DOI 10.5012/bkcs.2010.31.11.3371

Quantitative and Pattern Recognition Analyses for the Quality Evaluation of Magnoliae Flos by HPLC

Zhe Fang, Chang Min Shen, Dong Cheul Moon,[†] Kun Ho Son,[‡] Jong Keun Son,[§] and Mi Hee Woo[*]

College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Korea. [*]E-mail: woomh@cu.ac.kr
[†]College of Pharmacy, Chungbuk National University. Cheongju 361-763, Korea
[‡]College of Life Science, Andong National University. Andong 760-740, Korea
[§]College of Pharmacy, Yeungnam University. Gyeongsan 712-749, Korea
Received March 10, 2010, Accepted September 15, 2010

In this study, quantitative and pattern recognition analysis for the quality evaluation of Magnoliae Flos using HPLC/UV was developed. For quantitative analysis, eleven major bioactive lignan compounds were determined. The separation conditions employed for HPLC/UV were optimized using ODS C_{18} column (250 × 4.6 mm, 5 μm) with isocratic elution of acetonitrile and water with 1% acetic acid as the mobile phase at a flow rate of 1.0 mL/min and a detection wavelength of 278 nm. These methods were fully validated with respect to the linearity, accuracy, precision, recovery, and robustness. The HPLC/UV method was applied successfully to the quantification of eleven major compounds in the extract of Magnoliae Flos. The HPLC analytical method for pattern recognition analysis was validated by repeated analysis of twenty one reference samples corresponding to seven different species of Magnoliae Flos and nine samples purchased from market. The results indicate that the established HPLC/UV method is suitable for the quantitative analysis and quality control of multi-components in Magnoliae Flos.

Key Words: Mgnoliae Flos, HPLC, Pattern recognition, Quality control

Introduction

Herbal medicines have a long history in therapeutic field and they are attracting considerable attention because of low toxicity and excellent therapeutic benefit. Quality control in synthetic drugs is conducted by measuring their medicinal components whereas quality control in herbal medicines traditionally measuring a representative compound (a marker compound) contained in the herbal medicines. Herbal drugs, individually and in combination, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall pharmacological efficacy. Therefore, quantitation of one or few components will not be an adequate approach for quality control of herbal medicines. Fingerprint analysis/pattern recognition with multivariate statistical analysis can provide the information of overall chemical composition of herbal medicines including the marker compounds traditionally used for quality control.[1]

Magnoliae Flos is the dried flower bud of *Magnolia denudata* or the other species in the Korean Pharmacopeia (K.P.), of *Magnolia salicifolia* Maximowicz, *Magnolia kobus* De Candolle, *Magnolia biondii* Pampanini, *Magnolia sprengeri* Pampanini or *Magnolia denudata* Desrousseaux in the Japanese Pharmacopoeia (J.P.), and of *Magnolia biondii* Pamp., *Magnolia denudata* Desr., or *Magnolia sprengeri* Pamp in the Chinese Pharmacopoeia (C.P.). Magnoliae Flos is controlled to contain not less than 0.4 % of magnolin in C.P. There are ten different species of Magnoliae Flos: *M. denudata* Desr., *M. biondii* Pamp., *M. salicifolia*, *M. obovata Thunb*., *M. denudata* var. *purpurascens*, *M. quinquepeta* var. *gracilis*, *M. liliiflora* Desr., *M. kobus*, *M. sprengeri* Pamp, and *M. grandiflora* L. We collected seven different species of Magnoliae Flos for this study from Korea and China. The drug was collected between late winter and early spring before flowering, removed from branchlet, and dried in the shade. Pharmacological studies have revealed in to have neuromuscular bloking,[2] inotropic effect,[3] anti-inflammatory,[4-7] anti-allergy,[8] anti-angiogenic,[9] PAF receptor antagolist[10] and vasorelaxant activities.[11] HPLC-UV analytical methods for biondnoid I,[12] magnolin,[13] magnolin and fargesin,[14] and magnolin and anthricin[15] had been reported. However, quantitative and pattern recognition analyses more than two marker compounds from Magnoliae Flos have never been reported. Therefore we developed multi-compounds quantitative analysis and pattern analysis to control the quality of Magnoliae Flos.

Some HPLC/UV analytical methods have been developed for the analysis of Magnoiae Flos and its related products.[13,14,16] However, these studies were focused only quantitative analysis of selected marker compounds which are not promising approaches for the quality control of multi-component herbal drugs. In the present study, a simple, sensitive and precise reverse-phase HPLC/UV method has been developed for the quantitative determination of eleven marker lignan components, eudesmin (**1**), magnolin (**2**), lirioresinol dimethyl ether (**3**), epimagnolin (**4**), aschantin (**5**), kobusin (**6**), fargesin (**7**), burchellin (**8**), 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran (**9**), ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione (**10**) and (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran (**11**) along with pattern-recognition method for the quality control of Magnoliae Flos extract. The anti-platelet-activating factor activity of lignans and neolignans such as compounds **1**~**6**[17] from *M. biondii* and **7**~**11**[18] from *M. denudata* had been reported. Thus, these bioactive marker compounds are very useful to pattern recognition analysis. The twenty one Magnoliae Flos authentic samples collected from China and Korea and nine Magnoliae

Flos samples purchased from the market were analyzed by HPLC after extraction with methylene chloride. Quantitative analysis of magnolin single compound in C.P. would not be an adequate approach for quality control of Magnoliae Flos. Therefore in pattern recognition analysis we used four marker compounds as eudesmin (**1**), magnolin (**2**), aschantin (**3**) and burchellin (**4**). In pattern analysis with multivariate statistical analysis we used R program (downloaded from web http://www.r-project.org) to analyze twenty one authentic samples of Magnoliae Flos and nine commercial ones. Subsequent pattern analysis was applied to assess the comprehensive quality of Magnoliae Flos.

Experimental Section

Plant material. Magnoliae Flos samples collected in 2007 for this study include the following accessions: Twenty one authentic Magnoliae Flos samples corresponding to seven different Magnoliae Flos species were collected from China and Korea, and nine commercially available Magnoliae Flos samples were purchased from market of Korea (Table 1).

Reagents. All of the standard compounds were provided by Prof. K. H. Son, Andong National University, Andong, Korea. Their structures were unambiguously identified by NMR and MS data, with the published data, such as eudesmin,[19] magnolin,[20] lirioresinol dimethyl ether,[21] epimagnolin,[22] aschantin,[10] kobusin,[23] fargesin,[24] burchellin[25] 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran,[26] ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione[26] and (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxo-benzofuran.[26] Their purities were above 95% as determined by HPLC and LC-MS/MS analysis, and the standard compound structures were shown Fig. 1. Internal standard, propyl 4-hydroxy benzoate (**12**) was purchased from Sigma Chemicals (St. Louis, MO, USA). Methanol and acetonitrile of HPLC grade were purchased from Merck K GaA (Darmstadt, Germany). All other chemicals used were of analytical grade unless otherwise noted. Distilled water was prepared using Milli-Q purification system (Millipore, Bed-ford, MA, USA).

Sample preparation. To determine the content of eleven marker compounds and pattern recognition analysis of Magnoliae Flos samples, the dried flower bud powder were used for each extraction. Magnoliae Flos sample was powdered and through 50 mesh, and about 3 g of the powder were accurately weighed and added 50 mL of methylene chloride, accurately measured

Table 1. Twentyone Magnoliae Flos samples corresponding to seven different *Magnolia* species and nine commercial ones

Samples	Species	Source	Obtained
m1	*Magnolia biondii* Pam.	Henan, China,	collected (2007. 07)
m2	*Magnolia biondii* Pam.	Henan, China,	collected (2007. 09)
m3	*Magnolia biondii* Pam.	Henan, China,	collected (2007. 03)
m4	*Magnolia denudata* Desr.	Yangsan, Chungbuk, Korea	collected (2007. 03)
m5	*Magnolia denudata* Desr.	Gyeongju, Gyeongbuk, Korea	collected (2007. 03)
m6	*Magnolia denudata* Desr.	Yeongcheon, Gyeongbuk, Korea	collected (2007. 03)
m7	*Magnolia denudata* Desr.	Cheongju, Chungbuk, Korea	collected (2008. 04)
m8	*Magnolia denudata* Desr.	Cheongju, Chungbuk, Korea	collected (2008. 03)
m9	*Magnolia denudata* Desr.	Cheongju, Chungbuk, Korea	collected (2008. 04)
m10	*Magnolia denudata* Desr.	Catholic university of Daegu, Korea	collected (2008. 01)
m11	*Magnolia denudata* Desr.	Gyeongju, Gyeongbuk, Korea	collected (2007. 03)
m12	*Magnolia kobus* De Candolle	Seoul, Korea	collected (2007. 03)
m13	*Magnolia kobus* De Candolle	Cheongju, Chungbuk, Korea	collected (2008. 03)
m14	*Magnolia kobus* De Candolle	Cheongju, Chungbuk, Korea	collected (2008. 04)
m15	*Magnolia liliiflora* Desr. = *Magnolia quinquepeta (Beuchoz)* Dandy	Cheongju, Chungbuk, Korea	collected (2008. 04)
m16	*Magnolia liliiflora* Desr.	Catholic university of Daegu, Korea	collected (2008. 01)
m17	*Magnolia liliiflora* Desr.	Catholic university of Daegu, Korea	collected (2008. 01)
m18	*Magnolia liliiflora* Desr.	Catholic university of Daegu, Korea	collected (2008. 01)
m19	*Magnolia denudata* var. *purpurascens*	Yeongcheon, Gyongbuk, Korea	collected (2007. 04)
m20	*Magnolia quinquepeta* var. *gracilis* = *Magnolia liliiflora* Desr. var. *gracilis* (Salisb.) Rehder	Eumseong, Chungbuk, Korea	collected (2008. 04)
m21	*Magnolia salicifolia* Maximowicz	Daejeon, Korean	collected (2008. 04)
m22	*Magnolia biondii* Pam.	Yangyeongsi, Daegu, Korea	purchased (2007. 06)
m23	*Magnolia biondii* Pam.	Yangyeongsi, Daegu, Korea	purchased (2007. 04)
m24	*Magnolia biondii* Pam.	Gyeongdong market, Korea	purchased (2007. 09)
m25	*Magnolia biondii* Pam.	Gyeongdong market, Korea	purchased (2007. 09)
m26	*Magnolia biondii* Pam.	Gyeongdong market, Korea	purchased (2007. 09)
m27	*Magnolia biondii* Pam.	Gyeongdong market, Korea	purchased (2007. 09)
m28	*Magnolia biondii* Pam.	Yeongcheon market, Korea	purchased (2007. 10)
m29	*Magnolia biondii* Pam.	Yeongcheon market, Korea	purchased (2007. 10)
m30	*Magnolia biondii* Pam.	Yeongcheon market, Korea	purchased (2007. 10)

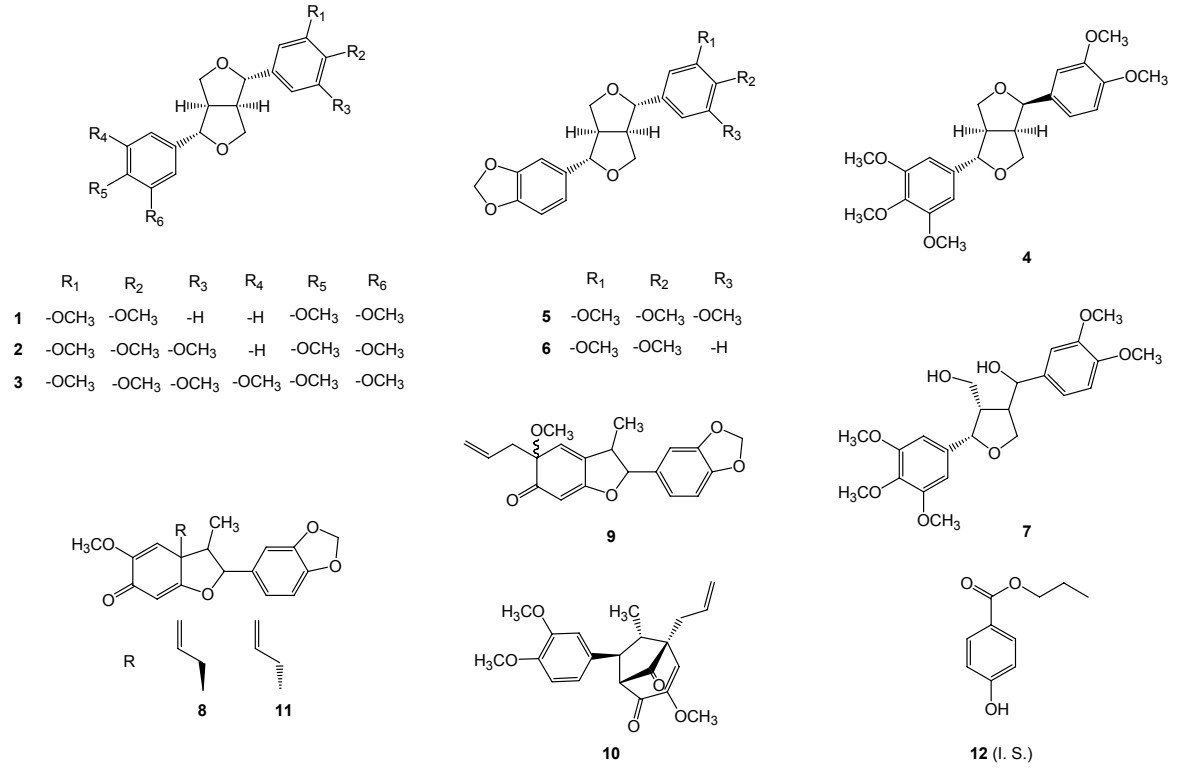

Figure 1. Chemical structures of standards. **1**. Eudesmin **2**. Magnolin **3**. Lirioresinol B dimethyl ether **4**. Epimagnolin **5**. Aschantin **6**. Kobusin **7**. Fargesin **8**. Burchellin **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran **12**. propyl 4-hydroxy benzoate (I.S.).

weigh and ultrasonicated for 45 min. at room temperature. The solution was cooled, weighed again, and made up the loss in weight with methylene chloride. The solution was filtered through a 0.45 μm membrane filter and the filtrate was used as the test solution. 10 μL of sample solution was subjected to injection into the HPLC system.

HPLC/UV condition. The HPLC equipment was a Waters HPLC system (Waters, Milford, MA, USA) with Waters 600 pumps, a Waters 486 UV detector and a Waters 717 autosampler. YMC ODS-H80 (250 × 4.6 mm, 4 μm), Shiseido capcell pak (250 × 4.6 mm, 5 μm) and Shodex ODS pak (250 × 4.6 mm, 5 μm) columns were tested with the guard columns filled with the same stationary phase. A mixture of 100% acetonitrile and 1% acetic acid of water (38:62, v/v %) was used as the mobile phase. The mobile phase was filtered under vacuum through a 0.45 μm membrane filter and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min with the detection wavelength set to 278 nm, and the total run time was 60 min. All compounds could be resolved with baseline separation at 278 nm with the maximum absorption. Hence, characteristic chromatographic patterns were obtained at 278 nm. The chromatograms were processed using software Empower pro system.

Analytical method validation.

Linearity: The calibration curves were made by diluting the stock solutions with 100% methanol. The reference solution of the eleven lignan compounds at concentrations of 620 ~ 50,000 ng/mL was analyzed by HPLC/UV. The regression equations were calculated in the form of $y = ax + b$, where y and x correspond to peak area and compound concentration, respectively.

Recovery, precision and accuracy: For the preparation of the crude extract, the powders of the dried flower bud of Magnoliae Flos were sieved through a 50 mesh. The recovery, precision and accuracy test were executed by mixing a powdered sample (3.0 g) with three control levels (20%, 50%, 100%) of the reference compounds. The mixture was then extracted by sonication with 50 mL of 100% methylene chloride at room temperature for 45 minutes. The extract solution was filtered through a 0.45 μm membrane. The HPLC/UV analysis experiments were performed in triplicate for each control level. The data was compared with those from the standard solution and extracted sample.

Pattern recognition analysis: The twenty one authentic samples of Magnoliae Flos were chosen as references for the quality control of Magnoliae Flos. To evaluate the phytochemical equivalency among the thirty samples corresponding to twentyone authentic and nine commercial ones, pattern recognition analysis was conducted. In this study we used four marker compounds (eudesmin (**1**), magnolin (**2**), aschantin (**3**) and burchellin (**4**)) for pattern recognition analysis.

Results and Discussion

Optimization of chromatographic condition. The HPLC conditions were selected by the requirement for obtaining the chromatograms with a better resolution of the adjacent peaks within a short retention time. For the optimization of chromatographic

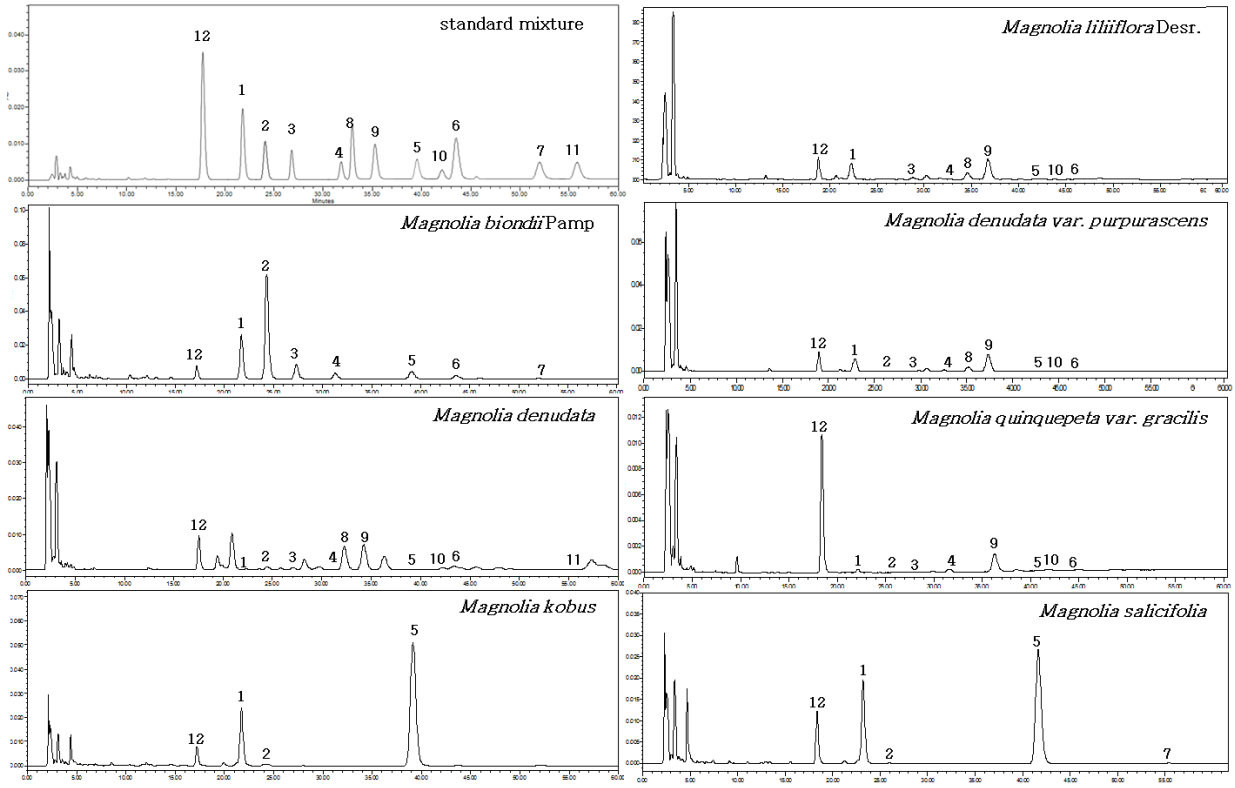

Figure 2. Chromatographies of standard mixture and seven different species of Magnoliae Flos. **1**. Eudesmin **2**. Magnolin **3**. Lirioresinol B dimethyl ether **4**. Epimagnolin **5**. Aschantin **6**. Kobusin **7**. Fargesin **8**. Burchellin **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran **12**. propyl 4-hydroxy benzoate (I.S.).

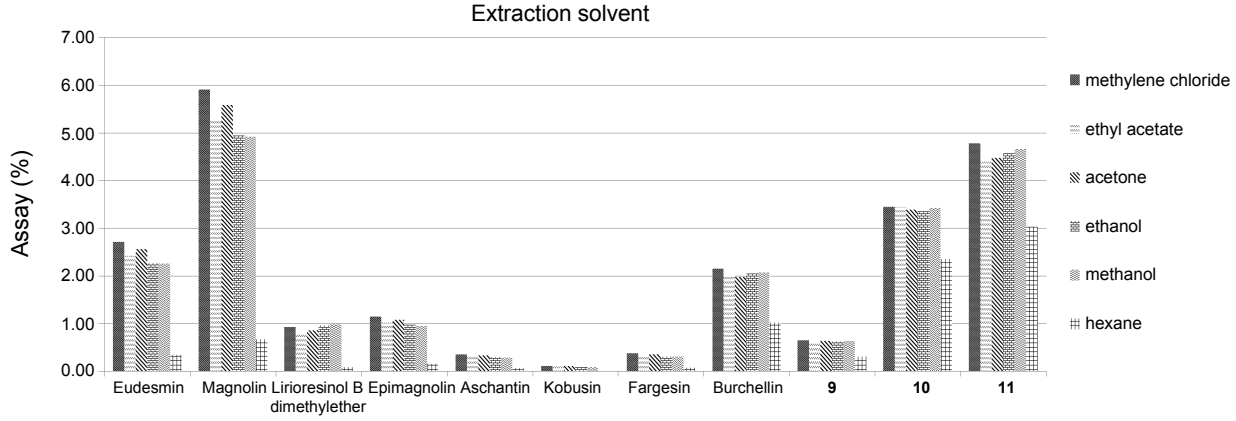

Figure 3. Comparison of the extraction solvents for extraction efficiencies of marker compounds. **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

condition, the effect of the composition of mobile phase on the separation was examined. Mobile phase of water-methanol did not result in the satisfactory separation of structurally similar compounds such as magnolin and eudesmin. Acetonitrile as an organic modifier demonstrated a significant improvement on separation. We had tested the addition of 0.1%, 1% and 10% acid (acetic acid, formic acid and phosphoric acid) to the mobile phase to do experiment. The addition of 1% acetic acid to the mobile phase to all of the compounds resulted in a good resolution, as well as satisfactory peak symmetry and shape. All compounds could be resolved with baseline separation at 278 nm with the maximum absorption. Hence, characteristic chromatographic patterns were obtained at 278 nm. The typical chromatograms of samples and standard mixture are shown in Fig. 2, from which one can observe that all target compounds and internal standard are completely separate within 60 minutes. Propyl 4-hydroxy benzoate (**12**) was selected as internal standard. The chromatographic peaks of the analytes in sample solution were identified by comparing their retention time with those of the references standards and further confirmed by spik-

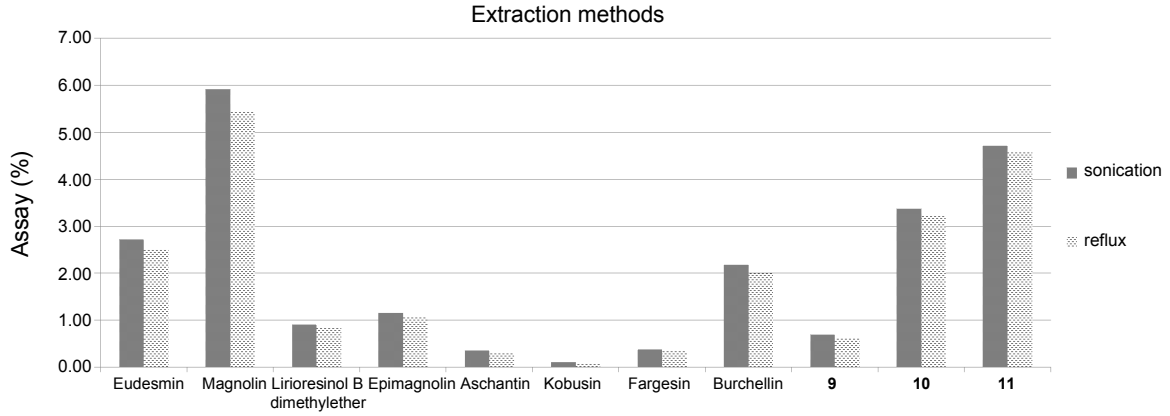

Figure 4. Comparison of the extraction methods (reflux and sonication) for extraction efficiencies of marker compounds. **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo [3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

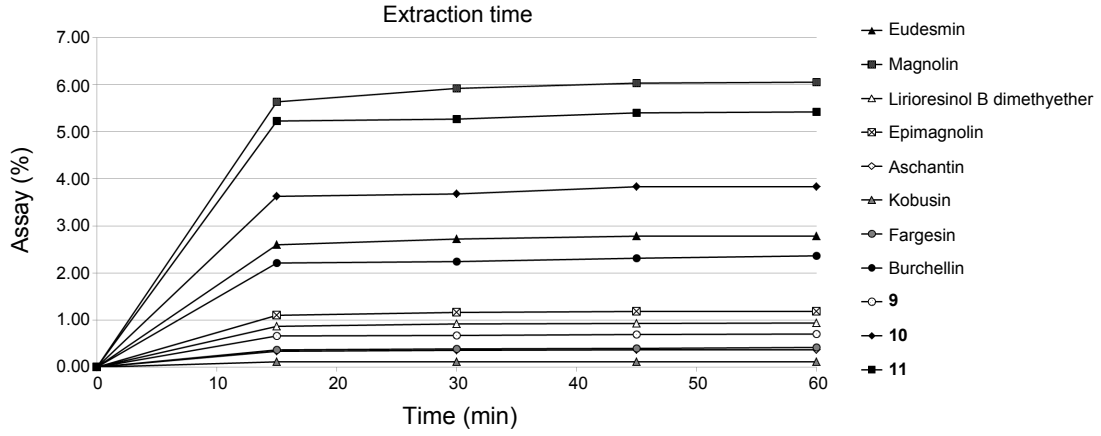

Figure 5. Comparison of the extraction time for extraction efficiencies of marker compounds. **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

Table 2. Calibration curve data, linear ranges, LOD and LOQ

Analytes	Linear range (ug/mL)	Slope (a)	Intercept (b)	Correlation coefficient (r)	LOD (ng/mL)	LOQ (ng/mL)
Eudesmin (**1**)	0.62 ~ 200	0.0489	0.0206	0.9999	12	36
Magnolin (**2**)	0.62 ~ 100	0.0256	0.0125	0.9999	13	39
Lirioresinol B dimethyl ether (**3**)	0.62 ~ 50	0.0810	−0.0021	0.9999	15	45
Epimagnoin (**4**)	0.62 ~ 50	0.0267	−0.0050	0.9999	12	36
Aschantin (**5**)	0.62 ~ 50	0.0501	0.0024	0.9999	11	33
Kobusin (**6**)	0.62 ~ 50	0.0272	−0.0069	0.9999	14	42
Fargesin (**7**)	0.62 ~ 50	0.0535	0.0200	0.9999	15	45
Burchellin (**8**)	0.62 ~ 200	0.0105	0.0071	0.9999	12	45
9	0.62 ~ 100	0.0566	0.0027	0.9999	13	41
10	0.62 ~ 200	0.0035	0.0060	0.9999	13	36
11	0.62 ~ 200	0.0061	0.0030	0.9999	15	33

9. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

ing samples with the reference compounds.

Optimization of sample preparation condition. Six extracting solvents, hexane, methylene chloride, ethyl acetate, acetone, ethanol and methanol were compared with regard to sample assays using sonication extraction. When sample was extracted with methylene chloride, the sample assay was higher than the other solvent samples. Therefore, we employed methylene chloride as an extracting solvent throughout this work (Fig. 3). Two

Table 3. Recovery of marker compounds through standard addition (n = 5)

Analyte	Fortified conc. (ug/mL)	Observed conc. (ug/mL)	Recovery mean (%)	RSD (%)	Analyte	Fortified conc. (ug/mL)	Observed conc. (ug/mL)	Recovery mean (%)	RSD (%)
Eudesmin	0	45.92	-	-	Fargesin	0	7.44	-	-
	20.0	64.94	95.07	0.49		3.5	10.94	100.02	0.76
	40.0	84.58	96.65	0.23		7.0	14.38	99.19	0.52
	80.0	122.28	95.45	0.44		14.0	21.34	99.28	0.81
Magnolin	0	113.55	-	-	Burchellin	0	71.02	-	-
	20.0	133.07	97.58	0.26		15	85.77	98.33	0.98
	50.0	164.74	102.37	0.40		35	107.16	103.26	0.50
	100.0	212.25	98.70	0.40		70	140.17	98.79	0.53
Lirioresinol B dimethyl ether	0	15.08	-	-	**9**	0	18.25	-	
	8.0	23.12	100.47	0.25		5.0	23.35	102.04	0.98
	16.0	31.03	101.37	0.28		10.0	28.60	103.52	0.95
	32.0	46.38	97.81	0.39		20.0	37.93	98.40	1.09
Epimagnolin	0	21.73	-	-	**10**	0	61.90	-	-
	10.0	32.22	104.88	0.37		15	76.20	95.33	1.26
	20.0	41.29	97.78	0.22		30	93.19	104.30	1.43
	40.0	60.72	97.45	0.45		60	120.22	97.21	0.57
Aschantin	0	6.55	-	-	**11**	0	116.84	-	-
	3.0	9.68	104.48	0.99		20	136.27	97.15	0.67
	6.0	12.57	100.26	0.58		50	167.93	102.18	0.76
	9.0	15.39	98.18	0.57		100	218.26	101.42	0.44
Kobusin	0	2.28	-	-					
	1.0	3.30	102.06	0.61					
	2.0	4.26	98.95	0.58					
	4.0	6.40	102.99	0.54					

9. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxy-phenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

extraction methods, ultra-sonication and reflux using methylene chloride extraction solvent, were compared with regard to sample assays. When used for sonication extraction method, the sample assay was higher than reflux one (Fig. 4). To determine the time needed to obtain complete extractions, extractions of a sample were performed for four different length of time (15, 30, 45 and 60 min). The rest of the variables employed were: room temperature, methylene chloride solvent and sonication extraction method. When extracted time was 45 min, the sample assay was same as 60 min, and higher than 15 and 30 min (Fig. 5). Therefore, when extracted time was 45 min, all of the compounds were sufficiently extracted.

Validation.

Linearity, LOD and LOQ: The linearity of the peak area ratio with respect to the concentration was examined under optimal HPLC/UV conditions and is described as a regression equation. Each coefficient of correlation (r^2) was > 0.999, as determined by least square analysis, suggesting good linearity between the peak areas and the compound concentrations over a wide concentration range. (Table 2) The limits of detection (LOD) were evaluated based on the lowest detectable peak in the chromatogram having asignal-to-noise (S/N) ratio of 3. Under our experimental conditions, we listed LOD and LOQ in Table 2. The obtained values for both LOD and LOQ for these eleven standards were shown to be low enough to detect traces of these compounds in either crude extract or its preparation.

Recovery: The extraction recovery test was performed by extracting a known amount of the eleven compounds from the Magnoliae Flos powder samples. A known amount of each standard compound at three different levels was mixed with the sample powder and extracted, as described in the experimental section. The % recovery of each standard ranged from 95.1 to 104.9%, and the RSD was less than 1.5% (Table 3). The average recovery was represented by the formula: R (%) = [(amount from the sample spiked standard – amount from the sample)/ amount from the spiked standard] × 100.

Precision and accuracy: Precision and accuracy were determined by multiple analysis (n = 3) of quality control samples prepared at lower, medium and higher concentration spanning the calibration range. Intra-assay precision and accuracy were determined from the variability of replicate analyses of quality control samples analyzed within the same analytical run. The remaining quality control samples had the intra-assay precision below 4.73% and accuracy between 92.58% and 105.13%. Inter-assay precision and accuracy were evaluated from the variability of triplicate analyses of quality control samples analyzed on single analytical run and extended for consecutive five days. The remaining quality control samples had the inter-assay precision lower than 3.28% and accuracy between 92.80% and 109.96%. The above data reflects that the developed method is highly reproducible and precision and accuracy data are presented in Table 4.

Robustness: The robustness was determined in order to evaluate the reliability of the established HPLC methods. All of the

Table 4. Precision and accuracy for determination of marker compounds

Analyte	Nominal conc. (ug/mL)	Intra-day (n = 3)				Inter-day (n = 3)			
		Observed	SD	Accuracy	Precision	Observed	SD	Accuracy	Precision
Eudesmin	20	19.01	0.17	95.07	0.89	19.02	0.12	95.11	0.63
	40	38.54	0.22	96.35	0.57	38.74	0.14	96.58	0.35
	80	77.16	0.54	96.45	0.70	77.50	0.19	96.88	0.24
Magnolin	20.0	19.35	0.09	96.75	0.47	19.16	0.27	98.47	1.37
	50.0	51.54	0.34	103.08	0.66	51.18	0.56	102.36	1.09
	100.0	98.40	0.48	98.40	0.49	98.94	0.24	98.94	0.24
Lirioreinol B dimethyl ether	8.0	8.18	0.15	102.28	1.86	8.24	0.11	102.95	1.28
	16.0	16.04	0.49	102.48	2.96	16.58	0.39	103.60	2.35
	32.0	31.52	0.39	98.51	1.25	31.65	0.68	98.90	2.16
Epimagnolin	10.0	9.44	0.32	94.37	3.44	9.54	0.31	95.40	3.28
	20.0	18.52	0.25	92.58	1.33	18.56	0.22	92.80	1.21
	40.0	37.90	0.57	94.80	1.51	38.04	0.24	95.10	0.62
Aschantin	3.0	2.90	0.07	96.83	2.45	2.26	0.02	95.42	0.92
	6.0	6.07	0.03	101.24	0.46	6.60	0.09	109.96	1.36
	9.0	9.00	0.03	100.00	0.31	8.74	0.05	97.91	0.56
Kobusin	1.0	1.01	0.05	101.45	4.73	1.04	0.03	104.01	2.46
	2.0	1.97	0.06	98.62	2.97	2.00	0.02	99.91	1.14
	4.0	4.11	0.06	102.86	1.56	4.13	0.04	103.26	0.99
Fargesin	3.5	3.37	0.06	96.30	1.92	3.39	0.09	96.74	2.75
	7.0	6.80	0.07	97.14	1.04	6.83	0.02	97.64	0.28
	14.0	13.69	0.07	97.78	0.53	13.86	0.02	99.01	0.15
Burchellin	15	14.85	0.17	99.00	1.14	14.65	0.12	97.67	0.82
	35	36.14	0.22	103.26	0.61	36.14	0.14	103.26	0.39
	70	69.16	0.54	98.80	0.78	69.14	0.19	98.77	0.27
9	5	4.96	0.01	99.2	0.20	5.25	0.11	105.0	2.10
	10	10.45	0.02	104.5	0.21	10.25	0.07	102.5	1.33
	20	19.67	0.02	98.35	0.10	19.70	0.13	98.5	0.66
10	15	14.35	0.09	95.67	0.63	14.25	0.27	95.00	1.89
	30	31.54	0.33	105.13	1.05	31.04	0.56	103.47	1.80
	60	58.4	0.43	97.33	0.74	58.25	0.24	97.08	0.41
11	20	19.18	0.15	95.90	0.78	19.68	0.11	98.4	0.56
	50	51.04	0.39	102.08	0.76	51.14	0.39	102.28	0.76
	100	101.52	0.21	101.52	0.21	101.32	0.68	101.32	0.67

9. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxy-phenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran.

parameters were maintained so there would not be any interference with the other peaks for the Magnoliae Flos extract. The experimental conditions, such as the column temperature and column species were purposely altered. The theoretical plate (N), capacity factor (k'), separation factor (α) and resolution (Rs) were evaluated. To evaluate the suitability of three different columns, YMC, Shiseido and Shodex, were compared with regard to four analytical factors (theoretical plate (N), capacity factor (k'), separation factor (α) and resolution (Rs)) on the column temperature of 25 °C. When used YMC column, the values of resolution and theoretical plate were higher than Shiseido and Shodex ones (Table 5). Therefore we selected YMC column to analyze samples throughout this work. Four different column temperatures, 25, 30, 35 and 40 °C, were compared with regard to four analytical factors using YMC column. When the column temperature was 25 °C, the values of resolution and theoretical plate were higher than the other column temperatures (Table 6). Therefore we selected column temperature as 25 °C.

Stability: The sample stability test was determined with a standard mixture solution at 0, 0.5, 1, 2, 5, 10, 15 and 30 days. During this period, the solution was stored at room temperature

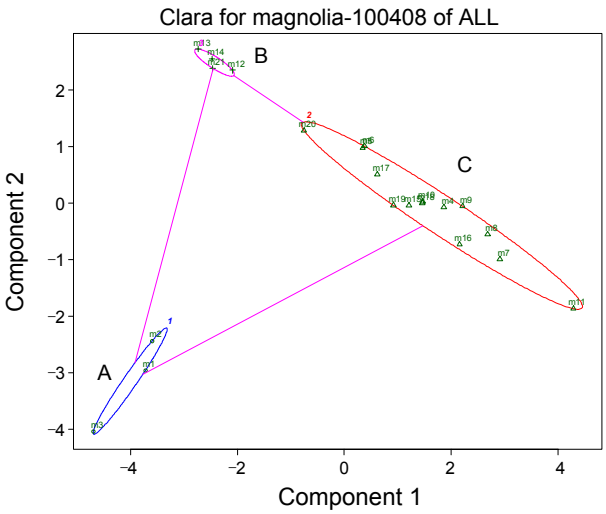

Figure 6. Clara of 21 authentic specimens of Magnoliae Flos. A (m1~m3: *M. biondii* Pamp.), B (m12~m14: *M. kobus*, m21: *M. salicifolia*.), C (m4~m11: *M. denudata* Desr., m15~m18: *M. liliiflora* Desr., m19: *M. denudata* var. *purpurascens*, m20: *M. quinquepeta* var. *gracilis*).

Table 5. Efficiency of marker compounds in different temperatures

		analytes										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Theoretical plate (*N*)												
25	mean	5624	5519	6714	12682	13951	15151	34565	11053	13304	14283	36489
	SD	67	98	128	258	227	298	169	249	233	268	148
30	mean	5236	4817	6560	11409	13718	16685	34419	12175	14007	14593	37321
	SD	51	106	102	308	143	315	318	266	273	277	103
35	mean	5054	4850	5318	11362	13824	16445	33876	11747	13686	14108	38542
	SD	62	96	125	475	170	171	179	234	251	314	117
40	mean	5591	5212	4615	12641	9978	14883	34436	12013	13712	14245	37913
	SD	56	72	108	284	327	413	297	296	301	329	156
Capacity factor (*k'*)												
25	mean	9.08	9.93	10.87	12.46	16.74	17.67	21.72	10.78	14.51	17.07	23.62
	SD	0.40	0.47	0.51	0.65	0.81	0.93	1.19	0.48	0.79	0.94	1.36
30	mean	8.85	9.78	10.86	12.59	16.08	17.71	20.75	10.51	14.73	17.31	23.96
	SD	0.62	0.67	0.74	0.87	1.05	1.11	1.41	0.52	0.62	0.81	1.44
35	mean	8.00	8.82	9.77	11.31	14.46	15.92	19.27	10.33	14.20	17.55	24.03
	SD	1.11	1.24	1.40	1.67	2.45	2.74	3.45	0.44	0.66	1.03	1.67
40	mean	8.85	9.80	10.88	12.59	16.04	17.68	20.69	11.02	14.38	17.85	23.44
	SD	0.61	0.66	0.71	0.86	1.11	1.16	1.47	0.57	0.71	1.07	1.51
Separation factor (α)												
25	mean	1.23	1.39	1.55	1.75	2.25	2.49	3.01	1.61	1.91	2.33	3.28
	SD	0.05	0.06	0.07	0.09	0.10	0.12	0.15	0.05	0.09	0.11	0.19
30	mean	1.23	1.36	1.51	1.75	2.24	2.47	2.89	1.63	1.96	2.38	3.41
	SD	0.03	0.04	0.04	0.04	0.02	0.02	0.16	0.03	0.11	0.17	0.15
35	mean	1.13	1.24	1.38	1.59	2.03	2.24	2.71	1.58	1.92	2.29	3.30
	SD	0.18	0.20	0.23	0.27	0.33	0.37	0.45	0.16	0.23	0.12	0.21
40	mean	1.24	1.38	1.53	1.77	2.25	2.48	2.91	1.66	1.99	2.34	3.38
	SD	0.04	0.05	0.05	0.05	0.03	0.04	0.17	0.04	0.05	0.21	0.18
Resolution (*Rs*)												
25	mean	3.74	2.68	2.85	3.51	6.97	2.92	7.20	3.44	4.53	2.93	7.41
	SD	0.78	0.06	0.19	0.37	0.41	0.27	0.47	0.38	0.41	0.21	1.11
30	mean	3.65	2.66	2.79	3.36	7.13	2.97	6.09	3.37	4.27	2.81	7.25
	SD	0.54	0.05	0.14	0.19	0.47	0.11	2.01	0.31	0.33	0.17	0.89
35	mean	3.46	2.69	2.67	3.12	6.37	2.62	7.10	3.19	4.63	2.67	7.52
	SD	0.72	0.05	0.04	0.22	1.03	0.28	0.95	0.22	0.48	0.29	1.25
40	mean	3.72	2.68	2.87	3.42	7.08	2.98	6.03	3.25	4.38	2.79	7.33
	SD	0.57	0.07	0.21	0.10	0.54	0.12	1.95	0.17	0.59	0.27	0.97

1. eudesmin **2**. magnolin **3**. lirioresinol B dimethyl ether **4**. epimagnolin **5**. aschantin **6**. kobusin **7**. fargesin **8**. burchellin **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran Column species: YMC column.

and 4 °C. the resulting data indicate that all marker analytes remained stable more than 98% during the experimental period.

Sample analysis. The developed HPLC/UV method was then applied to the simultaneous determination of the eleven compounds, eudesmin (**1**), magnolin (**2**), lirioresinol dimethyl ether (**3**), epimagnolin (**4**), aschantin (**5**), kobusin (**6**), fargesin (**7**), burchellin (**8**), 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran (**9**), ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione (**10**) and (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran (**11**) in the Magnoliae Flos. Twenty one authentic Magnoliae Flos samples corresponding to seven other Magnoliae Flos species and nine commercially available Magnoliae Flos samples were obtained from Korea and China. The developed analytical method was subsequently applied to the simultaneous determination of the eleven components in Magnoliae Flos extract. The quantity of each compound present in samples was determined and the results are summarized in Table 7. Each sample was analyzed in triplicate to ensure the reproducibility of the quantitative result. The results indicated that, magnolin (2.7 - 3.7%), eudesmin (0.5 - 1.7%) and epimagnolin (0.1 - 0.9%) were found to be the most abundant components in *M. biondii*, whereas eudesmin (0.4 - 0.8%) and aschantin (1.2 - 2.3%) in *M. kobus* and *M. salicifolia*, and burchellin (1.1 - 2.5%) and (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran (1.8 - 4.9%) in *M. denudata*. *M. liliiflora*, *M. denudata* var. *purpurascens* and *M. quinquepeta* var. *gracilis* contained the standard compounds **8**~**11**, therefore these three species of Magnoliae Flos clustered one

Table 6. Efficiency of marker compounds in different columns

		analytes										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Theoretical plate (*N*)												
YMC	mean	5412	5001	4982	15943	12686	17164	35455	12175	14007	14593	37321
	SD	64	79	127	397	270	466	270	266	273	277	103
Shiseido	mean	5054	4850	5318	14362	13824	16445	33876	11747	13686	14108	38542
	SD	62	96	132	475	170	171	179	234	251	314	117
Shodex	mean	5092	4861	4628	15807	10867	17132	35300	12013	13712	14245	37913
	SD	62	55	108	398	220	250	252	296	301	329	156
Capacity factor (*k'*)												
YMC	mean	9.02	9.98	11.03	12.86	16.55	18.24	22.10	10.51	14.73	17.31	23.96
	SD	0.61	0.67	0.81	0.82	0.90	0.98	1.10	0.52	0.62	0.81	1.44
Shiseido	mean	8.63	9.53	10.57	12.25	15.73	17.31	20.27	10.33	14.20	17.55	24.03
	SD	0.94	1.06	1.19	1.41	1.63	1.79	1.96	0.44	0.66	1.03	1.67
Shodex	mean	7.70	8.49	9.36	10.88	13.92	15.32	18.56	11.02	14.38	17.85	23.44
	SD	0.61	0.69	0.72	0.97	1.58	1.78	2.32	0.57	0.71	1.07	1.51
Separation factor (α)												
YMC	mean	1.26	1.39	1.54	1.79	2.31	2.54	3.08	1.63	1.96	2.38	3.41
	SD	0.00	0.00	0.01	0.00	0.04	0.05	0.10	0.03	0.11	0.17	0.15
Shiseido	mean	1.12	1.24	1.38	1.60	2.05	2.25	2.64	1.58	1.92	2.29	3.30
	SD	0.18	0.20	0.23	0.27	0.33	0.36	0.38	0.16	0.23	0.12	0.21
Shodex	mean	1.13	1.24	1.37	1.59	2.03	2.24	2.71	1.66	1.99	2.34	3.38
	SD	0.18	0.20	0.22	0.27	0.33	0.37	0.46	0.04	0.05	0.21	0.18
Resolution (*Rs*)												
YMC	mean	4.15	2.65	2.66	3.55	7.21	2.94	7.65	3.37	4.27	2.81	7.25
	SD	0.12	0.04	0.02	0.33	0.15	0.10	0.46	0.31	0.33	0.17	0.89
Shiseido	mean	3.37	2.68	2.76	3.35	7.14	2.82	6.00	3.19	4.63	2.67	7.52
	SD	0.66	0.08	0.14	0.20	0.46	0.19	1.93	0.22	0.48	0.29	1.25
Shodex	mean	3.38	2.69	2.67	3.32	6.43	2.67	6.98	3.25	4.38	2.79	7.33
	SD	0.59	0.05	0.04	0.55	1.11	0.36	0.77	0.17	0.59	0.27	0.97

1. eudesmin **2**. magnolin **3**. lirioresinol B dimethyl ether **4**. epimagnolin **5**. aschantin **6**. kobusin **7**. fargesin **8**. burchellin **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran Column temperature: 25 °C.

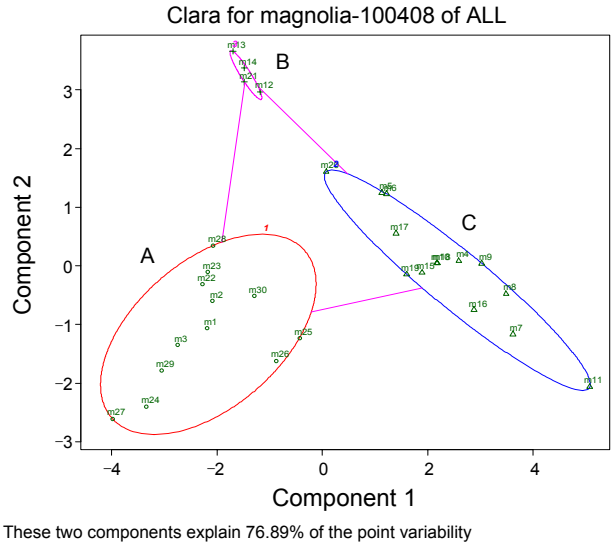

Figure 7. Clara of 30 specimens of Magnoliae Flos including 9 commercial samples. A (m1~m3: *M. biondii* Pamp. m22~m30: commercial Magnoliae Flos samples), B (m12~m14: *M. kobus*, m21: *M. salicifolia*.), C (m4~m11: *M. denudata* Desr., m15~m18: *M. liliiflora* Desr., m19: *M. denudata* var. *purpurascens*, m20: *M. quinquepeta* var. *gracilis*).

group, and the most abundant component was (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran (0.9 - 4.6%). In the quantitative analysis of Magnoliae Flos we indicated that the Magnoiae Flos samples clustered three groups same as mentioned below.

Pattern recognition analysis. From the pattern analysis of Clara (Fig. 6 and Fig. 7) and Hclust analyses (Fig. 8), we indicated that all of the samples were clustered to three groups (A~C); A: *M. biondii*, B: *M. kobus* and *M. salicifolia,* and C: *M. denudata*, *M. liliiflora*, *M. denudata* var. *purpurascens* and *M. quinquepeta* var. *gracilis*. In authentic specimen analysis, we can built three cluster (A~C), and all of the species were successfully clustered three groups (A~C). In Clara analysis (Fig. 6), 21 authentic specimen samples were clustered three groups (A~C). In Hclust analysis (Fig. 8) of 21 authentic specimen samples, we also found three clustering groups, exhibiting the same result as Clara analysis (Fig. 6). M1~m3 samples belong to the group A, m12~m14 and m21 samples belong to the group B, and another samples belong to the group C. In Clara analysis (Fig. 7), the commercial Chinese Magnoliae Flos samples (m22~m30) available from Korean markets were clustered together with the *M. biondii* group (m1~m3). The commercial samples were purchased at different market and have different

Table 7. Assay of compounds **1**~**11** in Magnoliae Flos (n = 3)

Samples	Assay (%)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
m1	0.5416	2.7908	1.1532	0.1442	0.1404	0.1315	0.0091	-	-	-	-
m2	1.2861	3.0632	0.4851	0.5603	0.1980	0.0624	0.1978	-	-	-	-
m3	1.6995	3.6960	0.5225	0.8894	0.2313	0.0670	0.3106	-	-	-	-
m4	0.0103	0.0808	0.9060	0.0018	0.0005	0.0312	0.0016	1.3307	0.4057	1.0015	3.4566
m5	0.0133	0.0334	0.4348	0.0020	0.0005	0.0027	0.0041	1.0761	0.2344	0.4197	1.8558
m6	0.0082	0.0352	0.2457	0.0023	0.0006	0.0308	0.0045	1.3125	0.2098	0.4718	1.7615
m7	0.0061	0.0281	0.8006	0.0022	0.0074	0.1771	0.0133	1.7844	0.7712	0.6056	3.2542
m8	0.0044	0.0272	0.8538	0.0015	0.0126	0.0740	0.0090	1.5575	0.7351	1.7587	2.5071
m9	0.0002	0.0190	0.5918	0.0021	0.0054	0.0792	0.0041	2.4891	0.4365	0.6372	3.4344
m10	0.0020	0.0280	0.6999	0.0015	0.0004	0.0876	0.0040	1.5408	0.3189	0.3809	2.7157
m11	0.0037	0.0293	1.1937	0.0021	0.0005	0.1654	0.0143	1.9554	0.5805	2.8963	4.5117
m12	0.3860	0.0077	0.0040	0.0029	1.2012	0.0040	0.0059	-	-	-	-
m13	0.8294	0.0045	0.0023	0.0017	2.2608	0.0023	0.0034	-	-	-	-
m14	0.6337	0.0103	0.0054	0.0039	1.8585	0.0053	0.0079	-	-	-	-
m15	0.0024	0.0179	0.5700	0.0015	0.0004	0.1338	0.0135	0.9487	0.5432	0.0455	1.6694
m16	0.0127	0.0316	0.8976	0.0016	0.0004	0.1172	0.0269	1.0263	0.6120	1.2559	2.2389
m17	0.0031	0.0091	0.3415	0.0014	0.0003	0.0913	0.0124	0.8900	0.3161	0.6565	1.1294
m18	0.0063	0.0222	0.5999	0.0016	0.0004	0.0941	0.0131	0.1167	0.4177	0.6092	4.6151
m19	0.0019	0.0097	0.5601	0.0017	0.0004	0.1415	0.0183	0.5978	0.3332	0.4130	1.6431
m20	0.0000	0.0029	0.1310	0.0012	0.0003	0.0253	0.0379	0.0044	0.1992	0.1783	0.8945
m21	0.7711	0.0056	0.0030	0.0022	1.5479	0.0029	0.0043	-	-	-	-
m22	1.3450	2.4343	0.3377	0.6899	0.1724	0.0457	0.2386	-	-	-	-
m23	1.1628	2.1526	0.2774	0.7193	0.1250	0.0397	0.2339	-	-	-	-
m24	2.3088	4.9755	0.6874	1.0514	0.3178	0.0921	0.3699	-	-	-	-
m25	1.4656	3.6986	0.5931	0.5643	0.2471	0.0795	0.2021	-	-	-	-
m26	0.7184	3.2687	1.1661	0.4837	0.1600	0.1257	0.0467	-	-	-	-
m27	1.2608	2.0739	0.3951	2.6970	0.1744	0.0596	0.7546	-	-	-	-
m28	0.9393	2.1593	0.0394	0.6118	0.1447	0.0457	0.2076	-	-	-	-
m29	1.9186	4.0571	0.5548	1.0339	0.2561	0.0759	0.3614	-	-	-	-
m30	0.7458	2.1629	0.6852	0.4463	0.1261	0.0762	0.1492	-	-	-	-

1. Eudesmin **2**. Magnolin **3**. Lirioresinol B dimethyl ether **4**. Epimagnolin **5**. Aschantin **6**. Kobusin **7**. Fargesin **8**. Burchellin **9**. 5-allyl-5-methoxy-3-methyl-2-piperonyl-2,3,5,6-tetrahydro-6-oxo-benzofuran **10**. ((1*S*,5*S*,6*S*,7*S*)-5-allyl-6-methyl-3-methoxy-7(3',4'-dimethoxyphenyl)-bicyclo[3.2.1]oct-3en-2,8-dione **11**. (2*R*,3*S*,3a*R*)-3a-allyl-5-methoxy-3-methyl-2-piperonyl 2,3,3a,6-tetrahydro-6-oxobenzofuran m1~m3: *M. biondii* Pamp., m4~m11: *M. denudata* Desr., m12~m14: *M. kobus*, m15~m18: *M. liliiflora* Desr., m19: *M. denudata* var. *purpurascens*, m20: *M. quinquepeta* var. *gracilis,* m21: *M. salicifolia,* m22~m30: commercial Magnoliae Flos samples.

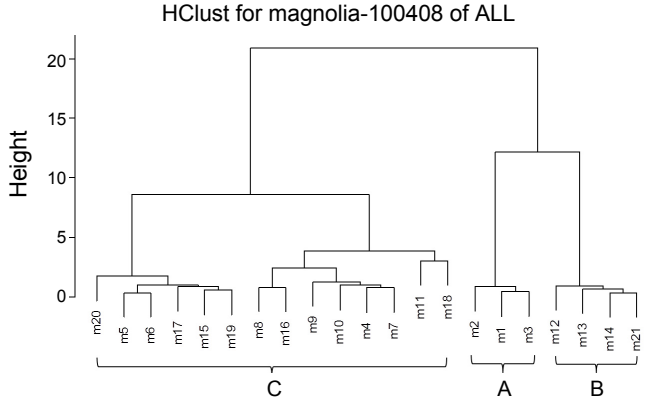

Figure 8. Hclust of 21 authentic specimens of Magnoliae Flos. A (m1~m3: *M. biondii* Pamp.), B (m12~m14: *M. kobus*, m21: *M. salicifolia*.), C (m4~m11: *M. denudata* Desr., m15~m18: *M. liliiflora* Desr., m19: *M. denudata* var. *purpurascens*, m20: *M. quinquepeta* var. *gracilis*).

producer, but m22~m30 were clustered with group A (m1~m3). Therefore, all of the commercial Chinese samples were *M. biondii*. It was due to only *M. biondii* among any other *Magnolia* species to contain not less than 0.4% of magnolin in C.P. Therefore, this result demonstrated that pattern recognition analysis can provide more comprehensive information for the chemical equivalency which can be omitted in the general simultaneous quantitative analyses. Thus, the pattern analysis result will be used to check the quality control of Magnoliae Flos. Also seven other *Magnolia* species had three different chromatographic patterns same as mentioned above (Fig. 2). Magnolin and eudesmin were found to be the major peaks in *M. biondii*, whereas eudesmin and aschantin in *M. kobus* and *M. salicifolia*, and burchellin and compound **9** in *M. denudata*. However compounds **1**-**2** and **4**-**7** were not nearly found in *M. denudata*, *M. liliiflora*, *M. denudata* var. *purpurascens* and *M. quinquepeta* var. *gracilis*. On the other hands, compounds **8**-**10** were not

found in *M. biondii*, *M. kobus* and *M. salicifolia*.

Conclusions

A rapid and optimized chromatographic method with UV detection was designed for the quality control of Magnoliae Flos, well-known Korean traditional medicine. Validation data indicates that the developed analytical methods are suitable to measure the concentration of eleven compounds to apply to pattern recognition analysis of Magnoliae Flos. The developed HPLC/UV method for quantitative analysis of major bioactive compounds, along with a pattern-recognition method, can provide the promising prospect to comprehensive quality control of Magnoliae Flos and its related herbal medicine. Our results confirm that eudesmin and magnolin can serve as the species-specific marker compounds to distinguish authentic *M. biondii*, aschantin to distinguish authentic *M. kobus* and *M. salicifolia,* and burchellin to distinguish authentic *M. denudata* from seven other *Magnolia* species in a pattern-recognition analysis. Therefore it is considered that magnolin, eudesmin, aschantin and burchellin are adequate as marker compounds of quality control to distinguish the different species of Magnoliae Flos. In the pattern recognition analysis we indicated that, all of the samples were clustered to three groups (A~C), and the commercial Chinese samples (m22~m30) available at Korean markets were clustered together with the *M. biondii* group (m1~m3). Therefore, all of the commercial Chinese Magnoliae Flos samples were *M. biondii*. HPLC fingerprinting analysis of the entire pattern of chromatographic profiles of the herbal products could serve as a complementary tool for quality control and for evaluation of quality consistency of herbal preparations. As herbal medicinal preparations have chemical complexity, it is very difficult to identify and determine all of their chemical components. Using both quantitative and fingerprint methods would be complementary approach for the quality control of the herbal preparations.

Acknowledgments. This research was supported by a grant (08182KFDA260) from Korea Food & Drug Administration in 2008.

References

1. Islam, M. N.; Lee, S. K.; Jeong, S. Y.; Kim, D. H.; Jin, C. B.; Yoo, H. H. *Bull. Korean Chem. Soc.* **2009**, *30*, 137.
2. Kimura, I.; Kimura, M.; Yoshizaki, M.; Yanada, K.; Kadata, S.; Kikuchi, T. *Planta Med.* **1983**, *48*, 43.
3. Watanabe, H.; Shimizu, H. *Japan. J. Pharmacol.* **1989**, *50*, 75.
4. Kimura, M.; Suzuki, J.; Yamada, T.; Yoshizaki, M.; Kikuchi, T.; Kadota, S.; Matsuda, S. *Planta Med.* **1985**, *50*, 291.
5. Wang, W. W.; Shen, Y. J.; Qi, Y. *Shanxi Yiyao Zazhi* **2000**, *29*, 206.
6. Wang, W. W.; Shen, Y. J.; Qi, Y.; Liu, P. P. *Zhonggo Shouyi Xuebao* **2005**, *25*, 301.
7. Lim, H. B.; Sohn, H. O.; Lee, D. W. *Korean J. Medicinal Crop Sci.* **2005**, *13*, 245.
8. Tsuruga, T.; Ebizuka, Y.; Nakajima, J.; Chun, Y. T.; Noguchi, H.; Iitaka, Y.; Sankawa, U. *Chem. Pharm. Bull.* **1991**, *39*, 3265.
9. Kobayash, S.; Kimura, I.; Kimura, M. *Biol. Pharm. Bull.* **1996**, *19*, 1304.
10. Pan, J. X.; Hensens, O.; Zink, D.; Chang, M. N.; Hwang, S. B. *Phytochemistry* **1987**, *26*, 1377.
11. Yin, M. H.; Kang, D. G.; Choi, D. H.; Kwon, T. O.; Lee, H. S. *J. Ethnopharmacol.* **2005**, *99*, 113.
12. Yu, Z. Y.; Sun, Z. F.; Su, B. Z.; Liu, Q. *Zhongcaoyao* **2004**, *35*, 574.
13. Fang, H.; Guo, Q.; Su, W.; Deng, F.; Wang, K. Q. *Yaowu Fenxi Zazhi* **2002**, *22*, 342.
14. Xu, L. H.; Cui, B. G.; Yu, Z. Y. *Yaowu Fenxi Zazhi* **2003**, *23*, 426.
15. Choi, J. Y.; Choi, E. H.; Oh, J. S.; Jung, H. W.; Kim, D. C.; Lee, H. S.; Jong, G.; Kim, J. A.; Son, J. K.; Lee, S. H. *Saengyak Hakhoechi* **2007**, *38*, 281.
16. Chen, G. F.; Feng, Y. F.; Meng, Q. *Guangdong Yaoxueyuan Xuebao* **2003**, *19*, 99.
17. Ma, Y. L.; Han, G. Q. *Zhongguo Zhongyao Zazhi* **1995**, *20*, 102.
18. Kuroyanagi, M.; Yoshida, K.; Yamamoto, A.; Miwa, M. *Chem. Pharm. Bull.* **2000**, *48*, 832.
19. Miyauchi, T.; Ozawa, S. *Phytochemistry* **1998**, *47*, 665.
20. Miyazawa, M.; Kasahara, H.; Kameoka, H. *Phytochemistry* **1995**, *39*, 1027.
21. Fish, F.; Waterman, P. G. *Phytochemistry* **1972**, *11*, 1527.
22. Pelter, A.; Ward, R. S.; Nishono, C. *Tetrahedron Lett.* **1977**, *18*, 4137.
23. Lida, T.; Nakano, M.; Ito, K. *Phytochemistry* **1983**, *22*, 211.
24. Yoshida, S.; Yamanaka, T.; Miyake, T.; Moritani, Y.; Ohmizu, H.; Iwasaki, T. *Tetrahedron* **1997**, *53*, 9585.
25. Lima, O. A.; Gottlieb, O. R.; Magalhaes, M. T. *Phytochemistry* **1972**, *11*, 2031.
26. Li, J.; Tanaka, M.; Kurasawa, K.; Ikeda, T.; Nohara, T. *Chem. Pharm. Bull.* **2005**, *53*, 235.