

Qualitative and quantitative analysis of nine major compounds in the Bozhougyiqi-Tang using a high-performance liquid chromatography coupled with a diode array detector and electrospray ionization mass spectrometer

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Submitted: 29-06-2012

Revised: 28-07-2012

Published: 11-06-2013

ABSTRACT

Background: Bozhougyiqi-Tang (BZYQT) is of traditional herbal medicine used for enhancement of digestive capacity. **Objective:** An accurate and reliable simultaneous determination using a HPLC-DAD and ESI-MS was developed and validated for the qualitative and quantitative analysis of 9 major compounds, ferulic acid (1), naringin (2), hesperidin (3), decursinol (4), glycyrrhizin (5), saikosaponin A (6), 6-gingerol (7), ginsenoside Rg3 (8), decursin (9), in traditional herbal medicine 'Bozhougyiqi-Tang.' **Materials and Methods:** The chromatographic separation of 9 compounds was performed on a SHISEIDO C₁₈ column (250 mm × 4.6 mm i.d., S-5 μm) using gradient elution with 0.1% trifluoroacetic acid and acetonitrile at a flow rate of 1.0 ml/min. The 9 compounds were identified based on peak retention time and UV spectrum and MS data of these compounds. **Results:** This developed method showed good linearity ($R^2 > 0.999$). The LOD and LOQ of the major compounds were less than 0.09 and 0.28 μg/ml, respectively. The intra - day and inter - day RSD values were within 2.06% and 1.64%, respectively. The mean recoveries were from 92.10% to 108.56% with less than 1.88%. The results indicated that established method had good precision and accuracy. **Conclusion:** The new method was successfully applied to the simultaneous analysis of 9 compounds in Bozhougyiqi-Tangs samples.

Key words: Bozhougyiqi-Tang, simultaneous determination, high-performance liquid chromatography-diode array detector, liquid chromatography-mass spectrometry, major compounds

INTRODUCTION

Bozhougyiqi-Tang (BZYQT) is one of traditional herbal medicines consisting of *Panax ginseng*, *Astragalus membranaceus*, *Angelica gigas*, *Bupleurum falcatum*, *Citrus unshiu*, *Glycyrrhiza uralensis*, *Atractylodes japonica*, *Zingiber officinale*, *Zizyphus jujube*, *Cimicifuga heracleifolia*. This herbal medicine has been widely used for enhancement of digestive capacity and clinical treatment of pulmonary tuberculosis. Recently, BZYQT has been reported various activities, including anti-allergy,

anti-inflammatory, anti-cancer, skin protection and so on.^[1,2] Traditional herbal medicines have a long history and have been widely used in many countries. Research about the multiple therapeutic effects of the traditional herbal medicines has been increasing. The therapeutic effects of herbal medicines are attributed to various compounds in it.^[3-5] However, because of change in content of many compounds in constituent herbs by many factors, such as cultural environment, processing and storage, it is difficult to evaluate quality and therapeutic efficacy of traditional herbal medicines. Thus, stable and reliable analytical method for simultaneous determination of various compounds will be helpful for evaluation of the quality of the traditional herbal medicine.

Generally, high-performance liquid chromatography

Access this article online

Website:

www.phcog.com

DOI:

10.4103/0973-1296.113291

Quick Response Code:**Address for correspondence:**

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(HPLC), one of the chromatography and relative techniques, is used to analyze the traditional herbal medicine. HPLC is able to separate complex compounds mixture.^[6] HPLC is quantitative analyzed and qualitative determined by combined various equipment such as diode array detector (DAD), evaporative light scattering detection (ELSD), and MS spectrum.^[7]

In this study, rapid, sensitive, and reliable simultaneous determination for the qualitative and quantitative analysis of 9 major compounds (ferulic acid in *Cimicifuga heracleifolia*, naringin and hesperidin in *Citrus unshiu*, decursinol and decursin in *Angelica gigas*, glycyrrhizin in *Glycyrrhiza uralensis*, saikosaponin A in *Bupleurum falcatum*, 6-gingerol in *Zingiber officinale*, ginsenoside Rg3 in *Panax ginseng*) of each herb in BZYQT was developed by HPLC coupled with DAD and ESI-MS [Figure 1]. In this technology, the major compounds were quantitatively determined by HPLC-DAD, and LC-MS is used to direct identification of major compounds. This established method was applied to BZYQT sample for quantitative analysis of their 9 compounds. In addition, BZYQT sample were fermented

to obtain the improved bioactivity by *Lactobacillus* strains, and to determine the quantitative change of contents of 9 compounds in fermented BZYQT samples, this HPLC method was applied.

EXPERIMENTAL

Materials

Reference compounds, decursin, saikosaponin A, hesperidin, naringin, glycyrrhizin, and 6-gingerol were obtained from the Korea Food and Drug Administration. Ferulic acid was purchased from Sigma (USA). Ginsenoside Rg3 was purchased from Chromadex (USA), and decursinol was purchased from Elcomscience (Korea). The purity of each compound was higher than 98% by HPLC. HPLC-grade water, acetonitrile, and methanol were purchased from J.T. Baker (USA). Analytical grade trifluoroacetic acid used buffer solution was purchased from DAE JUNG (Korea). BZYQT preparation (B-0) and fermented BZYQT samples (B-1~7) were supplied by the Korea Institute of Oriental Medicine.

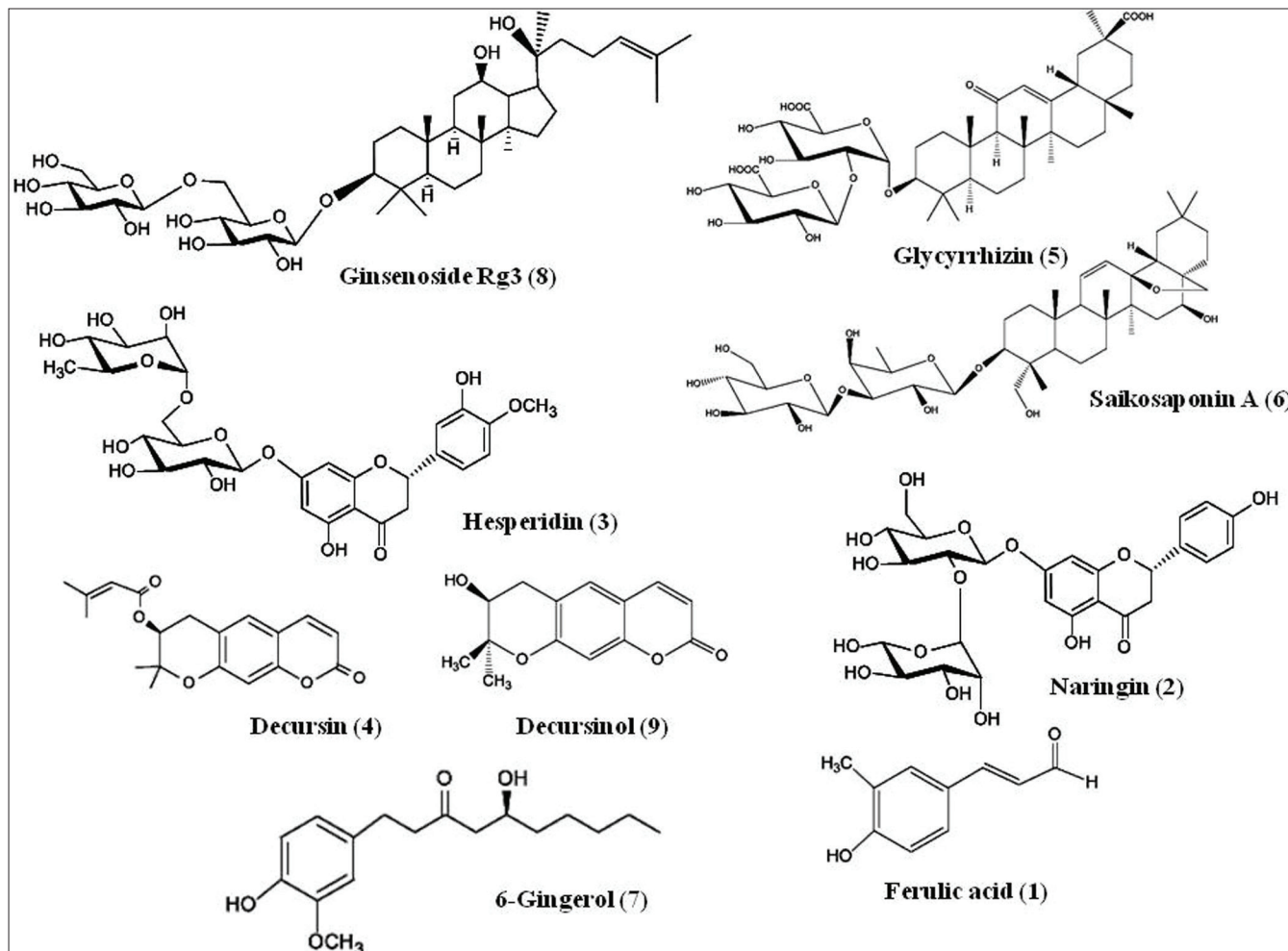


Figure 1: The chemical structures of the 9 standard compounds

Standard solutions and sample preparation

Standard stock solutions of accurately weighed 9 reference compounds were prepared at concentration of 250.00 µg/ml for ferulic acid (1), 143.75 µg/ml for naringin (2), 175.00 µg/ml for hesperidin (3), 210.00 µg/ml for decursinol (4), 400.00 µg/ml for glycyrrhizin (5), 330.00 µg/ml for saikosaponin A (6), 265.00 µg/ml for 6-gingerol (7), 250.00 µg/ml for ginsenoside Rg3 (8), 280.00 µg/ml for decursin (9) in methanol. Standard solutions of 9 reference compounds for HPLC-MS analysis were prepared in methanol at concentration of 10 µg/ml. These standard solutions were stored at 4°C until use. The standard working solution mixtures were obtained by diluting each stock solution to appropriate concentration.

The powder of BZYQT preparation (20.5 mg) was weighed precisely and dissolved in 10 ml of 60% methanol. Obtained fermented BZYQT preparations were prepared by same method. These sample solutions were filtered through a 0.45 µm membrane filter prior to HPLC analysis.

HPLC - DAD conditions

The HPLC analysis was performed on a Dionex Ultimate 3000 HPLC system (Dionex, Germany) consisting of a pump (LPG 3X00), an auto sampler (ACC-3000), a column oven (TCC-3000SD), and diode array UV/VIS detector (DAD-3000(RS)). Dionex Chromelon™ Chromatography Data System was used for instrument control and the chromatographic data acquisition. For chromatographic separation, SHISHEDO C₁₈ column (250 × 4.6 mm i.d., 5 µm) was used. The column temperature was set at 35°C. A linear gradient system consisted of mobile phase A (0.1% (v/v) trifluoroacetic acid) and mobile phase B (acetonitrile). The gradient program was conducted as follows: 15% (B) in 0-5 min, 15-35% (B) in 5-20 min, 35-50% (B) in 20-25 min, 50% (B) in 25-40 min, and 50-70% (B) in 40-50 min. The flow rate of mobile phase was 1.0 ml/min, and sample volume injected was 20 µL. The DAD detector recorded UV spectra in the range from 190 to 400 nm for peak characterization.

LC-MS conditions

LC-MS analysis was performed by using the TSQ Quantum Ultra Triple Stage Quadrupole Mass Spectrometer (Thermo) equipped with electrospray ionization (ESI) ion source in positive ion mode. The chromatographic separation was achieved on an Atlantis dC18 column (150 × 2.0 mm i.d., 3 µm) at a column temperature of 25°C with the same elution program of HPLC-DAD analysis. The injection volume was 20 µl. The flow rate was 200 µl/min. The MS operating condition were optimized as follows: The ion spray voltage set at 4,000 V, the vaporizer temperature at 100°C, capillary temperature at 320°C,

sheath gas pressure at 50 psi, aux gas pressure at 30 psi. Full scan mass spectra were recorded from m/z 100 to 1000 in positive ion modes.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Four different columns, Dionex C₁₈ column (150 mm × 4.6 mm i.d., 5 µm), LUNA C₁₈ column (250 × 4.6 mm i.d., 5 µm), SHISHEDO C₁₈ column (250 × 4.6 mm i.d., 5 µm), and XTerra™ RP18 (250 mm × 4.6 mm i.d., 5 µm) were compared to select the best column. The SHISHEDO C₁₈ column (250 × 4.6 mm i.d., 5 µm) exhibited good separation. The different mobile phases, such as water – acetonitrile and water – methanol were tested with various gradient programs to obtain the optimal separation condition. Water-acetonitrile had better solution and peak shape than water-methanol. Low concentration of trifluoroacetic acid was added to improve the peak shape and reduce the peak tailing.^[8] According to the higher UV absorption based on literature of 9 major compounds, we set a UV wavelength on HPLC analysis to obtain more detectable peaks. UV wavelengths of each compound were selected such as 205 nm for naringin (2), hesperidin (3), decursinol (4), saikosaponin A (6), 6-gingerol (7), ginsenoside Rg3 (8), and decursin (9), 250 nm for glycyrrhizin (5) and 330 nm for ferulic acid (1). The typical chromatograms for the standard mixtures separated using the optimized analysis condition are shown in Figure 2a and obtained a good separation. Figure 2b showed BZYQT sample HPLC chromatogram with UV spectrum of each standard peak.

Identification of standard compounds

Nine reference substances and BZYQT sample were qualitative analysis by using the LC-ESI-MS. The TIC chromatograms of 9 standards and BZYQT sample were shown in Figure 3. In MS spectra, the obtained molecular mass of the compounds applied for the structural identification of compounds. In positive ESI mode, the 9 compounds exhibited quasi-molecular ion $[M+H]^+$. The protonated molecular ions $[M+H]^+$ at m/z 195.13, 581.13, 611.14, 247.14, 823.30, 781.37, 277.22, and 329.17 for ferulic acid (1), naringin (2), hesperidin (3), decursinol (4), glycyrrhizin (5), saikosaponin A (6), 6-gingerol (7) and decursin (8), respectively, were observed, and ginsenoside Rg3 showed characteristic ion $[M+H-2glu]^+$ at m/z 459.46 [Table 1]. And these results were shown in Figure 4.

METHOD VALIDATION

Calibration curves, limits of detection, and quantification

Calibration curves were constructed by plotting the peak

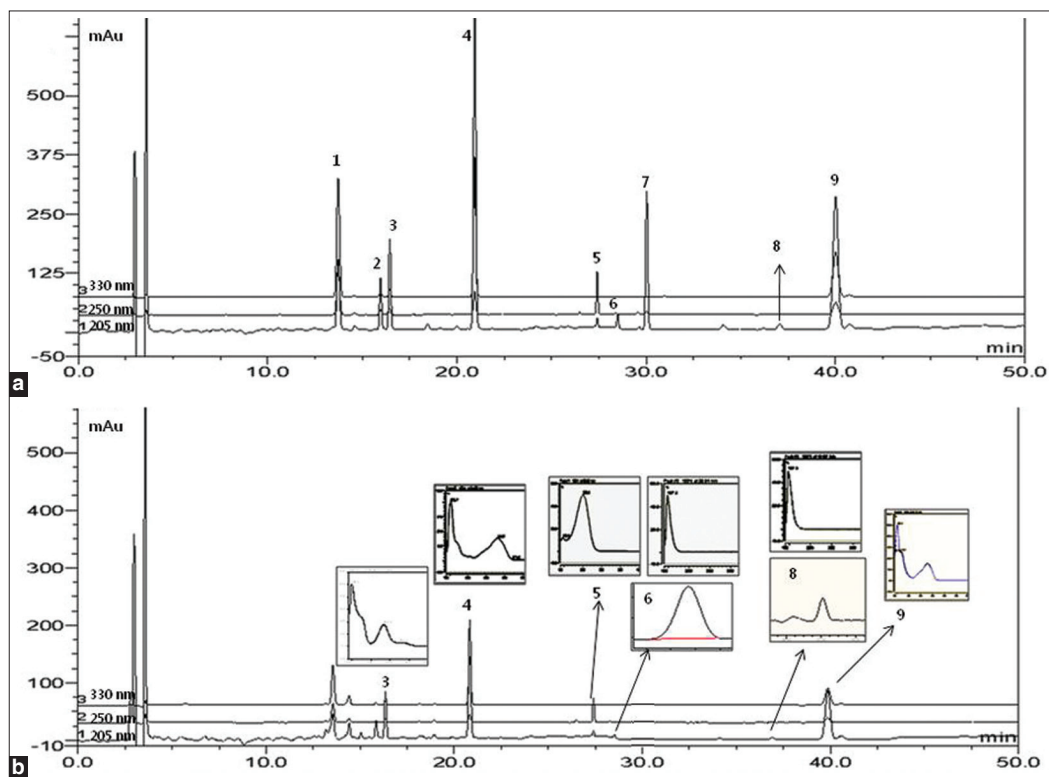


Figure 2: HPLC-DAD chromatograms of 9 standard compounds in BZYQT preparation and UV spectrum. (a) Mixed standards and (b) BZYQT preparation sample at 205, 250 and 330 nm. (1) ferulic acid, (2) naringin, (3) hesperidin, (4) decursinol, (5) glycyrrhizin, (6) saikosaponin A, (7) 6-gingerol, (8) ginsenoside Rg3, and (9) decursin

area against the corresponding 6 concentrations from the following concentration ranges; 0.347 – 27.778 $\mu\text{g/ml}$ for ferulic acid (1), 0.200 – 15.972 $\mu\text{g/ml}$ for naringin (2), 0.243 – 19.444 $\mu\text{g/ml}$ for hesperidin (3), 0.292 – 23.333 $\mu\text{g/ml}$ for decursinol (4), 0.556 – 44.444 $\mu\text{g/ml}$ for glycyrrhizin (5), 0.458 – 36.667 $\mu\text{g/ml}$ for saikosaponin A (6), 0.368 – 29.444 $\mu\text{g/ml}$ for 6-gingerol (7), 0.347 – 27.777 $\mu\text{g/ml}$ for ginsenoside Rg3 (8), and 0.389 – 31.111 $\mu\text{g/ml}$ for decursin (9).^[9] Correlation coefficient values indicated good linearity ($R^2 > 0.999$). The limits of detection (LOD) and limits of quantification (LOQ) were determined at the signal-to-noise ratio equal to 3 and 10, respectively.^[10] The range of LOD and LOQ for all compounds was from 0.0074 to 0.0931 ng/ml and 0.0224 to 0.2821 ng/ml, respectively. The detailed contents were exhibited in Table 2.

Precision and accuracy

The precision test of developed method was conducted by the intra-day and inter-day variation on 3 concentrations of 9 compounds.^[11] Intra-day variation was determined by analyzing 5 replicates within 1 day, and inter-day variation was assayed in 5 replicates for 3 sequential days (1,3,5 days). Variations were expressed by relative standard deviation (RSD). The intra-day precision for 9 compounds ranged from 0.19% to 1.91%, and the inter-day precision ranged from 0.05% to 1.64% [Table 3].

The recovery test was measured to evaluate the accuracy by the method of standard addition.^[12] Three different concentrations of 9 standard solutions were added to BZYQT sample and analyzed in triplicate. The accuracy varied between 92.57 and 108.56% with a maximum RSD of 1.98% [Table 4]. These results indicated that the established HPLC method was very sensitive, reliable, and accurate for simultaneous determination of the 9 compounds in BZYQT sample.

Analysis of samples

The developed method was subsequently applied to quantitative and qualitative analysis of 9 compounds, ferulic acid (1), naringin (2), hesperidin (3), decursinol (4), glycyrrhizin (5), saikosaponin A (6), 6-gingerol (7), ginsenoside Rg3 (8), and decursin (9) in obtained BZYQT samples (B-0) and 7 fermented BZYQT samples (B-1~7) from Korea Institute of Oriental Medicine. The sample chromatogram was shown Figure 2B, and the quantitative analytical results are summarized in Table 5. The contents of 9 compounds varied in different BZYQT samples (B-0~7). Glycyrrhizin (5) was abundant in the B-3, B-4, and B-5. Hesperidin (3) was not detected in the B-2, B-3, B-4, and B-5. Decursin (9) was not detected in the B-2. Decursinol was existed in B-0 and B-1. Ginsenoside Rg3 (8) was existed only in

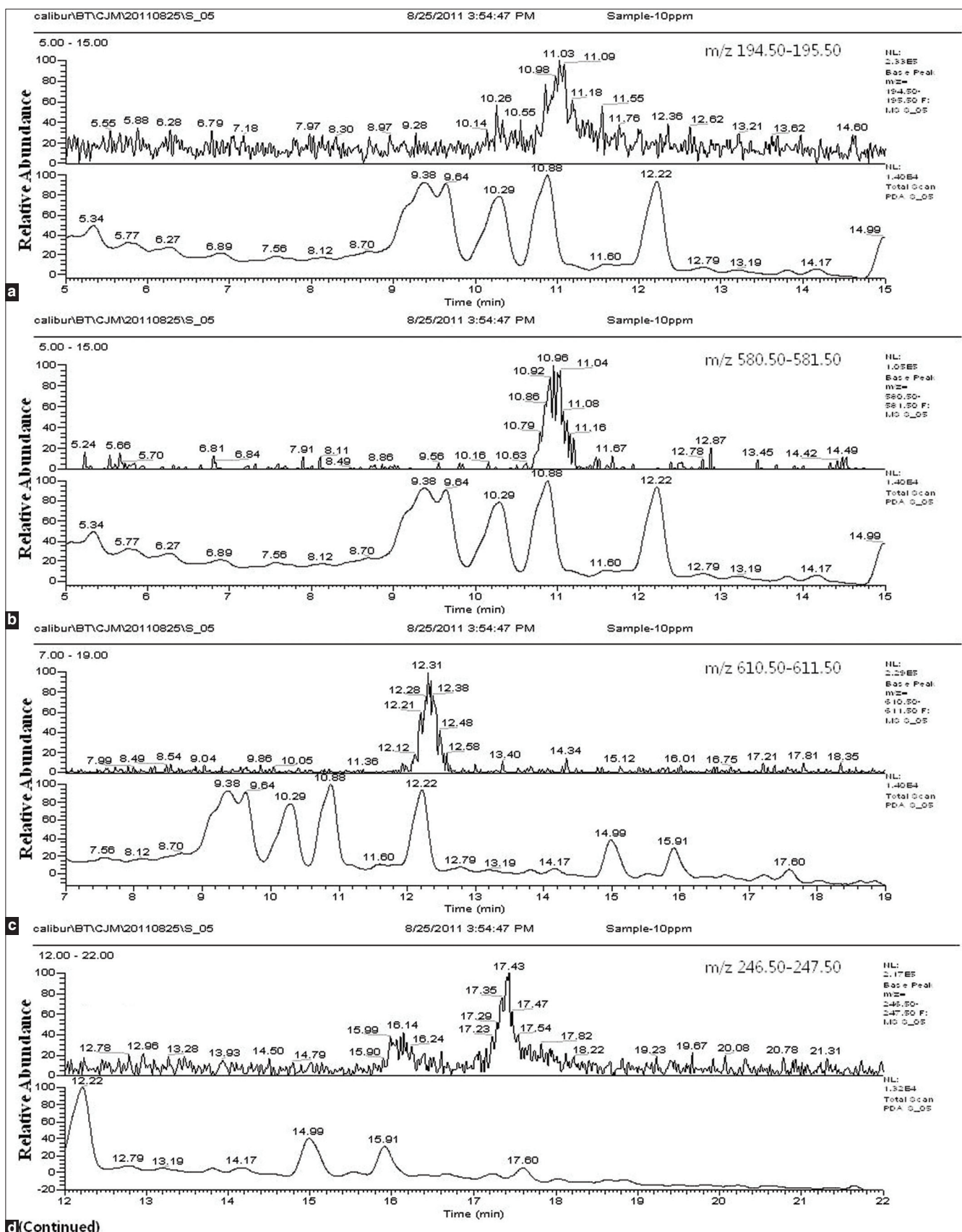
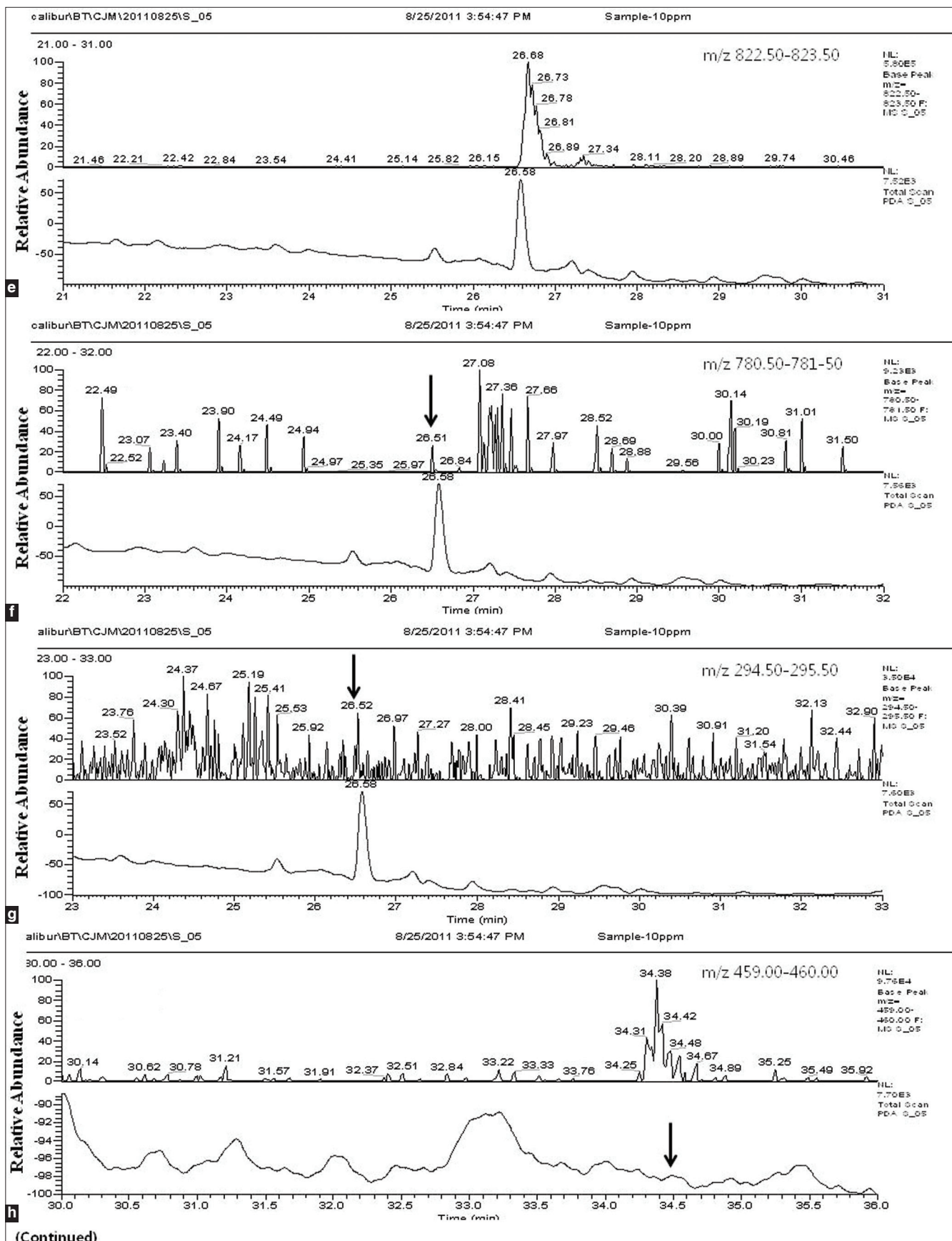


Figure 3: Total ion chromatogram of (I) mixed 9 standards and (II) BZYQT preparation sample. Representative SIM and PDA chromatograms of 9 standards in BZYQT preparation; (II)-a ferulic acid, (II)-b naringin, (II)-c hesperidin, (II)-d decursinol, (II)-e glycyrrhizin, (II)-f saikosaponin A, (II)-g 6-gingerol, (II)-h ginsenoside Rg3, and (II)-i decursin

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Figure 3: Contd...

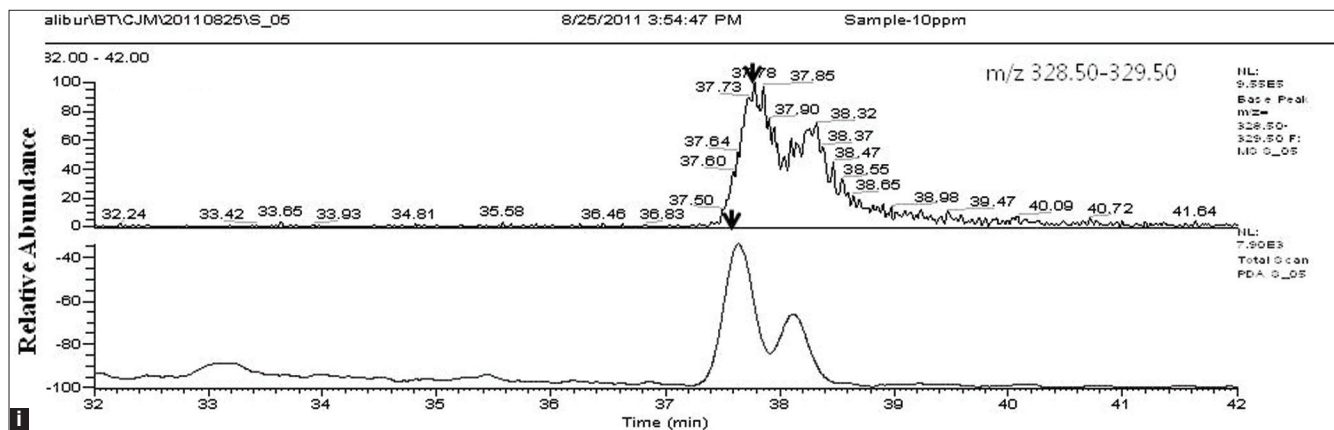


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Table 1: MS data for identification of the 9 components by LC-MS method

Compounds	MS data in positive ion (m/z)
Ferulic acid	195.13 [M+H] ⁺
Naringin	581.13 [M+H] ⁺
Hesperidin	611.14 [M+H] ⁺
Decursinol	247.14 [M+H] ⁺
Glycyrrhizin	823.30 [M+H] ⁺
Saikosaponin A	781.37 [M+H] ⁺
6-Gingerol	277.22 [M+H] ⁺
Ginsenoside Rg3	459.46 [M+H-2glu] ⁺
Decursin	329.17 [M+H] ⁺

Table 2: Linear regression data, limit of detection (LOD), and limit of quantification (LOQ) of 9 compounds

Compounds	Linear range (µg/ml)	Regression equation ^a	R ² (n = 5)	LOD (ng)	LOQ (ng)
Ferulic acid	0.347 ~ 27.778	Y = 1.576x + 0.044	1.000	30.5	36.9
Naringin	0.200 ~ 15.972	Y = 0.857x - 0.021	0.999	85.1	257.8
Hesperidin	0.243 ~ 19.444	Y = 1.247x - 0.048	0.999	63.4	192.0
Decursinol	0.292 ~ 23.333	Y = 4.532x + 0.122	0.999	7.4	22.4
Glycyrrhizin	0.556 ~ 44.444	Y = 0.187x - 0.009	1.000	27.3	82.8
Saikosaponin A	0.458 ~ 36.667	Y = 0.079x - 0.027	0.999	93.1	282.1
6-Gingerol	0.368 ~ 29.444	Y = 1.340x + 0.107	1.000	11.2	33.9
Ginsenoside Rg3	0.347 ~ 27.777	Y = 0.094x - 0.011	0.999	59.4	179.9
Decursin	0.389 ~ 31.111	Y = 2.884x + 0.121	1.000	20.9	63.2

^aY: peak area, x: concentration (µg/ml)

B-0. Ferulic acid (1), naringin (2), saikosaponin A (6), and 6-gingerol (7) were not detected in all samples (B-0~7). “Not detected” was defined as amount of each compound was lower than LOD. The results suggested that the differences of the content between BZYQT sample (B-0) and fermented BZYQT samples (B-1~7) were observed by bio-conversion such as fermentation of BZYQT preparation. In addition, absence of ferulic acid (1), naringin (2), saikosaponin A (6), and 6-gingerol (7) may attribute to the various factors, including

preparation process, condition of medicinal herbs that compose BZYQT.

CONCLUSION

In this study, we established a reliable, accurate, and sensitive analysis method using HPLC-DAD/LC-MS for simultaneous determination of BZYQT sample. Nine compounds, ferulic acid (1), naringin (2), hesperidin

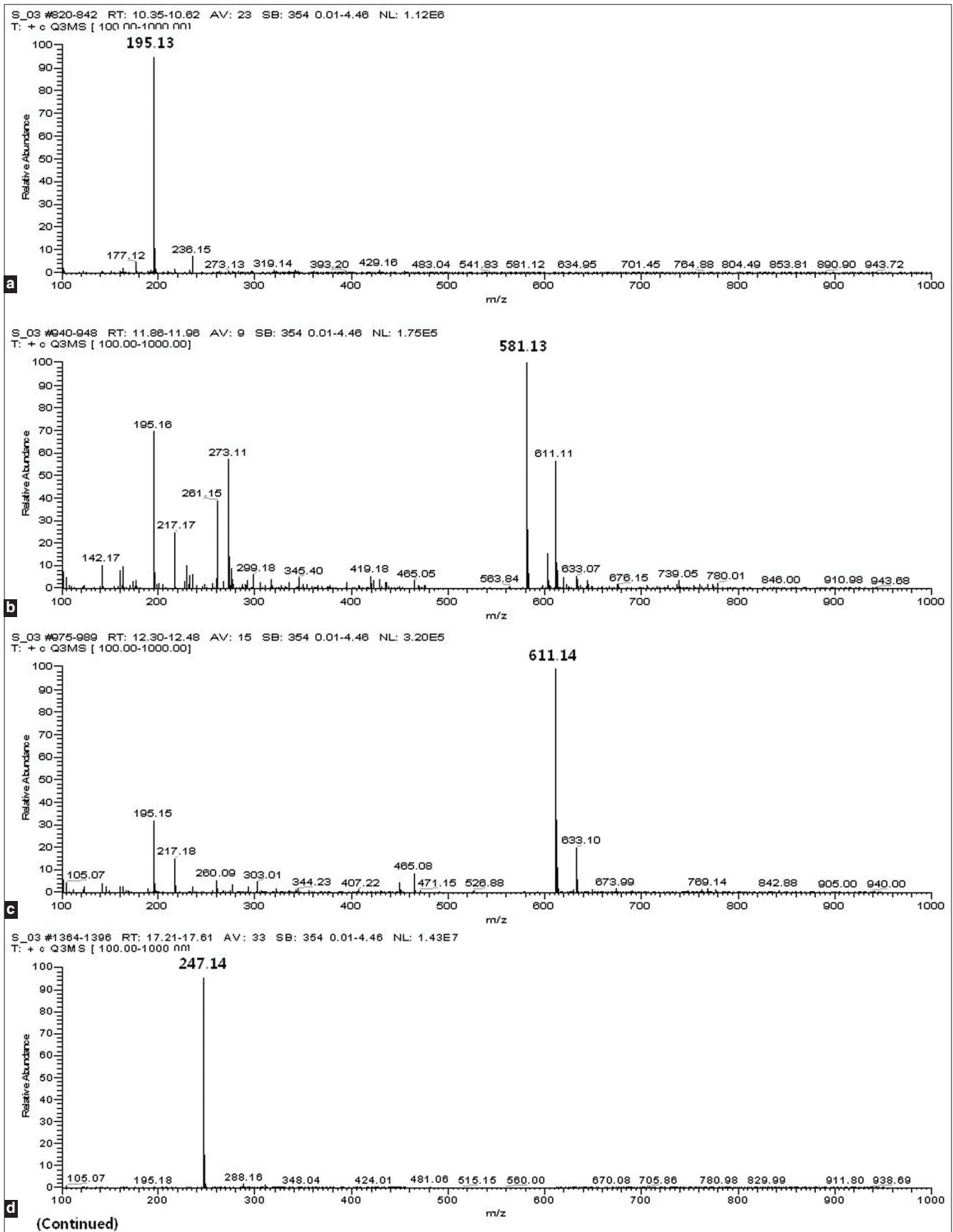


Figure 4: The MS spectra of 9 components in BZYQT preparation. For peak identification: (a) ferulic acid, (b) naringin, (c) hesperidin, (d) decursinol, (e) glycyrrhizin, (f) saikosaponin A, (g) 6-gingerol, (h) ginsenoside Rg3, and (i) decursin

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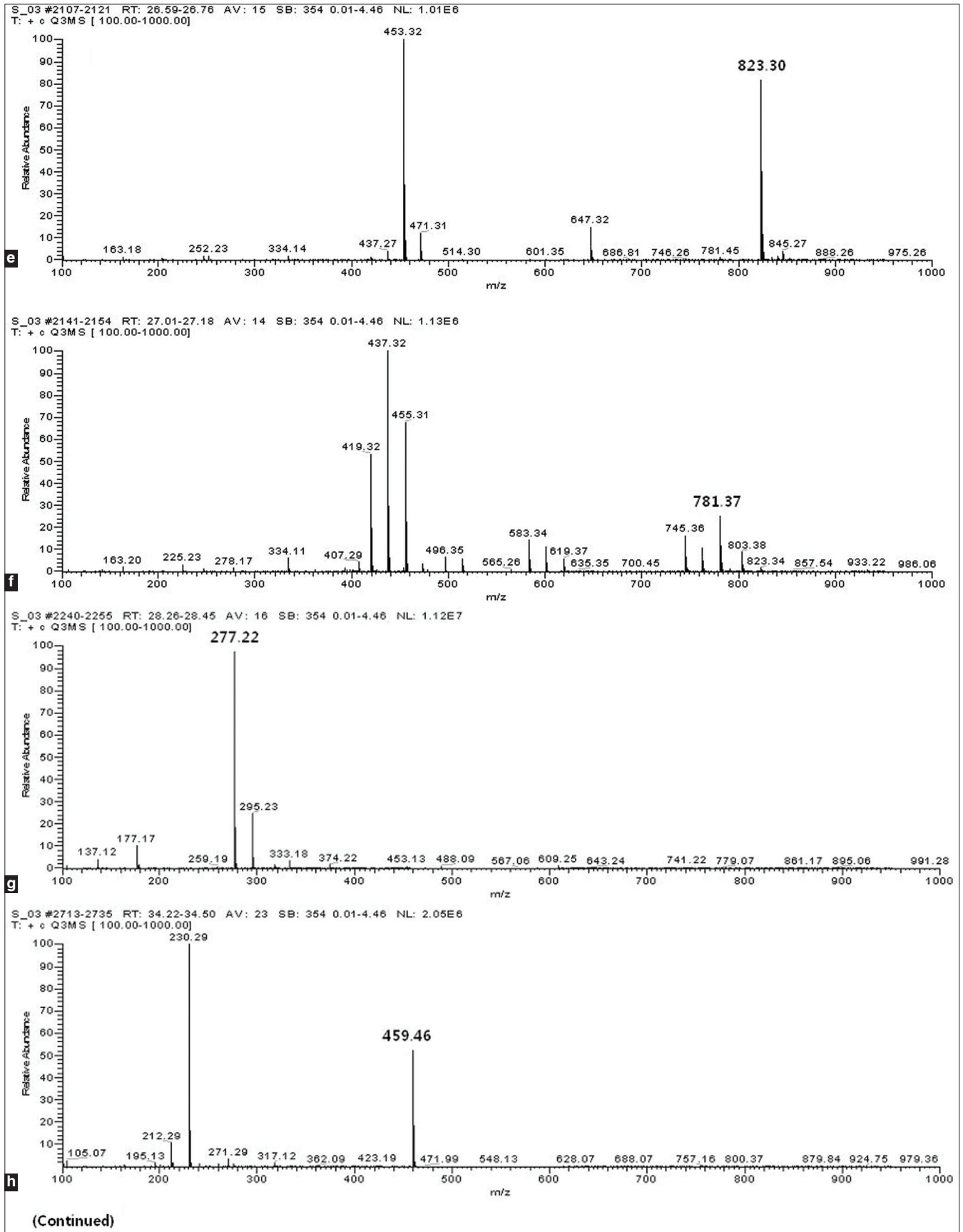


Figure 4: Contd....

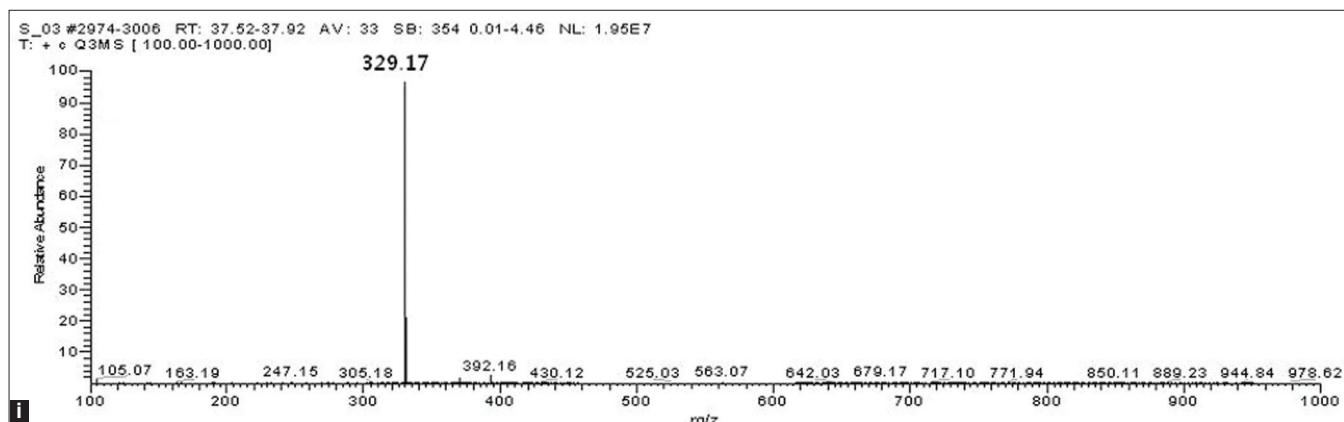


Figure 4: Contd....

Table 3: Intra-day and Inter-day accuracy of 9 compounds

Components	Concentration ($\mu\text{g/ml}$)	Intra-day (n = 5)			Inter-day (n = 5)		
		Mean \pm SD ($\mu\text{g/ml}$)	RSD ^a (%)	Accuracy (%)	Mean \pm SD ($\mu\text{g/ml}$)	RSD (%)	Accuracy (%)
Ferulic acid	3.47	3.46 \pm 0.01	0.40	99.64	3.52 \pm 0.05	1.56	101.25
	6.94	7.01 \pm 0.05	0.69	100.97	7.05 \pm 0.07	0.94	101.45
	13.89	13.97 \pm 0.16	1.11	100.70	14.03 \pm 0.02	0.11	101.03
Naringin	2.00	1.90 \pm 0.01	0.48	95.19	1.95 \pm 0.01	0.72	97.72
	4.00	3.94 \pm 0.03	0.71	98.88	3.93 \pm 0.02	0.38	98.34
	8.00	7.67 \pm 0.02	0.24	96.03	7.72 \pm 0.05	0.69	96.69
Hesperidin	2.43	2.46 \pm 0.04	1.68	101.40	2.46 \pm 0.04	1.56	101.41
	4.86	5.01 \pm 0.04	0.87	103.13	4.94 \pm 0.02	0.43	101.67
	9.72	9.96 \pm 0.04	0.40	102.53	9.78 \pm 0.01	0.05	100.59
Decursinol	2.92	2.94 \pm 0.02	0.58	100.73	2.96 \pm 0.03	1.14	101.56
	5.83	5.84 \pm 0.08	1.24	100.10	5.86 \pm 0.03	0.56	100.42
	11.67	11.80 \pm 0.05	0.42	101.13	11.81 \pm 0.05	0.38	101.19
Glycyrrhizin	5.56	5.80 \pm 0.03	0.56	104.35	5.81 \pm 0.07	1.22	104.54
	11.11	11.62 \pm 0.04	0.36	104.55	11.55 \pm 0.13	1.10	103.94
	22.22	22.88 \pm 0.08	0.35	102.97	23.04 \pm 0.38	1.64	103.67
Saikosaponin A	4.58	4.35 \pm 0.05	1.25	94.90	4.39 \pm 0.06	1.27	95.84
	9.17	9.05 \pm 0.02	0.19	98.77	8.56 \pm 0.10	1.20	93.34
	18.33	18.39 \pm 0.13	0.69	100.30	18.28 \pm 0.07	0.36	99.69
6-Gingerol	3.68	3.69 \pm 0.02	0.47	100.33	3.72 \pm 0.03	0.74	101.06
	7.36	7.49 \pm 0.05	0.66	101.72	7.46 \pm 0.09	1.18	101.36
	14.72	14.93 \pm 0.16	1.10	101.41	14.93 \pm 0.12	0.78	101.41
Ginsenoside Rg3	3.47	3.53 \pm 0.06	1.65	101.71	3.48 \pm 0.03	1.00	100.10
	6.94	7.05 \pm 0.01	0.07	101.49	7.18 \pm 0.13	1.80	103.38
	13.89	14.45 \pm 0.28	1.91	104.05	14.40 \pm 0.14	0.99	103.74
Decursin	3.89	3.90 \pm 0.01	0.32	100.37	3.96 \pm 0.01	0.35	101.90
	7.78	8.01 \pm 0.07	0.89	103.01	8.04 \pm 0.10	1.22	103.31
	15.56	16.00 \pm 0.14	0.89	102.88	16.07 \pm 0.19	1.19	103.31

^aRSD (%) = (SD of amount analyzed / mean of amount analyzed) \times 100 %

(3), decursinol (4), glycyrrhizin (5), saikosaponin A (6), 6-gingerol (7), ginsenoside Rg3 (8), and decursin (9) in BZYQT successfully identified by UV wavelength and MS data. The method showed good linearity, precision, and accuracy by validation test. The developed method used for quantitative analysis of BZYQT sample and fermented

BZYQT samples and proved that the differences in the content for compounds among different samples were obvious. Previous determination of glycyrrhizic acid in BZYQT by HPLC-DAD was reported.^[13] But, our analysis method analyzed more various compounds than previous method and used LC-MS detector. This study could provide

Table 4: Recovery of the 9 compounds

Components	Spiked Amount ($\mu\text{g/ml}$)	Measured Amount ($\mu\text{g/ml}$)	Recovery ^a (%)	RSD (%)
Ferulic acid	6.94	6.94 \pm 0.09	99.95	1.35
	3.47	3.49 \pm 0.04	100.59	1.06
	1.74	1.60 \pm 0.03	92.10	1.80
Naringin	3.99	3.95 \pm 0.02	98.83	0.44
	2.00	1.94 \pm 0.03	97.00	1.41
	1.00	0.96 \pm 0.02	95.93	1.79
Hesperidin	4.86	5.15 \pm 0.01	106.00	0.23
	2.43	2.59 \pm 0.02	106.65	0.63
	1.22	1.12 \pm 0.02	92.57	1.69
Decursinol	5.83	6.25 \pm 0.01	107.23	0.20
	2.92	3.14 \pm 0.02	107.77	0.50
	1.46	1.47 \pm 0.01	100.94	0.35
Glycyrrhizin	11.11	11.65 \pm 0.04	104.83	0.33
	5.56	5.77 \pm 0.04	103.86	0.72
	2.78	2.76 \pm 0.05	99.45	1.88
Saikosaponin A	9.17	9.42 \pm 0.04	102.80	0.45
	4.58	4.61 \pm 0.09	100.59	1.98
	2.29	2.28 \pm 0.04	99.32	1.80
6-Gingerol	7.36	7.99 \pm 0.02	108.56	0.20
	3.68	3.97 \pm 0.01	107.89	0.26
	1.84	1.84 \pm 0.01	99.79	0.30
Ginsenoside Rg3	6.94	6.67 \pm 0.03	96.09	0.39
	3.47	3.54 \pm 0.05	101.85	1.53
	1.74	1.74 \pm 0.03	100.04	1.86
Decursin	7.78	8.42 \pm 0.01	108.26	0.13
	3.89	4.13 \pm 0.02	106.33	0.52
	1.94	1.85 \pm 0.01	95.38	0.52

^aRecovery (%) = (concentration found – original concentration) / concentration spiked $\times 100$ %

Table 5: Contents ($\mu\text{g/mg}$) of the 9 compounds in the 8 BZYQT samples (B-0~7)

Sample	Content ($\mu\text{g/mg}$)								
	Ferulic acid	Naringin	Hesperidin	Decursinol	Glycyrrhizin	Saikosaponin A	6-Gingerol	Ginsenoside Rg3	Decursin
B-0	nd ^a	nd	2.79 \pm 0.08	0.07 \pm 0.01	8.86 \pm 0.04	nd	nd	0.14 \pm 0.02	0.57 \pm 0.01
B-1	nd	nd	3.23 \pm 0.02	0.09 \pm 0.01	11.24 \pm 0.01	nd	nd	nd	0.75 \pm 0.01
B-2	nd	nd	3.12 \pm 0.08	0.10 \pm 0.01	12.76 \pm 0.01	nd	nd	nd	0.72 \pm 0.01
B-3	nd	nd	3.20 \pm 0.10	nd	14.95 \pm 0.01	nd	nd	nd	0.82 \pm 0.02
B-4	nd	nd	3.15 \pm 0.07	nd	14.57 \pm 0.03	nd	nd	nd	0.80 \pm 0.01
B-5	nd	nd	3.16 \pm 0.04	nd	14.61 \pm 0.01	nd	nd	nd	0.81 \pm 0.01
B-6	nd	nd	3.26 \pm 0.03	nd	13.35 \pm 0.01	nd	nd	nd	0.83 \pm 0.01
B-7	nd	nd	3.25 \pm 0.04	nd	13.17 \pm 0.03	nd	nd	nd	0.80 \pm 0.01

nd^a: Not detected

effect quality control method of BZYQT and information on influence of the content differences of compounds on therapeutic effect of BZYQT sample. Further research on analysis of more novel compounds is required.

ACKNOWLEDGEMENT

This research was supported by a grant [K11050] from the Korea Institute of Oriental Medicine.

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Cite this article as: Weon JB, Ma JY, Yang HJ, Lee B, Yun B, Ma CJ. Qualitative and quantitative analysis of nine major compounds in the Bozhougyiqi-Tang using a high-performance liquid chromatography coupled with a diode array detector and electrospray ionization mass spectrometer. *Phcog Mag* 2013;9:271-82.

Source of Support: Supported by a grant [K11050] from the Korea Institute of Oriental Medicine. **Conflict of Interest:** None declared.