Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 931–936

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A combined HPLC-PDA and HPLC-MS method for quantitative and qualitative analysis of 10 major constituents in the traditional Chinese medicine Zuo Gui Wan

Xu Liang [a], Xi Zhang [a,*], Weixing Dai [a], Yonghai Lv [a], Shikai Yan [b], Weidong Zhang [a,b,*]

[a] School of Pharmacy, Second Military Medical University, Shanghai 200433, PR China
[b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200030, PR China

ARTICLE INFO

Article history:
Received 1 August 2008
Received in revised form 3 February 2009
Accepted 4 February 2009
Available online 13 February 2009

Keywords:
Zuo Gui Wan
HPLC-DAD
HPLC-MS
Qualitative evaluation
PLS-DA

ABSTRACT

A method using a high-performance liquid chromatograph coupled with a photodiode array UV detector (HPLC-DAD) and an electrospray ionization mass spectrometer (HPLC-ESI/MS) was developed for the quality assessment (fingerprinting and simultaneous quantification of 10 major constituents) of Zuo Gui Wan (ZGW), a Traditional Chinese Medicine (TCM). The compounds were identified on the basis of comparison of their mass spectra with literature data and standard samples and quantified by the HPLC-DAD method. Baseline separation was achieved on an ODS-100V C_{18} column (3.0 μm, 150 mm × 4.6 mm I.D.) with linear gradient elution of acetonitrile–0.1% formic acid. The method was validated for linearity ($r^2 > 0.9996$), repeatability (R.S.D. < 3.5%), intra- and inter-day precision (R.S.D. < 2.0%) with accuracy (91.8–99.7%), recovery (99.9–105.6%), limits of detection (1.4–22.0 ng), and limits of quantification (3.4–73.4 ng). The 10 compounds were selected for quality assessment of ZGW by using partial least squares-discrimination analysis (PLS-DA). The similarities of 20 batches of ZGW and their classification according to their manufacturers were based on the retention times and peak areas of the characteristic compounds.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Zuo Gui Wan (ZGW), a Traditional Chinese Medicine (TCM) has effect to invigorate the kidney and is widely used for deficiency of body fluid, night sweat, sore waist, nocturnal emission, mental fatigue and dry mouth [1]. Modern pharmacological studies showed that it also had contribution to promote bone marrow cells' forming into liver cells, delay aging, and protect from leucopenia induced by cyclophosphamide [2–4].

This formula is comprised of 200 g of prepared *Radix Rehmanniae*, 100 g of *Rhizoma Dioscoreae*, 100 g of *Fructus Lycii*, 100 g of *Fructus Corni*, 75 g of *Radix Acanthopanacis Bidentatae*, 100 g of *Colla Cornus Cervi*, 100 g of *Colla Plastri Testudinis* and 100 g of *Semen Cuscutae* [1], each of them containing many compounds that may be relevant to the medicine's putative activity. For example, gallic acid, 5-hydroxymethylfurfural (5-HMF), morroniside, sweroside, loganin, β-ecdysterone, rutin, hyperoside, quercetin, kaempferide etc., are proved to possess the effects of tonifying kidney, immunoloregulation, anti-inflammatory and anti-oxidation [5–8]. Therefore, an effective and reliable method, which can analyze as many bioactive constituents as possible in ZGW to ensure its safety and efficacy, is necessary for its qualitative and quantitative analysis.

Up to date, there is just one report for quality control of ZGW in which only a single active ingredient, loganin, is determined by HPLC [9]. However, the quality of TCM is closely related to the concentration of many of their chemical constituents, which can be slightly different according to their environmental conditions. Currently, many studies proved that chemometrics coupled with the HPLC fingerprint technique can be employed to solve this problem and this method has become one of the most frequently applied approaches to assist the recognition of the origin (area and manufacturer) and quality control of TCM. Partial least squares-discrimination analysis (PLS-DA) can be employed to construct a linear discrimination model and to classify samples on the basis of the HPLC chromatographic fingerprints which provide the whole profile of not only the marker compounds but also other active components [10–13]. In this paper, a combined high-performance liquid chromatograph coupled with a photodiode array UV detector (HPLC-DAD), HPLC-MS and chemometrics method was firstly established to the identification and quantification of 10 compounds in

* Corresponding authors at: School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China. Tel.: +86 21 25070386; fax: +86 21 25070386.
E-mail addresses: hkzx11@yahoo.com.cn (X. Zhang), WDZhangY@hotmail.com (W. Zhang).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.02.004

ZGW. This method was successfully applied to the quality assessment of various ZGW dosage forms.

2. Experiment

2.1. Chemicals and materials

HPLC-grade acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany) and Tedia (Fairfield, OH, USA). Deionized water was prepared by a Milli-Q_{50} SP Reagent Water System (Bedford, MA, USA). Other reagents were of analytical grade. All solvents and sample solutions were filtered through 0.45 μm membrane filters before injecting into HPLC.

The reference standards of gallic acid, 5-HMF, loganin, β-ecdysterone, rutin, hyperoside, quercetin, and kaempferide were obtained from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, http://www.nicpbp.org.cn/CL0001/), morroniside and sweroside were purchased from National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine (Jiangxi, China, http://www.berbfine.com). The purities of all the standards (Fig. 1) were not less than 98%.

Twenty batches of ZGW were collected from two pharmaceutical companies in China. The sample (S1–S10) made by Leiyunshang was concentrated pill, and that (S11–S20) made by Zhongjing was water-honeyed pill.

2.2. Standard solutions and sample preparation

Each accurately weighed standard was dissolved in methanol and diluted to provide a series of standard solutions for constructing the calibration curve. The solutions were stored at 4 °C.

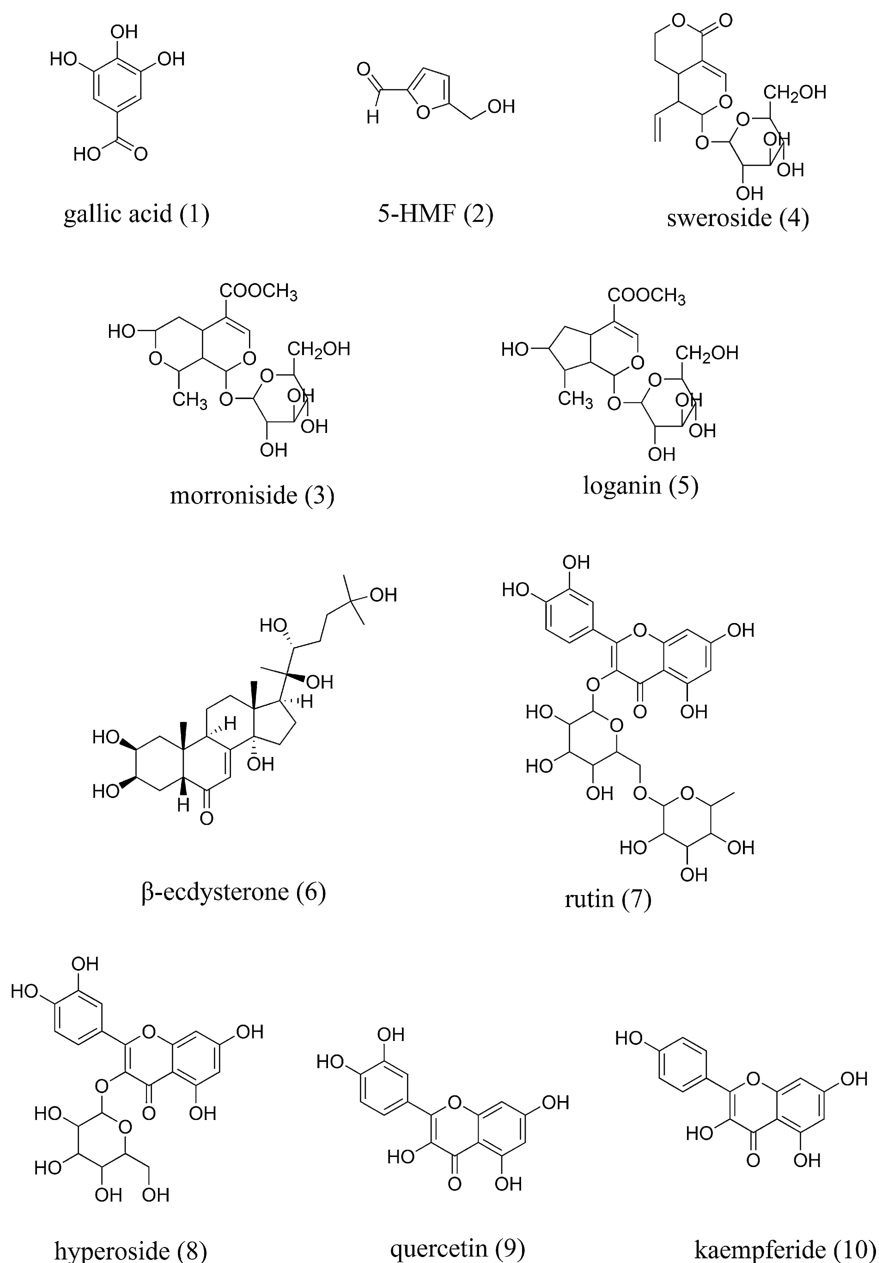

Fig. 1. Structures of the constituents identified in ZGW.

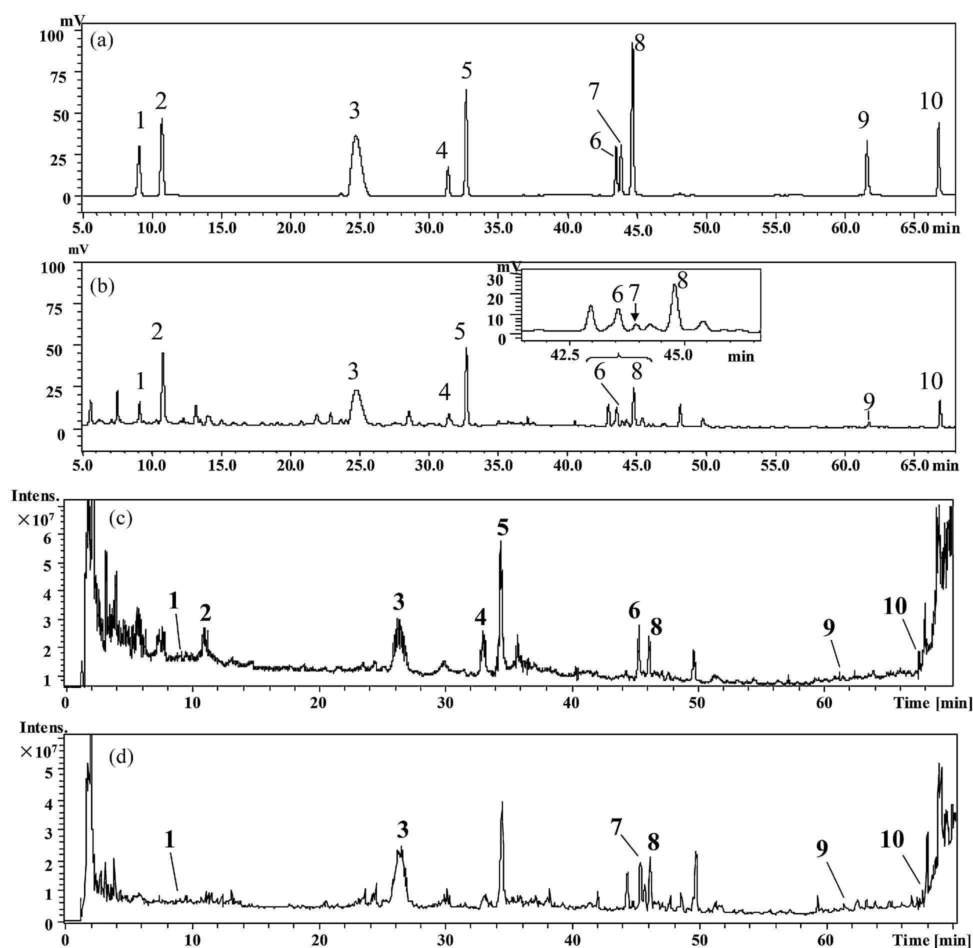

Fig. 2. HPLC-UV chromatograms of (a) 10 mixed bioactive markers, (b) ZGW (S4) and HPLC-MS TIC chromatograms both in positive (c) and negative (d) ion modes with the detection at 254 nm: (1) gallic acid, (2) 5-HMF, (3) morroniside, (4) sweroside, (5) loganin, (6) β-ecdysterone, (7) rutin, (8) hyperoside, (9) quercetin and (10) kaempferide.

The pills of ZGW were smashed into power. 2.0 g of pulverized samples was accurately weighed into centrifuge tube and ultrasonically extracted two times (2× 30 min) at room temperature with 10 ml methanol. After centrifuged for 5 min at 9000 × *g*, the supernatant was transferred into 10 ml volumetric flask and made up to volume with methanol.

2.3. Analytical method

LC2010A*HT* HPLC system coupled with DAD detector and LC solution workstation (Shimadzu, Kyoto, Japan) was used to acquire chromatograms and UV spectra. The separation was performed on a TSKgel ODS-100V C_{18} column (3.0 μm, 150 mm × 4.6 mm I.D.) (TOSOH, Tokyo, Japan). The mobile phase consisted of acetonitrile (A) and 0.1% (v/v) formic acid (B). The linear gradient was as follows: 0–30 min, 1–12% A; 30–32 min, 12–15% A; 32–55 min, 15–25% A; 55–60 min, 25–40% A; 60–70 min, 40–100% A at a flow rate of 1.0 ml/min. A pre-equilibration period of 20 min was set up between individual runs. The column temperature was 35 °C, detection wavelength was 254 nm and the injection volume was 10 μl.

Agilent 1100 series LC/MS ion trap mass spectrometer (Agilent, MA, USA) connected to HPLC system with ESI ion source was used for qualitative analysis. The chromatographic conditions were the same as those used for HPLC-PDA analysis. The conditions of MS analysis were as follows: both in the positive and negative ion mode from m/z 50 to 1000, drying gas N_2 flow rate, 10 l/min, gas temperature 350 °C, nebulizer, 35 psi; HV voltage 3.5 kV and fragmentor 1.00 V.

3. Results and discussion

3.1. Optimization of the chromatographic conditions and extraction

A small amount of acid was added into the mobile phase to inhibit the ionization of acidic ingredients in ZGW extracts thus improving the peak shape and restrain the peak tailing. 0%, 0.1% and 0.2% aqueous formic acid and acetic acid solutions were compared. The 10 compounds could be baseline separated when 0.1% formic acid solution was selected.

Monitoring the chromatograms at 254 nm was found to provide highest sensitivity and steady baseline (Fig. 2a and b).

Prior to sample analysis the extraction procedure was optimized. 2.0 g samples were extracted with different volumes and percentages of methanol and ethanol, respectively. The best results were found using 10 ml methanol. Investigating the dependence of the yield on the duration of the extraction (15, 20, 30 and 40 min) it was found that the 10 compounds were almost completely extracted when 2× 30 min extraction was used. The yield of a third extraction soaking for further 24 h was negligible.

3.2. Identification of the bioactive markers in fingerprint chromatograms

In the HPLC-ESI/MS spectra (Fig. 2c and d) most of investigated compounds exhibited their quasi-molecular ions $[M+H]^+$ or $[M+Na]^+$ in positive ion mode and $[M-H]^-$ in negative ion mode. Due to the addition of 0.1% formic acid in the mobile phase, the

adduct ions $[M+HCOO]^-$ were also detected. By comparing their retention times, UV data and MS spectra with those of standards, 10 target compounds in the extract of ZGW were unambiguously identified (Fig. 2 and Table 1).

The peak of betaine which was the main bioactive element of *Fructus Lycii* [14] did not appear because of the formic acid in mobile phase, but it appeared when pure water was used as mobile phase. The amino acids, trace elements, allantoin, choline and diosgenin which had no or weak UV absorption present in *Colla Plastri Testudinis*, *Colla Cornus Cervi* and *Rhizoma Dioscoreae*, respectively [15–17], were difficult to be evaluated by this method.

3.3. Validation of the quantitative analysis

3.3.1. Linearity, limits of detection and limits of quantification

The linear calibration curves were constructed by at least six different concentrations of chemical markers. Each concentration was analyzed in triplicate. The limits of detection (LOD) and limits of quantification (LOQ) were measured on the basis of the signal-to-noise ratio of 3 and 10 as criteria, respectively. Good linear correlation and high sensitivity at these chromatographic conditions were confirmed by the correlation coefficients ($r^2 > 0.9996$), LOD (1.4–22.0 ng), and LOQ (3.4–73.4 ng) (Table 1).

3.3.2. Accuracy, precision and repeatability

The mixture standard solution was analyzed under the optimal conditions five times both in 1 day for intra-day variation and on 3 successive days for inter-day variation to evaluate the precision and accuracy. The intra- and inter-day precisions were within 1.0% and 2.0%, respectively, with accuracy from 91.8% to 99.7%. In order to check the repeatability, five different solutions made from the same sample (S4) were determined. The R.S.D. of repeatability were less than 3.5%. These results indicated that the developed method had acceptable precision, accuracy and repeatability (Table 2).

3.3.3. Recoveries

In order to evaluate the recovery of this method, three different concentration levels (approximately equivalent to 0.8, 1.0 and 1.2 times of the concentration of the matrix) of the reference standards were added into the sample S4 in triplicate. The solutions were extracted and quantified as described before. The results showed that the assay was satisfactory with the mean recovery from 99.9% to 105.6% with R.S.D. less than 4.6% for the 10 components (Table 3).

3.4. Sample analysis

The described method was applied to analyze the 10 compounds in 20 batches of ZGW. The variations of their contents (Table 4) were great, even in the 10 batches from the same company. Among them, morroniside, quercetin and kaempferide were even hardly detected in a few samples probably because the content of these bioactive markers was also affected by the year of the plant cultivation, harvest time, climate and environment. The content of 5-HMF might be influenced by the extent of processing of processed *Radix Rehmanniae* in this formula. There was much doubt about whether all the products will give rise to the same efficacy. Therefore, the supply and quality of medicinal substances and the quality standard of preparations should be regulated in the future to ensure the safety of ZGW.

3.5. Similarity analysis

Professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Ver-

Table 1
Linear regression equation analysis in the determination of the 10 compounds and HPLC-MS identification.

t_R (min)	Compound	λ max (nm)	Linear range (μg/ml)	Slope (a)	Intercept (b)	r^2 ($n=6$)	LOD (ng)	LOQ (ng)	Molecular ions (m/z)	
9.131	Gallic acid	216, 271	1.96–392.00	5.99×10^6	18552.28	0.9998	4.0	13.1	$[M+H]^+$ 171	$[M-H]^-$ 169
10.775	5-HMF	282	10.72–670.00	5.23×10^6	−20052.13	0.9997	10.8	35.8	$[M+H]^+$ 127	–
24.791	Morroniside	241	22.00–1375.00	6.69×10^6	−34700.33	0.9998	22.0	73.4	$[M+Na]^+$ 429	$[M-H]^-$ 405, $[M+HCOO^-]^-$ 451
31.440	Sweroside	246	0.92–115.00	1.01×10^7	−3357.37	0.9998	1.9	6.2	$[M+H]^+$ 359	–
32.739	Loganin	238	11.76–735.00	5.01×10^6	−11432.73	0.9998	11.8	39.2	$[M+Na]^+$ 413	$[M+HCOO^-]^-$ 435
43.542	β-Ecdysterone	248	0.48–48.00	9.52×10^5	3153.81	0.9997	2.0	4.8	$[M+H]^+$ 481	–
43.897	Rutin	255, 353	0.58–58.00	8.16×10^6	3042.74	0.9997	2.4	5.8	–	$[M-H]^-$ 609
44.729	Hyperoside	255, 353	1.38–276.00	1.56×10^7	−14734.22	0.9996	5.6	13.8	$[M+H]^+$ 465, $[M+Na]^+$ 487	$[M-H]^-$ 463
61.647	Quercetin	254, 370	0.34–34.00	1.86×10^7	1985.82	0.9999	1.4	3.4	$[M+H]^+$ 303	$[M-H]^-$ 301
66.785	Kaempferide	264, 366	0.50–50.00	1.42×10^7	735.42	0.9999	2.0	5.0	$[M+H]^+$ 287	$[M-H]^-$ 285

In the regression equation $y = ax + b$, y refers to the peak area (A), x concentration of the reference (μg/ml) and r^2 the correlation coefficient of the equation.

Table 2
Statistical results of precision, accuracy and repeatability of the 10 compounds.

Compound	Intra-day		Inter-day		Repeatability	
	Accuracy (R.E., %)	Precision (R.S.D., %)	Accuracy (R.E., %)	Precision (R.S.D., %)	Content (μg/g)	R.S.D. (%)
Gallic acid	95.9	0.3	91.8	1.0	127	0.9
5-HMF	93.45	0.6	99.1	2.0	764	2.1
Morroniside	93.0	0.9	97.3	1.2	1102	2.7
Sweroside	96.3	1.0	96.8	1.4	55	2.1
Loganin	95.6	0.2	94.7	1.3	763	2.0
β-Ecdysterone	94.0	0.8	94.6	0.8	19	2.7
Rutin	98.9	0.3	97.5	0.4	21	3.1
Hyperoside	94.7	0.4	91.8	1.5	119	3.5
Quercetin	99.7	0.1	99.6	0.5	10	2.8
Kaempferide	94.6	0.4	93.7	1.0	26	1.7

Table 3
Statistical results of recovery of the 10 compounds ($n=3$).

Compound	Added amount (μg)			Recorded amount (μg)			Mean recovery (%)	R.S.D. (%)
Gallic acid	210 ± 10	257 ± 12	310 ± 10	476 ± 7	510 ± 11	564 ± 13	101.8 ± 3.6	3.6
5-HMF	1197 ± 21	1487 ± 21	1787 ± 21	2715 ± 49	3046 ± 026	3396 ± 39	101.9 ± 2.7	2.6
Morroniside	1767 ± 12	2200 ± 60	2567 ± 70	3863 ± 63	4123 ± 9	4638 ± 120	101.5 ± 4.7	4.6
Sweroside	96 ± 0	120 ± 0	144 ± 0	207 ± 20	222 ± 1	258 ± 4	99.9 ± 4.6	4.6
Loganin	1333 ± 31	1800 ± 310	1840 ± 69	2952 ± 36	3412 ± 340	3464 ± 98	105.6 ± 1.4	1.4
β-Ecdysterone	36 ± 0	45 ± 0	54 ± 0	74 ± 1	85 ± 1	91 ± 1	103.6 ± 3.0	2.9
Rutin	36 ± 0	45 ± 0	54 ± 0	78 ± 2	89 ± 2	97 ± 2	100.2 ± 4.3	4.3
Hyperoside	188 ± 0	235 ± 0	282 ± 0	434 ± 3	.487 ± 5	520 ± 3	103.6 ± 3.0	2.9
Quercetin	20 ± 0	25 ± 0	30 ± 0	40 ± 1	46 ± 2	51 ± 1	104.5 ± 4.0	3.8
Kaempferide	44 ± 0	55 ± 0	66 ± 0	96 ± 1	109 ± 3	118 ± 2	100.0 ± 3.5	3.5

sion 2004A) was used to achieve the correlation coefficient between a test sample and a reference sample in evaluating the similarities of different chromatograms. Mean HPLC-UV chromatograms of two groups of ZGW were generated firstly by importing twenty chromatograms. The correlation coefficient of each sample to their mean chromatogram which was taken as a reference and that between mean chromatogram was calculated. The mean correlation coefficients of every origin were more than 0.974. The correlation coefficient between mean chromatograms of the samples from different origins was 0.421. The result showed that the chromatograms of samples from the same origin were generally consistent and stable, and those from different origins were a little dissimilar. However, this result was not efficient enough for distinguishing different groups of samples because the complex multivariate data of fingerprint chromatograms can lead to neglecting minor differences among the samples.

To solve this problem, the PLS-DA was performed on the data of HPLC-UV chromatogram fingerprints. It enabled us to calculate discriminant functions of the selected descriptors, which maximize the interspecific difference. Peak areas and retention times of 98 common compounds including the 10 target compounds in 20 samples were used as input data. All programs were coded in Matlab7.0. After applying PLS-DA, the 20 batches of samples were clearly classified into two groups (Fig. 3). Furthermore, gallic acid, 5-HMF, morroniside, sweroside, loganin, β-ecdysterone, rutin, hyperoside, quercetin and kaempferide were found to have more significance

Table 4
Contents (mg/g) of the 10 compounds in the 20 samples ($n=3$).

Sample no.	Content of each compound in 20 samples (μg/g)									
	Gallic acid	5-HMF	Morroniside	Sweroside	Loganin	β-Ecdysterone	Rutin	Hyperoside	Quercetin	Kaempferide
S1	447 ± 4.0	256 ± 5.4	1043 ± 28.2	53 ± 1.1	579 ± 11.6	17 ± 0.5	21 ± 0.7	92 ± 3.2	11 ± 0.3	214 ± 3.7
S2	59 ± 0.5	320 ± 6.7	798 ± 21.5	60 ± 1.3	515 ± 10.3	12 ± 0.3	32 ± 1.0	77 ± 2.7	10 ± 0.3	16 ± .3
S3	132 ± 1.2	569 ± 11.9	922 ± 24.9	49 ± 1.0	634 ± 12.7	18 ± 0.5	24 ± 0.7	110 ± 3.9	9 ± 0.3	418 ± 7.2
S4	127 ± 1.1	764 ± 15.7	1102 ± 29.8	55 ± 1.2	763 ± 15.3	19 ± 0.5	21 ± 0.7	119 ± 4.2	10 ± 0.3	26 ± 0.4
S5	115 ± 1.0	582 ± 12.2	1072 ± 28.9	58 ± 1.2	777 ± 15.6	16 ± 0.4	29 ± 0.9	137 ± 4.8	11 ± 0.3	36 ± 0.6
S6	78 ± .0.7	410 ± 8.6	983 ± 26.5	48 ± 1.0	697 ± 13.9	19 ± 0.5	28 ± 0.9	117 ± 4.1	10 ± 0.3	42 ± 0.7
S7	123 ± 1.1	729 ± 15.3	979 ± 26.4	48 ± 1.0	681 ± 13.6	21 ± 0.6	24 ± 0.7	141 ± 4.9	12 ± 0.3	45 ± 0.8
S8	191 ± 1.7	398 ± 8.4	546 ± 14.7	39 ± 0.8	431 ± 8.6	12 ± 0.3	15 ± 0.5	99 ± 3.5	8 ± 0.2	50 ± 0.9
S9	259 ± 2.3	644 ± 13.5	815 ± 22.0	57 ± 1.2	636 ± 12.7	18 ± 0.5	19 ± 0.6	132 ± 4.6	11 ± 0.3	68 ± 1.2
S10	325 ± 2.9	542 ± 11.4	677 ± 18.3	53 ± 1.1	500 ± 10.0	12 ± 0.3	12 ± 0.4	88 ± 3.1	9 ± 0.3	39 ± 0.7
S11	236 ± 2.1	1937 ± 40.7	–	50 ± 1.1	388 ± 7.8	11 ± 0.3	10 ± 0.3	22 ± 0.8	–	18 ± 0.3
S12	142 ± 1.3	2044 ± 42.9	–	49 ± 1.0	220 ± 4.4	5 ± 0.1	3 ± 0.1	15 ± 0.5	3 ± 0.1	–
S13	270 ± 2.4	2913 ± 61.2	–	46 ± 1.0	318 ± 6.4	5 ± 0.1	7 ± 0.2	19 ± 0.7	–	9 ± 0.2
S14	220 ± 2.0	2112 ± 44.4	144 ± 3.9	57 ± 1.2	352 ± 7.0	13 ± 0.4	6 ± 0.2	18 ± 0.6	4 ± 0.1	5 ± 0.1
S15	256 ± 2.3	2706 ± 56.8	176 ± 4.8	70 ± 1.5	428 ± 8.6	17 ± 0.5	8 ± 0.2	21 ± 0.8	3 ± 0.1	6 ± 0.1
S16	179 ± 1.6	2126 ± 44.6	130 ± 3.5	48 ± 1.0	321 ± 6.4	11 ± 0.3	5 ± 0.2	17 ± 0.6	–	12 ± 0.2
S17	206 ± 1.9	1789 ± 37.6	466 ± 12.6	75 ± 1.6	370 ± 7.4	31 ± 0.8	35 ± 1.1	62 ± 2.2	11 ± 0.3	14 ± 0.2
S18	466 ± 4.0	3017 ± 63.4	–	58 ± 1.2	379 ± 7.6	10 ± 0.3	11 ± 0.3	20 ± 0.7	3 ± 0.1	6 ± 0.1
S19	74 ± 0.7	1301 ± 27.3	136 ± 3.7	45 ± 0.9	175 ± 3.5	12 ± 0.3	13 ± 0.4	27 ± 0.9	7 ± 0.2	13 ± 0.2
S20	170 ± 1.5	1411 ± 29.6	121 ± 3.3	45 ± 0.9	267 ± 5.3	14 ± 0.4	21 ± 0.7	23 ± 0.8	2 ± 0.1	14 ± 0.2

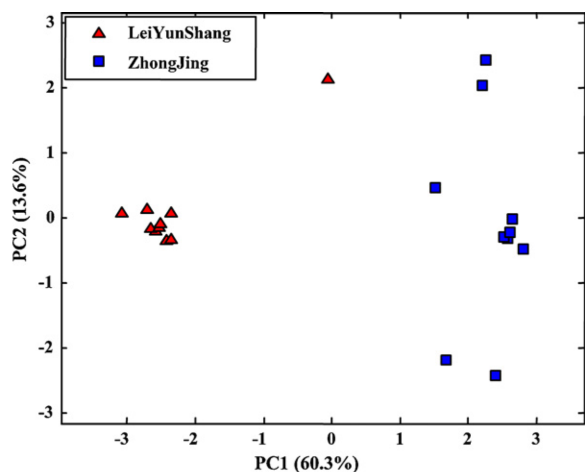

Fig. 3. Scores plot from PLS-DA model classifying samples from different companies: each point representing a particular sample, is automatically shape-coded according to its origin.

on the separation between ZGW samples, which could be applied to accurate discrimination and quality control of ZGW.

Acknowledgements

This work was supported by program for Changjiang Scholars and Innovative Research Team in University (PCSIRT), NCET Foundation, NSFC (30725045), National 863 Program (2006AA02Z338), China Postdoctoral Science Foundation (20070410711), "973" program of China (2007CB507400), Shanghai Leading Academic Discipline Project (B906) and in part by the Scientific Foundation of Shanghai China (07DZ19728, 06DZ19717, 06DZ19005).

References

[1] Drug Standards of Ministry of Public Health of the People's Republic of China, vol. 1, People's Medical Publishing House, Beijing, 1964.
[2] H.Y. Li, X. Gao, X.S. Yan, A.P. Ming, Y.Q. Peng, J.J. Li, World Chin. J. Digestol. 13 (2005) 2818–2822.
[3] W.W. Dai, G.Q. Jin, X.L. Zhang, L.A. Yao, P.C. Xu, Chin. J. Gerontol. 26 (2006) 1006–1069.
[4] J.C. Cai, R. Yu, M.H. Yao, Chin. J. Clin. Pharm. 15 (2006) 350–353.
[5] W.F. Du, H. Cai, M.Y. Wang, X. Ding, H. Yang, B.C. Cai, J. Pharm. Biomed. Anal. 48 (2008) 194–197.
[6] M. Ye, Y. Li, Y.N. Yan, X.H. Ji, J. Pharm. Biomed. Anal. 28 (2002) 621–628.
[7] X. Jiang, Foreign Med. Sci. 27 (2005) 105–106.
[8] Liu., Y.Q. Zhou, C. Peng, J. AnHui TCM College 24 (2005) 43–44.
[9] C.W. Li, Herald Med. 26 (2007) 419–420.
[10] L.Z. Yi, D.L. Yuan, Y.Z. Liang, P.S. Xie, Anal. Chim. Acta 588 (2007) 207–215.
[11] H.M. Lu, Y.Z. Liang, S. Chen, J. Ethnopharmacol. 105 (2006) 436–440.
[12] Y. Chen, S.B. Zhu, M.Y. Xie, S.P. Nie, W. Liu, C. Li, X.F. Gong, Y.X. Wang, Anal. Chim. Acta 623 (2008) 146–156.
[13] C.J. Xu, Y.Z. Liang, F.T. Chau, Y.V. Heyden, J. Chromatogr. A 1134 (2006) 253–259.
[14] The State Pharmacopoeia Commission of People's Republic of China, Pharmacopoeia of People's Republic of China, vol. 1, Chemical Industry Press, Beijing, 2005.
[15] L. Zhang, Y.H. Liu, G.N. Chen, J. Chromatogr. A 1043 (2004) 317–321.
[16] F. Li, H.J. Hou, Chin. Tradit. Patent Med. 18 (1996) 12–14.
[17] B.S. Huang, LiShiZhen Med. Mater. Med. Res. 12 (2001) 487.