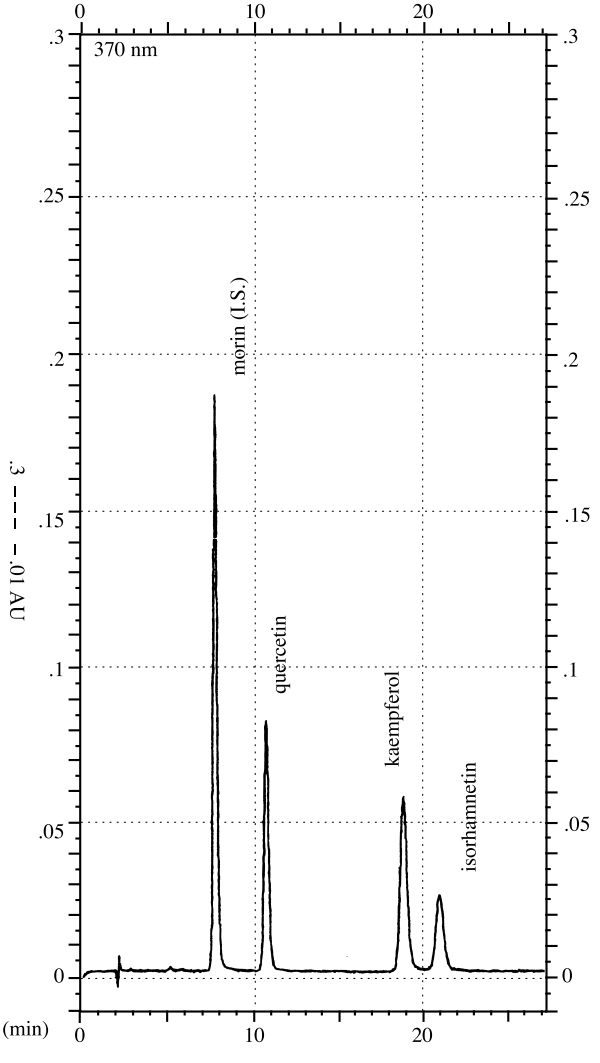

Figure 3. Chromatogram of quercetin, kaempferol, isorhamnetin and morin, Column: Cosmosil 5C_{18}-AR, Mobile phase : MeOH: 0.3% H_3PO_4 (1:1).

IV. *Precision of Analysis*

Quercetin, kaempferol, and isorhamnetin were analyzed

Table 3. Relative yields of flavonoid aglycones from *Ginkgo biloba* extract treated by various concentrations of hydrochloric acid (HCl)

flavonoid aglycone	Yields (%)				
	2.5%	5.5%	10%	20%	25%
quercetin	62.2	84.2	91.9	100	91.9
kaempferol	58.8	78.3	90.5	100	90.0
isorhamnetin	55.8	71.4	87.1	100	92.5

Table 4. Relative yields of flavonoid aglycones from *Ginkgo biloba* extract treated with 5.5% HCl for different length of time

flavonoid aglycone	Yields (%)			
	15 min	30min	1 hr	2 hr
quercetin	90.1	96.3	100.0	93.8
kaempferol	91.0	100.0	100.0	91.0
isorhamnetin	94.9	100.0	89.7	79.5

Table 5. Relative yields of flavonoid aglycones from *Ginkgo biloba* extract treated with 20.0% HCl for different length of time

flavonoid aglycone	Yields (%)			
	15 min	30min	1 hr	2 hr
quercetin	100.0	85.5	89.2	84.3
kaempferol	100.0	86.1	86.1	84.7
isorhamnetin	100.0	86.8	71.1	81.6

Table 6. Comparison of flavonoid aglycone contents of a liquid product after two different conditions of hydrolysis

flavonoid aglycone	Content (mg/mL)	
	5.5% HCl , 30 min	20% HCl , 15 min
quercetin	0.050	0.051
kaempferol	0.038	0.041
isorhamnetin	0.036	0.036

Table 7. Inter-day and Intra-day relative standard deviation for quercetin, kaempferol and isorhamnetin

flavonoid aglycone	concentration (mg/mL)	peak area of marker substance / peak area of morin		mean ± S.D.[a] (R.S.D.%)	
		inter-day	intra-day	inter-day	intra-day
quercetin	0.11	1.78	1.81	1.80 ± 0.017 (0.95)	1.81 ± 0.005 (0.29)
		1.81	1.81		
		1.78	1.82		
		1.78	1.82		
		1.81	1.81		
kaempferol	0.10	1.75	1.77	1.76 ± 0.011 (0.85)	1.78 ± 0.005 (0.28)
		1.78	1.78		
		1.75	1.77		
		1.76	1.78		
		1.78	1.78		
isorhamnetin	0.054	0.41	0.42	0.42 ± 0.007 (1.67)	0.42 ± 0.008 (1.90)
		0.43	0.42		
		0.41	0.41		
		0.42	0.43		
		0.42	0.43		

[a]n = 5.

Table 8. Recoveries of quercetin, kaempferol and isorhamnetin from *Ginkgo biloba* extrat

flavonoid aglycone	added (mg/mL)	found (mg/mL)	relative recovery (%)	mean ± S.D.[a]	(R.S.D.%)
quercetin	0.528	0.464	87.9	87.4 ± 1.27	(1.45)
	1.056	0.920	87.1		
	2.112	1.812	85.8		
	3.168	2.812	88.8		
keampferol	0.496	0.468	94.4	94.0 ± 1.40	(1.49)
	0.992	0.924	93.1		
	1.984	1.840	92.7		
	2.976	2.852	95.8		
isorhamnetin	0.268	0.188	70.1	72.4 ± 1.84	(2.54)
	0.536	0.388	72.4		
	1.072	0.564	72.6		
	1.608	0.880	74.6		

[a]n = 5.

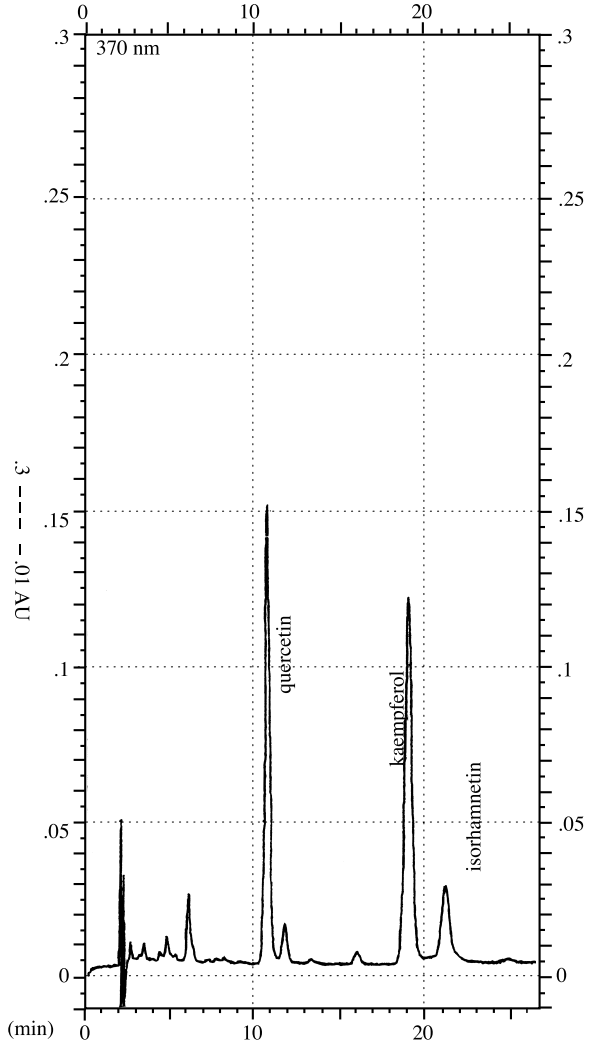

Figure 4. Chromatogram of a Ginkgo product.

5 times/day and 1 time/day for 5 consecutive days. The relative standard deviations of intra-day and inter-day were listed in Table 7. As the result shown, the relative standard deviations for the intra-day analyses for quercetin, kaempferol, and isorhamnetin were 0.29%, 0.28%, and 1.90%, respectively; inter-day analyses were 0.95%, 0.85%, and 1.67%, respectively. These data indicated the assays were precise.

V. *Recovery Test*

As described above, the diluted *Ginkgo biloba* extract was spiked with various concentrations of reference standards. After HPLC analysis, the recoveries of these reference standards were calculated and listed in Table 8. The average recoveries for quercetin, kaempferol, and isorhamnetin were 87.4%, 94.0%, and 72.4%, respectively. The lower recovery of isorhamnetin is probably due to the minor content of this compound in *Ginkgo biloba*.

VI. *HPLC Analysis of the Commercial Products*

The commercial *Ginkgo biloba* products include film-coated tablet, sugar-coated tablet, tablet, oral solution, drop, and injection, among them, film-coated tablet is the most popular product. Twelve commercial products were purchased from local stores and analyzed using the currently developed HPLC method. For different products, their excipients are also dissimilar and may interfere with HPLC analysis. However, this method was able to separate quercetin, kaempferol, and isorhamnetin well and without any interference. The chromatogram of film-coated tablet was shown in Figure 4.

CONCLUSIONS

I. Based on the comparisons of retention time (t_R), relative retention time (α), asymmetry of peaks (As), resolution (Rs), and plate number (N), application of Cosmosil $5C_{18}$-AR column and methanol:0.3% H_3PO_4 (1:1) as the mobile phase resulted in the best resolution.

II. Samples treated with 20% HCl solution and refluxed in an 85˚C water bath for 15 min resulted in a similar hydrolysis level as that with 5.5% HCl solution and refluxed at the same water bath for 30 min.

III. The correlation coefficients of linear regressions for

quercetin, kaempferol and isorhamnetin were 0.9999, 0.9999 and 0.9989, respectively. The relative standard deviations for intra-day assay of quercetin, kaempferol and isorhamnetin were 0.29%, 0.28%, and 1.90%, respectively; inter-day assay were 0.95%, 0.85%, and 1.67%, respectively. These results indicated the assays were precise.

IV. The HPLC method developed in this study could apply to analyze quercetin, kaempferol, and isorhamnetin in *Ginkgo biloba* extract and other relevant products including film-coated tablets, sugar-coated tablet, tablet, drop, and oral solution with excellent separation and without the interference of excipients.

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銀杏浸膏及其製劑中三種類黃酮啟元定量方法之評估

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摘　要

本研究針對有關銀杏葉浸膏原料及製劑內qucercetin, kaempferol及 isorhamnetin之HPLC定量方法進行評估，結果顯示，分析條件使用Cosmosil 5C_{18}-AR層析管，以MeOH：0.3%H_3PO_4（1:1）為移動相，分離效果良好。各成分檢量線之相關係數分別為quercetin ｒ = 0.9999，kaempferol ｒ = 0.9999，isorhamnetin ｒ = 0.9989，而同日內檢測之相對標準偏差分別為0.29%，0.28%，及1.90%，異日間檢測之相對標準偏差分別為0.95%，0.85%及1.67%，其添加回收率則分別為87.4%，94.0%及72.4%。另外檢體前處理之方法，使用20%HCl溶液經15分鐘加熱迴流，與使用5.5%HCl溶液經30分鐘加熱迴流，可得到相近之水解效果。

關鍵詞：銀杏葉浸膏，高效液相層析法，quercetin，kaempferol，isorhamnetin