

Studies on the Component Analysis and Quality Control in Tonic Wine Preparation of King-Mon-Long-Fong-Jyo

HORNG-LIANG LAY^{1*}, SHIOW-CHYN HUANG², CHIA-CHI CHEN¹ AND TIAN-SHUNG WU³

¹. Department of Plant Industry, National Ping-Tung University of Science & Technology,

No.1, Syuefu Rd., Neipu Township, Pingtung County 912, Taiwan (R.O.C.)

². Department of Pharmacy, Chia-Nan University of Pharmacy and Science,

No.60, Sec. 1, Erren Rd., Rende Township, Tainan County 711, Taiwan (R.O.C.)

³. Department of Chemistry, National Cheng-Kung University, No.1, Dasyue Rd., East District, Tainan City 701, Taiwan (R.O.C.)

(Received: August 30, 2002; Accepted: April 22, 2003)

ABSTRACT

An HPLC method for simultaneous determination of seven marker substances was established for the quality control in tonic wine preparation of "King-Mon-Long-Fong-Jyo". These marker substances were gomisin A and schizandrin from *Schizandrae* Fructus, loganin from *Corni* Fructus, cinnamic acid and cinnamaldehyde from *Cinnamomi* Cortex, scopoletin and ferulic acid from *Angelicae* Radix. Different rice wine extraction volume and extraction temperature conditions were performed to evaluate quality of King-Mon-Long-Fong-Jyo.

Extracted samples were run through the HPLC column (Inertsil 5 ODS-2, 4.6 I.D. × 250 mm.) at 30°C and the column was developed with a mixture of 20% acetonitrile and 70% acetonitrile (adjusted to pH 3.0 with phosphoric acid) aqueous solution and then employed linear gradient elution method at a flow-rate of 1.0 mL/min. An UV 250 nm was used for the detection of the marker substances.

Relative standard deviations of intra- and inter-day analysis were less than 5%. This separation method could be successfully applied for the simultaneous determination of seven marker substances in "King-Mon-Long-Fong-Jyo".

Key words: King-Mon-Long-Fong-Jyo, gomisin A, schizandrin, loganin, cinnamic acid, cinnamaldehyde, scopoletin, ferulic acid.

INTRODUCTION

In Taiwan, the Department of Health (DOH) has been promoting the HPLC method for quantitative analysis of ingredients in Chinese medicinal preparation since 2001. Especially when its manufacturer's license expires and needs to be renewed⁽¹⁾, these documents should be also included. Therefore, good method for marker substances analysis is an important factor in upgrading the qualities of Chinese medicinal preparations.

In recent years, a number of analytical methods for Chinese medicinal preparations have been established in our laboratory⁽²⁻⁷⁾. In this study, we selected King-Mon-Long-Fong-Jyo, a popular tonic wine preparation of Chinese medicine in Taiwan. This wine contains *Schizandrae* Fructus, *Corni* Fructus, *Morindae* Radix, *Cistanchis* Herba, *Cinnamonomi* Cortex and *Angelicae* Radix. The tonic wine is known to nourish yin and enrich the kidney etc. Analytical methods for these Chinese crude drugs were reported⁽⁸⁻²⁶⁾, but no analytical methods for King-Mon-Long-Fong-Jyo has been reported. The goal of this particular study is to determine the seven marker substances in King-Mon-Long-Fong-Jyo simultaneously by HPLC method. These substances include gomisin A and

schizandrin from *Schizandrae* Fructus, loganin from *Corni* Fructus, cinnamic acid and cinnamaldehyde from *Cinnamomi* Cortex, as well as scopoletin and ferulic acid from *Angelicae* Radix. While searching for the optimum analytical conditions, we found that a 20~70% acetonitrile aqueous solvent is an effective eluent to perform good analysis for this tonic wine preparation.

MATERIALS AND METHODS

I. Materials

The materials for preparation of King-Mon-Long-Fong-Jyo preparation are *Schizandrae* Fructus, *Corni* Fructus, *Morindae* Radix, *Cistanchis* Herba, *Cinnamonomi* Cortex and *Angelicae* Radix. They were all purchased from herbal market, and were all pulverized through a #8 mesh sieve (2.36 mm) for use.

II. Chemicals and Reagents

The structures of marker substances are shown in Figure 1. Cinnamic acid and cinnamaldehyde were purchased from Fluka Chemie AG (Switzerland), and Scopoletin and ferulic acid were purchased from Sigma

* Author for correspondence. Tel:886-8-7703202 ext. 6345; Fax:886-8-7740415; E-mail:layhl@mail.npust.edu.tw

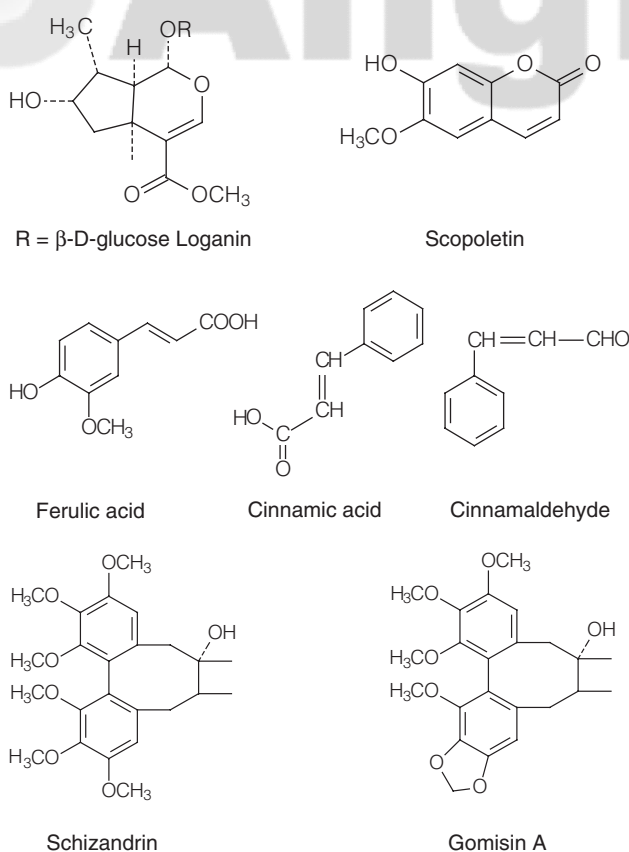


Figure 1. Structures of the compounds studied.

Chemical Co. (St. Louis, Mo, USA). Gomisin A, schizandrin, loganin, and internal standard nobiletin were purified from *Schizandrae Fructus*, *Corni Fructus*, and *Citri Leiocarpae Exocarpium*, respectively, in our laboratory. Mass spectrometry, NMR spectrometry and infrared spectrometry were applied to determine compound characteristics. All analytical values obtained agreed with previous investigations⁽²⁷⁻³⁰⁾.

Rice wine (19.5% ethanol) and 95% ethanol were purchased from Taiwan Tobacco and Wine Board (R.O.C.). Acetonitrile and methanol (HPLC grade) were obtained from Mallinckrodt, Inc. (USA), and phosphoric acid (analytical-reagent grade) from Kanto Chemical (Japan). Ultrapure distilled water with a resistivity greater than 18 M Ω was prepared with a mini-Q system (Millipore, Bedford, MA, USA). Samples for HPLC were filtered through a 0.45 μ m membrane filter (Millipore, Bedford, MA, USA). All other reagents were analytical grade.

III. Preparation of Standard and Internal Standard Solutions

(I) Preparation of standard solution

15.0 mg, 2.5 mg, 1.0 mg, 2.0 mg, 3.6 mg, 10.0 mg and 4.0 mg of loganin, scopoletin, ferulic acid, cinnamic acid,

cinnamaldehyde, schizandrin, and gomisin A were dissolved in 70% methanol to give sequential concentrations 600.0 μ g/mL, 100.0 μ g/mL, 40.0 μ g/mL, 160 μ g/mL, 144.0 μ g/mL, 400.0 μ g/mL and 160.0 μ g/mL, respectively.

(II) Preparation of internal standard solution

To prepare the internal standard solution, 20.0 mg of nobiletin was dissolved in 70% methanol with a final volume of 250 mL (40 μ g/mL).

IV. Preparation of Test Solution

According to Reference 8, Chinese crude drugs containing 6.3 g of *Schizandrae Fructus*, 12.5 g of *Corni Fructus*, 6.3 g of *Morindae Radix*, 12.5 g of *Cistanchis Herba*, 2.5 g of *Cinnamomi Cortex* and 3.8 g of *Angelicae Radix* were used to make King-Mon-Long-Fong-Jyo. The above-mentioned Chinese crude drugs were extracted twice with 800 mL of 50% ethanol by refluxing at 90°C for 3 hr each time, combined the extract and concentrated under vacuum and adjusted to 50 mL by adding 80% methanol. A 1.0 mL sample of the solution was diluted to 5 mL by 80% methanol solution, while internal standard nobiletin was added to each solution to a concentration of 20.0 μ g/mL at the same time. This was the test solution used for subsequent HPLC analysis after filtration through a 0.45 μ m membrane filter.

V. Preparation of Sample Solutions

(I) Preparation of sample solutions with different volumes of rice wine

The pulverized Chinese crude drugs listed above were weighed with 43.9g and extracted respectively with 87.8 mL (2-fold), 175.6 mL (4-fold), 351.2 mL (8-fold), 526.8 mL (12-fold), 702.4 mL (16-fold) and 878.0 mL (20-fold) of rice wine under room temperature for 3 weeks. Each extract was evaporated under vacuum and adjusted to 50 mL by adding 80% methanol. Then, 1 mL sample of each of the solution was adjusted to 5 mL by adding 80% methanol, while internal standard nobiletin was added to each solution to a concentration of 20.0 μ g/mL at the same time. After filtration through a 0.45 μ m membrane filter, the solutions were used for quantification.

(II) Preparation of solution by different extraction conditions

The pulverized Chinese crude drugs listed above were weighed with 43.9 g and extracted under four different conditions: addition of 800 mL of rice wine to each sample and store the mixture either at room temperature (25°C) for 3 weeks, reflux at 45°C for 24 hr, at 60°C for 12 hr, or at boiling temperature (90°C) for 3hr. Each extract was evaporated under vacuum and adjusted to 50 mL by adding 80%

methanol. Then, 1 mL sample of each solution was adjusted to 5 mL by adding 80% methanol, while internal standard nobiletin was added to each solution to a concentration of 20.0 µg/mL at the same time. These sample solutions were used for quantification after filtration through a 0.45 µm membrane filter.

VI. HPLC Analysis

HPLC was conducted by a Hitachi system equipped with a degasser DG-2410, Pump L-7100, UV/Vis Detector L-7420, photodiode array Detector L-4500 and Autosampler L-7200. Peak areas were calculated with a D-7000 HSM software. The analysis conditions were listed as follows:

Column: Inertsil 5 ODS-2, 4.6 mm I.D. × 250 mm.

Column temperature: 30°C.

Mobile phases:

A: 20% acetonitrile (adjusted to pH 3.0 with phosphoric acid).

B: 70% acetonitrile (adjusted to pH 3.0 with phosphoric acid).

The mixtures of A and B aqueous solutions used in the linear gradient elution are shown in Table 1.

Flow rate: 1.0 mL/min.

Injection volume: 20 µL.

Detection wavelength: UV 250 nm (λ_{max} were obtained as the following: 250 nm for loganin, 240 nm for scopoletin, 250 nm for ferulic acid, 240 nm for cinnamic acid, 240 nm for cinnamaldehyde, 250 nm for schizandrin and 260 nm for gomisin A. Therefore, we selected the middle value, UV250 nm, as the detection wavelength).

20 µL of each sample solution prepared as described above was injected into the HPLC column for analysis. The results were quantified by interpolating into the linear regression plot made from standard solution.

VII. Calibration Method

The standard solutions of each marker substance described in section III (I) were diluted by 80% methanol to give the following sequential concentrations:

(I) loganin: 18.75, 37.5, 75.0, 150.0, 300.0 µg/mL.

(II) scopoletin: 3.125, 6.25, 12.5, 25.0, 50.0 µg/mL.

Table 1. Gradient elution program using mobile phase A and B.

Time (min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0	1.0	100	0
10	1.0	95	5
15	1.0	90	10
25	1.0	80	20
50	1.0	0	100
60	1.0	0	100
70	1.0	100	0

A: 20% acetonitrile (adjusted to pH 3.0 with phosphoric acid).

B: 70% acetonitrile (adjusted to pH 3.0 with phosphoric acid).

(III) ferulic acid: 1.25, 2.5, 5.0, 10.0, 20.0 µg/mL.

(IV) cinnamic acid: 2.5, 5.0, 10.0, 20.0, 40.0 µg/mL.

(V) cinnamaldehyde: 4.5, 9.0, 18.0, 36.0, 72.0 µg/mL.

(VI) schizandrin: 12.5, 25.0, 50.0, 100.0, 200.0 µg/mL.

(VII) gomisin A: 5.0, 10.0, 20.0, 40.0, 80.0 µg/mL.

Each dilution contained the internal standard solution, nobiletin, at 20 µg/mL. After filtering through a 0.45 µm membrane filter, 20 µL of each concentration was injected into the HPLC column for analysis. The calibration curve was plotted by using the ratio of the peak areas that corresponded to each standard solution and the internal standard solution on the Y-axis, versus each concentration on the X-axis. Linear regression method was used to evaluate the parameters of $y = ax + b$ and the correlation coefficient.

RESULTS AND DISCUSSION

I. Separation of Marker Substances by HPLC

The HPLC chromatograms of the 50% ethanol extract of King-Mon-Long-Fong-Jyo are shown in Figure 2A. The chromatogram indicated that the peaks of Loganin, scopoletin, ferulic acid, cinnamic acid, cinnamaldehyde, schizandrin, gomisin A, and the internal standard had been well separated. The respective retention times were as follows: 18.09 min for loganin, 27.72 min for scopoletin, 29.69 min for ferulic acid, 42.21 min for cinnamic acid, 44.48 min for cinnamaldehyde, 51.17 min for schizandrin, 53.87 min for gomisin A, and 49.77 min for the internal standard, nobiletin.

The peak purity of components in the King-Mon-Long-Fong-Jyo were quantified by HPLC with photodiode array detector. High purity of each peak was shown for each component (Figure 3). The standard compound was shown together with the components of the three kinds of solutions, which were prepared with the deletion of one material of *Corni Fructus*, *Angelicae Radix*, *Cinnamonomi Cortex*, and *Schizandrae Fructus*, respectively. As shown in Figure 2B to 2E, no peaks of the deleted material were observed at retention times corresponding to the respective marker substances. Apparently, there was no interaction between components of King-Mon-Long-Fong-Jyo. Therefore, the above conditions can be used for quantification of the marker substances.

II. Calibration Line

The regression equations and correlation coefficients of calibration lines for those marker substances were as follows:

(I) Loganin in the concentration range of 18.75~300.0 µg/mL,

$$y = 0.0201x + 0.0267, r = 1.0000 (n = 5).$$

(II) Scopoletin in the concentration range of 3.125~50.0 µg/mL,

$$y = 0.0268x - 0.0058, r = 0.9999 (n = 5).$$

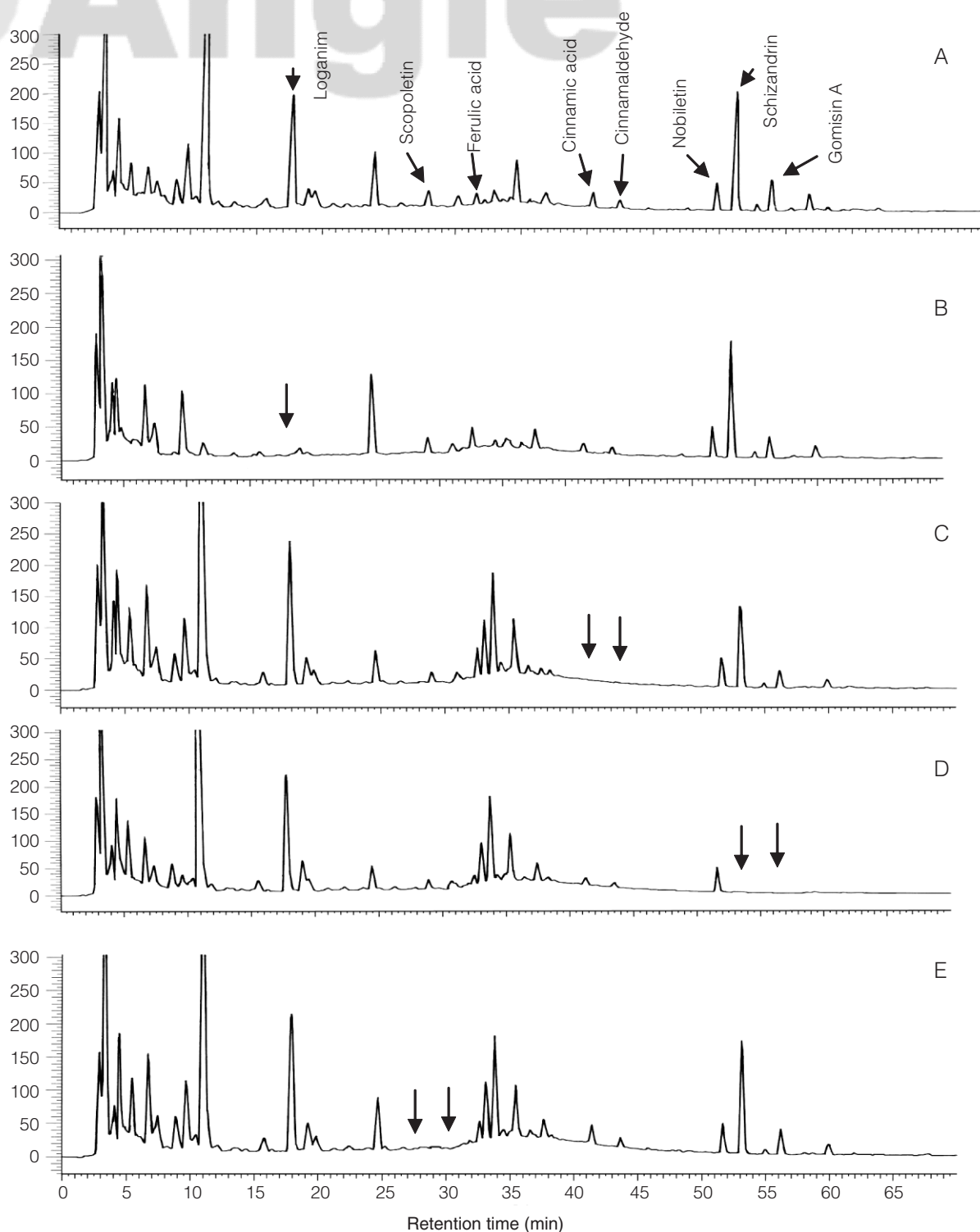


Figure 2. Chromatograms of marker substances in standard solutions of King-Mon-Long-Fong-Jyo made from incomplete materials.

A: King-Mon-Long-Fong-Jyo standard solution containing nobiletin as internal standard.

B: King-Mon-Long-Fong-Jyo standard solution without Corni Fructus.

C: King-Mon-Long-Fong-Jyo standard solution without Cinnamonomi Cortex.

D: King-Mon-Long-Fong-Jyo standard solution without Schizandrae Fructus.

E: King-Mon-Long-Fong-Jyo standard solution without Angelicae Radix.

(III) Ferulic acid in the concentration range of
1.25~20.0 $\mu\text{g/mL}$,
 $y = 0.0527x - 0.0693$, $r = 0.9944$ ($n = 5$).

(IV) Cinnamic acid in the concentration range of
2.5~40.0 $\mu\text{g/mL}$,
 $y = 0.0464x + 0.0331$, $r = 0.9996$ ($n = 5$).

- (V) Cinnamaldehyde in the concentration range of 4.5~72.0 $\mu\text{g/mL}$,
 $y = 0.0201x + 0.0212$, $r = 0.9998$ ($n = 5$).
 (VI) Schizandrin in the concentration range of 12.5~200.0 $\mu\text{g/mL}$,
 $y = 0.0272x + 0.0909$, $r = 0.9997$ ($n = 5$).
 (VII) Gomisin A in the concentration range of 5.0~80.0 $\mu\text{g/mL}$,
 $y = 0.0322x + 0.0337$, $r = 0.9995$ ($n = 5$).

III. Reproducibility Test

Using the standard solutions with various concentrations of loganin (18.75, 75.0, and 300.0 $\mu\text{g/mL}$), scopoletin (3.125, 12.5, and 50.0 $\mu\text{g/mL}$), ferulic acid (1.25, 5.0, and

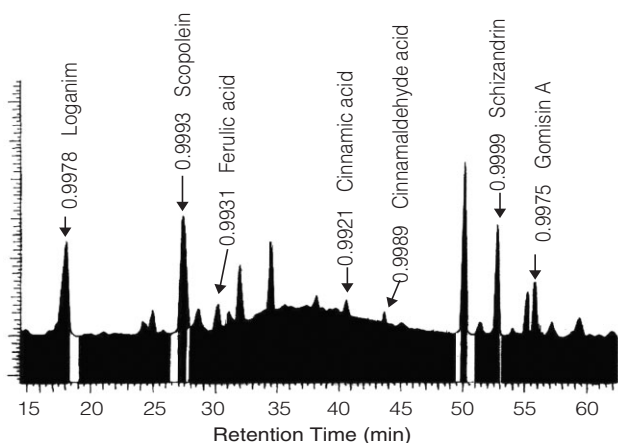


Figure 3. Peak purity of each marker substance in standard by HPLC photodiode array detector solutions of King-Mon-Long-Fong-Jyo.

20.0 $\mu\text{g/mL}$), cinnamic acid (2.5, 10.0, and 40.0 $\mu\text{g/mL}$), cinnamaldehyde (4.5, 18.0, and 72.0 $\mu\text{g/mL}$), schizandrin (12.5, 50.0, and 200.0 $\mu\text{g/mL}$), and gomisin A (5.0, 20.0, and 80.0 $\mu\text{g/mL}$), intra-day test (injecting each concentration five times during 24 hr) and inter-day test (injecting each concentration four times during 7 days with each injection separated by at least 24 hr) were used to check reproducibility. The coefficients of variation (C.V. %) were calculated from the experimental results as shown in Table 2. The intra-day relative standard deviations were 0.73~0.76%, 1.69~3.03%, 2.72~4.90%, 1.37~3.17%, 0.99~3.28%, 0.79~3.17%, and 0.45~4.67% for loganin, scopoletin, ferulic acid, cinnamic acid, cinnamaldehyde, schizandrin, gomisin A, respectively. Likewise, the inter-day relative standard deviations were 2.38~4.18%, 1.73~4.30%, 3.42~4.56%, 1.10~5.32%, 2.98~4.73%, 1.82~2.72%, and 1.68~3.74%, respectively.

C.V. values of different concentrations of the seven marker substances in the inter-day test were slightly higher, and the C.V. values increased at very low concentrations. The C.V. values were less than 5%, suggesting that method had very good reproducibility.

IV. Recovery Test

The 50% ethanol extraction of King-Mon-Long-Fong-Jyo was divided into four portions, with one portion used as a control group and the rest spiked with different concentrations of standard solutions to obtain various concentrations of loganin (18.75, 75.0, and 300.0 $\mu\text{g/mL}$), scopoletin (3.125, 12.5, and 50.0 $\mu\text{g/mL}$), ferulic acid (1.25, 5.0, and 20.0 $\mu\text{g/mL}$), cinnamic acid (2.5, 10.0, and 40.0 $\mu\text{g/mL}$), cinnamaldehyde (4.5, 18.0, and 72.0 $\mu\text{g/mL}$), schizandrin

Table 2. Reproducibilities of intra-day, inter-day and recovery analysis of King-Mon-Long-Fong-Jyo.

Compound	Concentration ($\mu\text{g/mL}$)	CV (%)		Recovery (%)
		Intra-day (n = 5)	Inter-day (n = 4)	
Loganin	18.750	0.76	4.18	106.18 \pm 4.65
	75.000	0.74	2.38	101.04 \pm 2.18
	300.000	0.73	2.47	103.74 \pm 4.42
Scopoletin	3.125	3.03	4.30	97.93 \pm 4.06
	12.500	2.89	2.58	102.35 \pm 2.27
	50.000	1.69	1.73	100.62 \pm 0.98
Ferulic acid	1.250	4.90	4.56	107.11 \pm 4.12
	5.000	3.46	3.95	103.04 \pm 4.57
	20.000	2.72	3.42	101.06 \pm 4.57
Cinnamic acid	2.500	3.17	5.32	103.11 \pm 4.09
	10.000	1.37	3.05	101.85 \pm 1.77
	40.000	1.83	1.10	100.12 \pm 0.68
Cinnamaldehyde	4.500	3.28	4.73	101.86 \pm 4.89
	18.000	0.99	2.98	99.90 \pm 3.36
	72.000	1.69	3.15	99.58 \pm 1.16
Schizandrin	2.500	3.17	2.72	111.69 \pm 4.60
	50.000	0.79	2.37	116.44 \pm 2.20
	200.000	1.75	1.82	113.67 \pm 3.97
Gomisin A	5.000	4.67	3.74	98.70 \pm 2.60
	20.000	2.37	2.86	102.18 \pm 1.29
	80.000	0.45	1.68	96.53 \pm 4.56

Table 3. Content of marker substance in six different rice wine extraction volumes of King-Mon-Long-Fong-Jyo.

Compound	2-fold	4-fold	8-fold	12-fold	16-fold	20-fold
Loganin	63.12 ± 4.87	65.60 ± 4.45	66.70 ± 2.83	67.70 ± 3.81	63.80 ± 4.99	62.82 ± 4.20
Scopoletin	6.60 ± 5.90	6.98 ± 3.77	7.80 ± 4.46	7.72 ± 3.50	7.80 ± 4.19	7.28 ± 6.20
Ferulic acid	3.60 ± 1.97	3.48 ± 2.96	4.20 ± 5.35	3.56 ± 5.07	3.52 ± 3.21	3.36 ± 2.54
Cinnamic acid	1.36 ± 1.53	2.14 ± 3.27	3.52 ± 4.92	3.74 ± 4.19	3.60 ± 3.32	3.70 ± 5.31
Cinnamaldehyde	1.38 ± 6.25	2.38 ± 6.10	4.96 ± 7.10	5.06 ± 4.99	4.68 ± 7.67	5.04 ± 2.88
Schizandrin	21.10 ± 3.38	28.60 ± 5.93	30.44 ± 5.22	31.16 ± 4.06	31.76 ± 5.09	31.46 ± 2.47
Gomisin A	6.50 ± 5.58	6.94 ± 4.99	7.44 ± 2.15	7.80 ± 4.47	8.04 ± 3.91	8.00 ± 5.27

※ Data represented as mean (mg/one dose) ± C.V. value (%).

One dose: *Schizandrae* Fructus 6.3 g, *Corni* Fructus 12.5 g, *Morindae* Radix 6.3 g, *Cistanchis* Herba 12.5 g, *Cinnamonomi* Cortex 2.5 g and *Angelicae* Radix 3.8 g.

Table 4. Content of marker substance in four different extract conditions of King-Mon-Long-Fong-Jyo.

Compound	Room temp. (25°C) 3 weeks	45°C · 24 hr	60°C · 12 hr	Boiling (90°C) · 3 hr
Loganin	59.32 ± 3.07	57.82 ± 1.54	62.28 ± 4.52	73.02 ± 2.54
Scopoletin	6.46 ± 4.19	10.20 ± 2.36	14.38 ± 1.67	16.68 ± 3.14
Ferulic acid	2.80 ± 6.21	3.42 ± 6.38	3.68 ± 1.70	4.48 ± 1.88
Cinnamic acid	2.22 ± 5.79	2.46 ± 6.41	2.42 ± 2.86	4.58 ± 2.91
Cinnamaldehyde	1.20 ± 7.24	1.82 ± 4.62	1.44 ± 6.04	2.04 ± 6.27
Schizandrin	27.64 ± 6.44	28.44 ± 4.84	27.34 ± 3.98	22.12 ± 1.07
Gomisin A	8.80 ± 3.61	6.74 ± 1.36	5.56 ± 5.41	4.20 ± 7.09

※ Data represented as mean (mg/one dose) ± C.V. value (%).

One dose: *Schizandrae* Fructus 6.3 g, *Corni* Fructus 12.5 g, *Morindae* Radix 6.3 g, *Cistanchis* Herba 12.5 g, *Cinnamonomi* Cortex 2.5 g and *Angelicae* Radix 3.8 g.

(12.5, 50.0, and 200.0 µg/mL), and gomisin A (5.0, 20.0, and 80.0 µg/mL). Internal standard nobiletin was added to each solution to a concentration of 20.0 µg/mL. All samples were filtered through a 0.45 µm membrane filter, injected into the HPLC column for analysis and the recovery calculated.

Good recovery of the analysis were obtained as follows: 101.04 ± 2.18~106.18 ± 4.65% for loganin, 97.93 ± 4.06~102.35 ± 2.27% for scopoletin, 101.06 ± 4.57~107.11 ± 4.12% for ferulic acid, 100.12 ± 0.68~103.11 ± 4.09% for cinnamic acid, 99.58 ± 1.16~101.86 ± 4.89% for cinnamaldehyde, 111.69 ± 4.60~116.44 ± 2.20% for schizandrin, and 96.53 ± 4.56~102.18 ± 1.29% for gomisin A, respectively (Table 2).

V. Analysis of the Sample Solutions

(I) Different rice wine extraction volume

Table 3 demonstrates content of each marker substance in six different rice wine extraction volumes of King-Mon-Long-Fong-Jyo. The optimized extraction volume is 8 or 12 times.

(II) Different extraction conditions

The quantitative analysis indicated that with the increase of temperature, the concentration of marker substances such as loganin, scopoletin, ferulic acid, cinnamic acid, cinnamaldehyde increased, whereas schizandrin and gomisin A are decreased (Table 4). This is probably

because the schizandrin and gomisin A one unstable under high temperature condition.

CONCLUSIONS

A multi-component HPLC method was developed for the simultaneous quantification of seven marker substances in King-Mon-Long-Fong-Jyo. A matrix of 20% acetonitrile and 70% acetonitrile, which were both adjusted to pH 3.0 with phosphoric acid, was used as the mobile phase in a gradient elution program, with an ODS column for the stationary phase. UV 250 nm was used for the detection of the marker substances. The internal standard used to determine the calibration line resulted in a precise and reliable quantification method. The results of the quantitative analysis showed that the optimal extraction condition of King-Mon-Long-Fong-Jyo was 8 or 12 times extraction volume (351.2 or 526.8 mL), refluxing at boiling temperature (90°C) for 3 hr. This method can be used to establish the standards for quality control to ensure accuracy, efficiency and manufacturing process of King-Mon-Long-Fong-Jyo in the future.

ACKNOWLEDGMENTS

This study was supported by a grant from the Committee on Chinese Medicine and Pharmacy, Department of Health, Executive Yuan, Republic of China (CCMP90-RD-009). The content of this paper does not

necessarily represent the opinion of Committee on Chinese Medicine and Pharmacy.

REFERENCES

1. Department of Health, Executive Yuan. 2000. The application of registration and license extension of following ten items should submit at least two of primary components. No. 89040256, R.O.C.
2. Lay, H. L., Chan, H. J. and Lin, C. F. 1997. Simultaneous analysis of six components in "Chai-Hu-Kuei-Chih-Tang" by high performance liquid chromatography. *J. Food Drug Anal.* 5(4): 381-390.
3. Lay, H. L., Denq, S. M., Liu, S. Y. and Sheu, B. W. 1999. Studies on the identification of Chinese drug material in yams (*Dioscorea* spp.). *J. Food Drug Anal.* 7(4): 313-325.
4. Lay, H. L. and Chen, C. C. 2000. Simultaneous analysis of eight components in "Pin-Wei-San" by high performance liquid chromatography. *J. Liquid Chromatogr. Rel. Technol.* 23(9): 1439-1450.
5. Lay, H. L., Sheu, C., Wu, Y. S. and Kuo, J. H. 2000. The development of manufacturing and analytical method of Hwan-Shio-Dan softgel. *J. Food Drug Anal.* 8(1): 35-43.
6. Lay, H. L., Shih, I. J., Yeh, C. H., Lin, C. F. and Liang, J. W. 2000. Simultaneous determination of five constituents in "Tzyy-Yun-Gau" medicine by high performance liquid chromatography. *J. Food Drug Anal.* 8(4): 304-308.
7. Lay, H. L., Liu, H. J., Liao, M. H., Chen, C. C., Liu, S. Y. and Sheu, B. W. 2001. Identification of Chinese drug materials in yams (*Dioscorea* spp.) by RAPD Analysis. *J. Food Drug Anal.* 9(3): 132-138.
8. Milena, B., Lubomír, O., Vladimír, C. and Helena, S. 2002. Liquid chromatographic analysis of supercritical carbon dioxide extracts of *Schizandra chinensis*. *J. Chromatogr. B* 770 (1-2): 283-289.
9. Tadashi, A. and Eiji, I. 2003. Use of synthetic adsorbents in preparative normal-phase liquid chromatography. *J. Chromatogr. A* 989(1):19-29.
10. Wu, H. K., Chuang, W. C. and Sheu, S. J. 1998. Separation of nine iridoids by capillary electrophoresis and high-performance liquid chromatography. *J. Chromatogr. A* 803(1-2): 179-187.
11. El-Sayed, N. H., Awaad, A. S., Hifnawy, M. S. and Mabry, T. J. 1999. A flavonol triglycoside from *Chenopodium murale*. *Phytochemistry* 51(4): 591-593.
12. Heavner, D. L., Morgan, W. T. and Ogden, M. W. 1996. Determination of volatile organic compounds and respirable suspended particulate matter in New Jersey and Pennsylvania homes and workplaces. *Environ. Int* 22(2): 159-183.
13. Lockwood, G. B. 1979. The major constituents of the essential oils of *Cinnamomum cassia* Blume growing in Nigeria. *Planta Med.* 36 (4): 380-381.
14. Kakinuma, K., Koike, J., Kotami, K., Ikekawa, N., Kada, T. and Nomoto, M. 1984. Cinnamaldehyde: identification of an antimutagen from a crude drug, cinnamoni cortex (*Cinnamomum cassia*). *Agric. Biol. Chem.* 48 (7): 1905-1906.
15. Takahashi, S., Hikino, H. and Sasaki, Y. 1958. Studies on Umbelliferous plants. IX. Studies on "Toki". (9). Components of the Root of *Angelica acutiloba* and *A. acutiloba* var. *sugiyamae*. *Yakugaku Zasshi* 79: 1156-1159.
16. Mitsuhashi, H., Nagai, U. and Muramatsu, T. 1961. Studies on the coctituents of Umbelliferae plants. III. Structure of ligustilide. *Chem. Pharm. Bull.* 9: 115-119.
17. Mitsuhashi, H., Muramatsu, T., Nagai, U., Nakano, T. and Ueno, K. 1963. Studies on the coctituents of Umbelliferae plants. VIII. Distribution of alkylphthalides in Umbelliferae plants. *Chem. Pharm. Bull.* 11(10): 1317-1319.
18. Bohrmann, H., Stahl, E. and Mitsuhashi, H. 1967. Studies on the coctituents of Umbelliferae plants. XIII. Chromatographic studies on the coctituents of *Cnidium officinale* Makino. *Chem. Pharm. Bull.* 15(10): 1606-1608.
19. Ruo, T. I., Wu, T. Y. and Yang, Y. Y. 1967. Comparison of chemical composition and effect on animal feeding of native and imported. *J. Chin. Agric. Chem. Soc.* 5(3,4): 93-99.
20. Yamagishi, T., Kaneshima, H., Kinoshita, Y. and Mori, M. 1974. Studies on the standardization of crude drugs produced in Hokkaido (part 5). On the existence of Ligustilide in *Angelicae Radix* (Touki). *Ann. Rep. Hokkaido Metr. Res. Lab. P. H.* 24: 47-51.
21. Yamagishi, T., Kaneshima, H., Kinoshita, Y. and Honma, S. 1975. Studies on the standardization of crude drugs produced in Hokkaido (part 7). The ether soluble components of *Angelicae Radix* (Touki). *Ann. Rep. Hokkaido Metr. Res. Lab. P. H.* 25: 21-24.
22. Yamagishi, T., Kaneshima, H., Kinoshita, Y. and Honma, S. 1975. Studies on the standardization of crude drugs produced in Hokkaido (part 8). The comparison quality and components of Touki cultivated in different places. *Ann. Rep. Hokkaido Metr. Res. Lab. P. H.* 25: 25-29.
23. Lin, M., Ju, C. D., Sun, C. M. and Fang, C. C. 1979. Studies on the chemical component of *Angelica sinensis* Diels. *Acta Pharm. Sinica.* 14(9): 529-534.
24. Fang, H. G., Lu, R. M., Liu, G. S. and Liu, T. C. 1979. Studies on the components of essential oils. *Acta Pharm. Sinica.* 14(10): 617-623.
25. Lu, R. M., Ho, L. Y., Fang, H. G. and Zhang, X. Q. 1980. Thin layer chromatography and densitometry of ligustilide in Umbelliferae plants. *Acta Pharm. Sinica.* 15(6): 371-374.
26. Watanabe, T., Yamamoto, A., Nagai, S. and Terabe, S. 1998. Micellar electrokinetic chromatography as an alternative to high-performance liquid chromatography for separation and determination of phenolic

- compounds in Japanese spirituous liquor. *J. Chromatogr. A* 793(2): 409-413.
27. Department of Health, Executive Yuan. 2001. Notes for unified formulas in tonic wine preparation of Chinese herb medicine. No. 0900002545, R.O.C.
28. Endo, T. and Taguchi, H. 1973. Study on the constituents of *Cornus officinalis* Sieb. *Et Zugg. Yakugaku Zasshi* 93(1): 30-32.
29. Ikeya, Y., Taguchi, H., Yosioka, I. and Kobayashi, H. 1979. The constituents of *Schizandra Chinensis* Bail, I. Isolation and structure determination of five new lignans, gomisin A, B, C, F and G, and the absolute structure of schizandrin. *Chem. Pharm. Bull.* 27(6): 1383-1394.
30. Tatum, J. H. and Berry, R.E. 1972. Six new flavonoids from *Citrus*. *Phytochemistry* 11: 2283-2288.