Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 436–442

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A strategy for the detection of quality fluctuation of a Chinese herbal injection based on chemical fingerprinting combined with biological fingerprinting

Yongshen Ren[a,b,1], Ping Zhang[a,c,1], Dan Yan[a,*], Jiabo Wang[a], Xiaoxi Du[d], Xiaohe Xiao[a,*]

[a] Military Institute of Chinese Materia Medica, 302th Military Hospital of China, Beijing 100039, PR China
[b] College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, PR China
[c] Postgraduate Team, Military Medical Postgraduate School of China, Beijing 100853, PR China
[d] Center for Drug Reevaluation, State Food and Drug Administration, Beijing 100045, PR China

ARTICLE INFO

Article history:
Received 5 January 2011
Received in revised form 7 May 2011
Accepted 9 May 2011
Available online 13 May 2011

Keywords:
Herbal injection
Yinzhihuang injection
Quality fluctuation
Chemical fingerprinting
Biological fingerprinting

ABSTRACT

Herbal injections are powerful preparations of traditional Chinese medicines. However, the quality control (QC) of these formulations is difficult to establish. Recently, chemical fingerprinting (CF) has been recommended as a potential strategy for the QC of herbal injections. However, some constituents cannot be detected by chromatographic methods. To establish a comprehensive QC process, biological fingerprinting (BF) was combined with CF to detect the fluctuation in quality of a herbal injection from chemical and biological aspects. Yinzhihuang injection was selected as a representative herbal injection. Ten batches of normal samples and six batches of artificially abnormal samples were collected. High-performance liquid chromatography and thermal activity monitoring were applied to develop CF and BF, respectively. The CF and BF of normal samples were similar, with good stability and consistency, but the abnormal samples were not. The results were analyzed by hierarchical clustering analysis: all abnormal samples could be correctly distinguished when CF and BF were combined. The recognition ratio was higher for CF (87.5%) than for BF (93.75%). The key factors of quality fluctuation were bacterial contamination, high temperature, lighting, and sterilizing conditions. The described method could be used for early prediction of adverse drug events, which could help improve the safety of herbal injections.
© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Herbal injections have been widely employed in China owing to their rapid and powerful therapeutic effects [1,2]. However, the quality control (QC) of such herbal injections has been difficult to establish [3,4].

Chemical fingerprinting (CF) and assaying of multiple ingredients have been demonstrated to be powerful techniques for the QC of herbal injections [5,6]. More advanced analytical methods have also been applied, including high-performance liquid chromatography (HPLC) [7], gas chromatography [8], capillary electrophoresis [9], and mass spectroscopy [10] as well as their hyphenated techniques [11]. These modern analytical instruments are powerful tools to identify more compounds in herbal injections, and have improved the QC of herbal injections.

Nevertheless, many substances cannot be detected by chromatographic methods because of a lack of absorption in spectra, in particular certain biological active ingredients, biopollutants, and pharmaceutical adjuvants [12]. This has been a problem in the QC of herbal injections because unknown substances have resulted in adverse drug reactions and adverse drug events [13]. These include pyrogenic reactions, anaphylactic reactions, allergic shock, or even death [14,15]. Hence, it has become necessary to establish novel approaches for the QC of herbal injections from a biological viewpoint [16–18].

The methods for the biological QC of herbal injections should be sensitive and specific, and have "fingerprint" qualities [19]. Herein, a quantitative and versatile bioanalytical method ("biothermodynamics") was adopted to establish biological fingerprinting (BF) for the QC of herbal injections. This method has been used to investigate heat metabolism along with the growth processes of organisms using a microcalorimeter, and has been widely applied in pharmaceutical analyses, biotechnology, and environmental evaluation [20,21]. In suitable conditions, the power–time curve of each biosystem (e.g., *Staphylococcus aureus*) was species-specific and consistent [22,23]. When conditions changed, the curve shifted and was different from the standard curves. This is helpful to check the consistency and stability of herbal injections.

Yinzhihuang injection (YZHI) is derived from the traditional Chinese medicine Yin Chen Hao Tang. This agent is used for

* Corresponding authors. Tel.: +86 10 66933322; fax: +86 10 63879915.
E-mail addresses: pharmacy302@126.com (D. Yan), pharmacy302@126.com (X. Xiao).
[1] These authors contributed equally to this work.

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.05.006

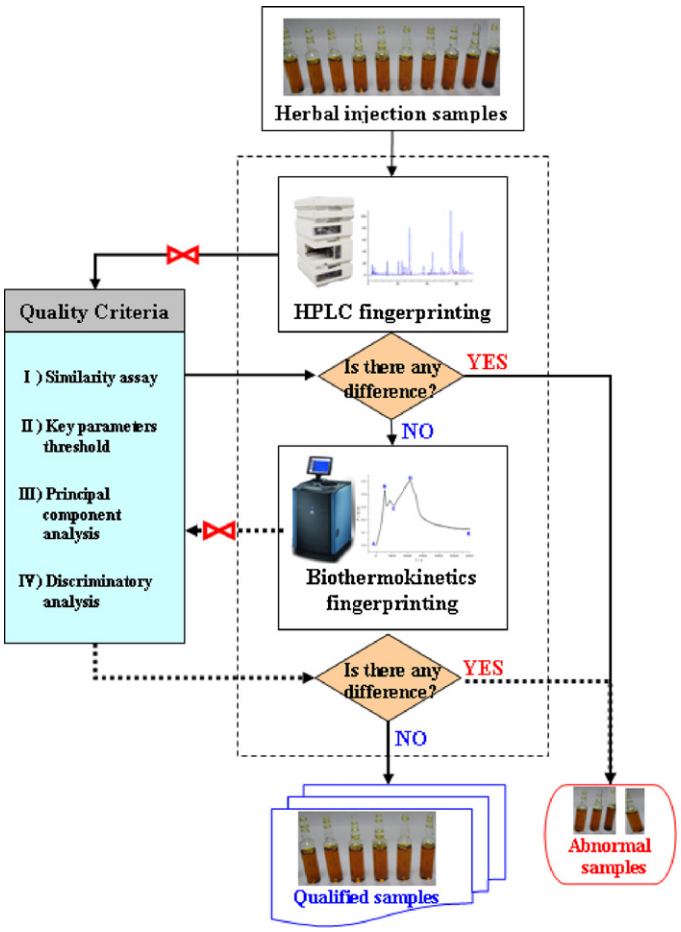

Fig. 1. Flowchart for the detection of the quality fluctuation of herbal injections via a combination of HPLC and biothermokinetic fingerprinting.

the treatment of chronic jaundice and hepatitis [24,25]. The main constituents of YZHI are coumarin from *Herba Artemisiae* Scopariae (Yinchen), chlorogenic from *Flos Lonicerae* (Jinyinhua), gardenoside from *Fructus* Gardeniae (Zhizi) and baicalin from *Radix Scutellariae* (Huangqin), and they have detoxification and antimicrobial properties [26,27]. However, only the content of baicalin has been adopted for the QC of YZHI (http://baike.baidu.com/view/564926.htm#sub564926).

In this work, a feasible approach for the QC and safety of a herbal injection was established based on the QC and stability detection by a combination of CF by HPLC and BF by thermal activity monitoring (TAM). Herein, YZHI was selected as a representative of herbal injections.

Ten batches of normal samples and 6 batches of artificial abnormal samples were collected to establish CF and their effect on sensitive strains of *S. aureus* chosen to establish BF. The procedures are shown in Fig. 1. The results were analyzed by multivariate statistical analysis methods, and reported below.

2. Experimental

2.1. Reagents and materials

Chromatographic-grade methanol was purchased from Fisher Chemicals (Pittsburgh, PA, USA). All other chemicals were of analytical grade and purchased from Beijing Chemical Factory (Beijing, China). Water was purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA).

Reference substances (geniposide, caffeic acid, chlorogenic acid, jasminoidin, baicalin, wogonoside, baicalein, wogonin) were purchased from the National Institutes for Food and Drug Control (NIFDC) (Beijing, China). Their purity was >99.8%. *S. aureus* (CMCC B 26 003) was also obtained from the NIFDC.

2.2. Preparation of culture medium

The nutrient broth culture medium contained (per 1000 mL) (pH 7.0): 5 g NaCl, 10 g peptone, and 6 g beef extract. Lactose broth (LB) culture medium contained (per 1000 mL) (pH 7.0): 10 g peptone, 5 g yeast extract and 5 g NaCl. These broths were sterilized at high-pressure (0.1 MPa) steam at 121 °C for 30 min.

2.3. Preparation of samples

Ten batches of normal YZHI samples were purchased from a Chinese pharmaceutical factory (Shineway, Shijiazhuang, China). 6 batches of abnormal samples were prepared to expose and magnify the data on quality fluctuation according to influencing factors such as: sterilization condition, high temperature, highlight and exposure to air. These samples are classified in Table 1 according to their sources and grouping.

2.4. CF assay

2.4.1. Preparation of standard solutions

Standard solutions (geniposide, caffeic acid, chlorogenic acid, jasminoidin, baicalin, wogonoside, baicalein, wogonin) were prepared by adding an accurately weighed amount of each standard stock into a volumetric flask and dissolving with 100 mL MeOH to make a final concentration of 0.118, 0.111, 0.144, 0.108, 0.126, 0.138, 0.121 and 0.115 mg mL^{-1}, respectively. These were then filtered through a 0.45-μm micropore film to yield the standard solution.

2.4.2. Preparation of sample solution

Two milliliters YZHI were accurately obtained and diluted to 10 mL using methanol. This was then filtered through a 0.45-μm micropore film to yield the sample solution.

2.4.3. Apparatus and conditions

CF of YZHI were undertaken using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA). This included a vacuum degasser, quaternary pump, autosampler manager, column compartment, and diode array detector (DAD).

The chromatographic separation was carried out using a Kromasil™ C_{18} column (250 mm × 4.6 mm; 5 μm), operated at 25 °C. The mobile phase was water/phosphoric acid (100/0.05, A) and methanol (B). Gradient elution was done using the following linear gradient: 0–54 min, 10%–46% B; 54–79 min, 46%–60% B; 79–85 min, 60% B. The mobile flow rate was 1.0 mL min^{-1}. The sample injection volume was 10 μL. The detection wavelength was set at 238 nm. CF of each sample was repeated thrice and mean values used for analyses.

2.4.4. Similarity analyses of CF

A similarity evaluation system for the CF of traditional Chinese medicines (TCMs; Chinese Pharmacopoeia Committee, Version 2004A) was applied to evaluate the consistency of the chemical materials of normal samples and the fluctuation of abnormal samples. Common peaks were defined, and a representative standard chemical fingerprint (RSCF) generated by using the mean value of 10 batches of normal samples. All samples were compared with the

Table 1
Samples used in this work.

Sample	Source	Group	Sample	Sources	Group
S01	SW06060811	Normal	S09	SW07070942	Normal
S02	SW06060813	Normal	S10	SW07040341	Normal
S03	SW06060912	Normal	S11[a]	Non-sterilized samples	Abnormal
S04	SW07010242	Normal	S12[b]	High-temperature accelerated samples	Abnormal
S05	SW07010243	Normal	S13[c]	Light-accelerated samples	Abnormal
S06	SW07021042	Normal	S14[d]	Samples exposed to the environment	Abnormal
S07	SW07021141	Normal	S15[e]	Insufficiently sterilized samples	Abnormal
S08	SW07021142	Normal	S16[f]	Over-sterilized samples	Abnormal

[a] S11: semi-product without sterilization, the same batch with S10.
[b] S12: kept S10 in a 60 °C calorstat for 7 days.
[c] S13: kept S10 under 1×10^4 lx illumination for 7 days.
[d] S14: broke off the ampoule of S10 and kept in a sterile room for 15 days.
[e] S15: sterilized S11 by a 100 °C flowing steam for 10 min.
[f] S16: sterilized S11 by moist heat at 126 °C for 30 min.

RSCF. The relative retention time (RRT) and the relative peak area (RPA) of common peaks were then calculated.

2.5. BF assay

2.5.1. Preparation of sample solution

In sterile surroundings, a 20-mL ampoule was used to inject a 100 μL YZHI sample and 4.9 mL LB culture medium. The concentration of the *S. aureus* suspension was 2×10^6 cells mL^{-1}.

2.5.2. Apparatus and conditions

An eight-channel twin microcalorimeter, 3114 thermal active monitor, and an air isothermal calorimeter (Thermometric AB, Stockholm, Sweden) were used to measure the metabolic power–time curves of the growth of *S. aureus* affected by YZHI. The instrument was thermostated at 37 °C. The sample ampoule was put into the sample compartment of the calorimeter, and another homogenous ampoule containing 5 mL sterile water was put into its twin side as a static reference. The experiment was ended when the curve returned to the baseline. The BF of the growth of *S. aureus* at 37 °C as influenced by YZHI was obtained. The BF of each sample was repeated thrice and mean values used for analyses.

2.5.3. Similarity analyses of BF

According to the similarity evaluation of CF, a representative standard biological fingerprint (RSBF) was generated by taking the mean values of 10 batches of normal samples. The similarity of all samples with the RSBF was then calculated.

2.5.4. Hierarchical clustering analysis (HCA)

HCA was used to sort samples into groups. This technique comprises an unsupervised classification procedure that involves measuring the distance or similarity between the objects to be clustered. The similarity or dissimilarity between samples (objects) is usually represented in a dendrogram for ease of interpretation. In the present study, HCA of samples S01–S16 was undertaken using SAS statistical software (SAS for Windows 8.0, SAS, Chicago, IL, USA). The between-groups linkage method as the amalgamation rule and the squared Euclidean distance as the metric were used to establish clusters.

3. Results and discussion

3.1. Chromatographic fingerprint analyses of YZHI

3.1.1. Methodology validation

To obtain a stable and repeatable chromatographic fingerprint of YZHI for QC, a method validation of HPLC fingerprint analyses was undertaken on the basis of retention time and peak area. One sample (S06) was chosen randomly and successively injected into the HPLC system six times at 25 °C by automatic sampling. The relative standard deviations (RSD) for the precision of retention times and peak areas of all peaks were ≤3% and ≤5%, respectively. The stability test was done with S06 for 0 h, 2 h, 4 h, 6 h, 8 h and 24 h at 25 °C by automatic sampling. The RSD of peak areas was <5%. This indicated that the sample solution was stable within 24 h. All results indicated that the method of HPLC for the fingerprint analysis was valid and satisfactory. The HPLC fingerprints of YZHI were obtained under optimized conditions.

3.1.2. Similarity analyses

The RSCF was generated from 10 batches of normal YZHI (Fig. 2). Thirty-seven peaks (which contributed >98% peak area and which were in each chromatogram of normal YZHI samples) were selected as the common characteristic peaks. Peak 25 of baicalin at retention time 56.27 min was selected as the reference peak (S). The similarities between the atlas of 10 batches of qualified samples and the reference atlas were all as high as 1.000. This indicated a high consistency and stability between batches.

Fig. 3 shows that the number of common peaks decreased to 15 (peak numbers 1, 8, 9, 11, 12, 15, 19, 21, 22, 25 (S), 28, 29, 30, 31, 33) when compared with six batches of abnormal YZHI samples and 10 batches of normal samples.

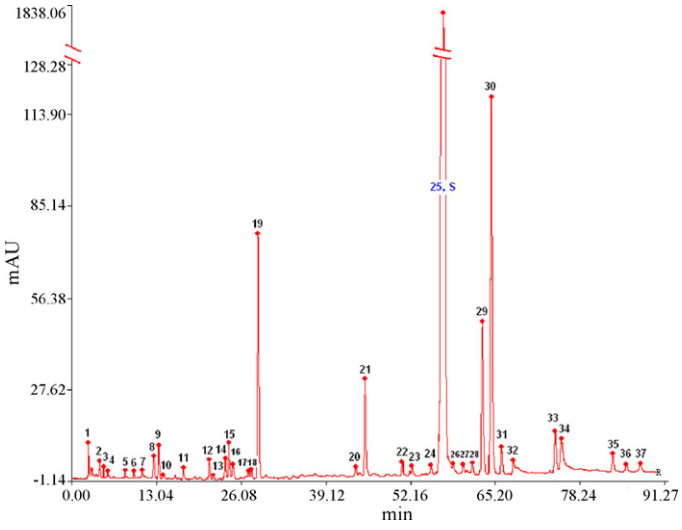

Fig. 2. Reference atlas from 10 batches of qualified YZHI. The reference atlas was generated from 10 batches of qualified YZHI chromatograms using a similarity evaluation system for the chromatographic fingerprint of traditional Chinese medicines (TCMs) (Chinese Pharmacopoeia Committee, Version 2004A) using the median method.

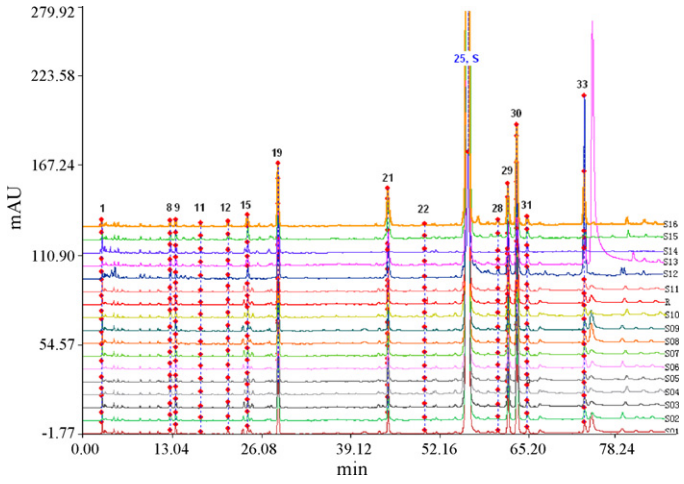

Fig. 3. HPLC fingerprints of 10 batches of qualified YZHI (S01–S10), reference standard chromatographic fingerprints and six batches of abnormal samples (S11–S16).

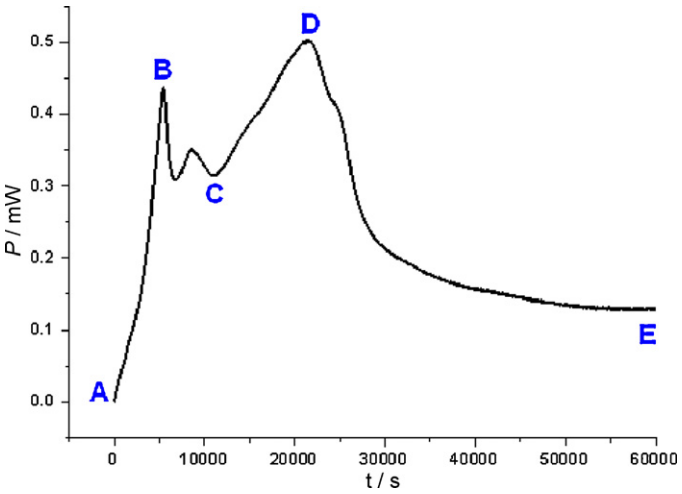

Fig. 4. Power–time curve of the growth of *S. aureus* at 37 °C. The power–time curves of SA growth at 37 °C were obtained using a 3114 TAM air isothermal calorimeter. They could be divided into four phases: first exponential (AB), lag (BC), second exponential (CD) and decline (DE). The biothermokinetic parameters were: B (T^1_m, P^1_m), D (T^2_m, P^2_m), Q_t (AUC_{AE}), k_1 and k_2 (slope rate from linear equation $\ln P_t$–t of section AB and CD).

When we compared chromatographic peaks in sample fingerprints with standard substances at the same retention time, eight constituents present in normal and abnormal samples were identified: geniposide (P12), caffeic acid (P15), chlorogenic acid (P19), jasminoidin (P21), baicalin (P25, S), wogonoside (P29), baicalein (P30) and wogonin (P33).

The similarities between the atlas of six batches of abnormal samples and the RSCF were 1.000, 0.996, 0.975, 0.827, 1.000 and 0.705. The RRT and RPA of common peaks from the RSCF and six batches of abnormal samples are shown in Table 2.

Peak 33 from S12 and peak 34 from S13 were enhanced. This suggested that these peaks were sensitive to temperature and light. The RPA of S14 (samples exposed to the environment) degraded acutely, indicating exhaustion of these materials. Peak 25 of S16 divided into two peaks (peak 25 and 25′), indicating that this constituent had decomposed. Several peaks disappeared from the CF of S12–S16, suggesting quality fluctuation in these samples.

3.1.3. Results of HCA

The HCA for CF of samples S01–S16 was undertaken using SAS statistical software (Fig. S1 in Supporting Information). This showed that the samples could be divided into two clusters: samples S01–S11 and S15 were in cluster I; samples S12, S13, S14 and S16 in cluster II. Apart from S11 (non-sterilized samples) and S15 (insufficiently sterilized samples), the other samples in cluster I were all normal, and the squared Euclidean distance was <4. In cluster II, all samples were far from cluster I. The distance between samples increased gradually, just like the change in CF. This indicated that CF could be used to discriminate certain abnormal samples from qualified samples if the chemical composition changed. However, non-sterilized samples and insufficiently sterilized samples could not be detected by CF. The prevalence of mis-classification was as high as 12.5% (two errors in classification out of 16 samples). Therefore, further investigation should be applied to complement chemical-based QC methods.

3.2. BF analyses of YZHI

3.2.1. BF and biothermokinetic parameters

The power–time curve of *S. aureus* growth at 37 °C is shown in Fig. 4. The shape of these fingerprints could be divided into four phases: first exponential (AB), lag (BC), second exponential (CD) and decline (DE). The quantitative parameters P^1_m and P^2_m were the maximum power output of the first and second exponential phases, respectively; T^1_m and T^2_m were the appearance time of P^1_m and P^2_m; Q_t was the total thermogenesis of *S. aureus* calculated from the area under the curve (AUC) of the power–time curve were obtained.

The growth rate constants k_1 and k_2 were calculated by fitting $\ln P_t$ of the exponential growth phase (section AB and CD in Fig. 4) with t to a linear equation according to the equation $P_t = P_0 \exp(k \cdot t)$ or $\ln P_t = \ln P_0 + k \cdot t''$ [28], where P_0 represents the heat power of the beginning of the baseline and P_t represents the power at time t.

3.2.2. Methodological validation

To evaluate the methodological validation of BF for the QC of YZHI, sample S06 was chosen randomly and used in eight channels simultaneously at 37 °C. The RSD of k_1 from each channel was <1.5%, indicating good homogeneity among channels. The stability test was undertaken: S06 was detected in the same channel six times at 37 °C. The RSD of k_1 was <3%, which indicated that the system was stable under this condition. The results indicated that the method for BF of YZHI was valid and satisfactory. Under the optimized conditions, BF of YZHI was established and biothermokinetic parameters extracted: the results are shown in Fig. 5 and Table 3.

3.2.3. Similarity analyses

The similarity of BF was calculated using the vector angle cosine model. The RSBF was generated by taking the mean values of 10 batches of normal samples. The similarity of all samples with the RSBF is shown in Table 3. The similarity of normal samples with the RSBF was >0.98, whereas most of the abnormal samples (except for S16, which was 0.982) were <0.98. This suggested that similarity analysis alone was inadequate for the discrimination of abnormal samples. The BF of normal samples was homogeneous and stable, yet the BF of abnormal samples fluctuated (Fig. 5). There were "ghost peaks" in the BF of S11 (non-sterilized samples), S14 (samples exposed to the environment) and S15 (insufficiently sterilized samples), indicating potential bacterial contamination of these samples. The P^2_m enhancement of S11, S12, and S15 indicated a reduction of the antibacterial effect. The P^2_m decrease and T^2_m prolongation of S13 and S14 indicated that the inhibition effect increased in them.

3.2.4. Results of HCA

The HCA for the BF of samples S01–S16 was undertaken using SAS statistical software (Fig. S2 in Supporting Information). This

Table 2
Mean RRT and RPA of common peaks from abnormal samples ($n = 3$).[d]

Peak no.	Mean RPA[c] of common peak								
	RRT[a]	RSCF[b]	RSCF[e]	S11	S12	S13	S14	S15	S16
1	0.05	75.51	1	1.36	1.14	0.88	3.97	1.04	1.04
2	0.08	40.48	1	1.24	1.42	–	1.56	0.53	0.43
3	0.09	26.25	1	0.70	2.99	–	1.53	1.61	1.35
4	0.10	14.96	1	1.38	2.00	–	–	1.03	0.99
5	0.15	21.12	1	0.76	3.13	–	1.38	1.53	1.64
6	0.17	21.57	1	0.78	1.64	–	1.22	1.15	1.17
7	0.19	26.19	1	1.13	1.03	–	–	–	–
8	0.23	128.81	1	0.70	1.04	0.83	1.19	0.78	0.78
9	0.24	120.04	1	1.12	0.78	1.05	0.91	0.97	1.00
10	0.25	22.73	1	1.05	1.24	0	0	2.15	1.28
11	0.30	49.06	1	0.80	1.27	1.23	1.28	1.21	1.15
12	0.37	87.38	1	1.14	0.77	1.08	0.65	1.01	1.12
13	0.39	17.62	1	0.88	–	–	–	–	–
14	0.42	87.47	1	1.14	–	–	0.81	–	–
15	0.43	148.71	1	0.93	1.51	1.60	1.15	1.53	1.61
16	0.44	74.37	1	1.18	–	0.75	0.65	0.70	0.70
17	0.48	30.63	1	0.70	–	–	2.93	2.73	1.24
18	0.48	38.68	1	1.07	–	–	–	1.15	1.12
19	0.51	1115.49	1	0.92	1.16	1.07	1.25	1.05	1.08
20	0.77	61.78	1	1.17	1.02	–	–	1.38	0.30
21	0.79	523.36	1	0.92	1.44	1.45	0.25	1.78	2.07
22	0.89	67.93	1	1.08	0.32	0.87	0.80	0.75	0.55
23	0.92	51.97	1	0.84	–	–	0.73	0.85	0.48
24	0.97	54.02	1	0.95	0.66	–	0.65	1.05	0.45
25	1.00	49349.7	1	1.00	0.99	1.04	0.04	1.00	0.50
25′	1.02			–	0.02	0.0022	–	–	0.49
26	1.03	74.32	1	1.01	2.10	1.04	–	1.83	3.47
27	1.05	42.54	1	1.00	8.20	2.37	–	2.41	1.52
28	1.08	60.68	1	0.86	5.77	1.83	0.58	2.46	2.12
29	1.10	909.87	1	1.02	1.21	1.08	0.08	1.05	1.07
30	1.13	2199.20	1	1.00	1.07	1.08	0.24	1.06	1.05
31	1.15	161.80	1	1.01	1.81	1.33	0.81	1.22	1.23
32	1.19	112.85	1	0.86	0.77	0.61	–	0.53	0.59
33	1.30	258.21	1	1.01	17.26	2.53	0.39	0.66	5.12
34	1.32	297.22	1	0.73	0.20	39.47	–	0.22	0.24
35	1.46	120.37	1	0.74	1.78	1.60	–	1.57	1.53
36	1.50	50.99	1	0.98	4.09	1.74	–	1.83	2.97
37	1.54	58.67	1	0.84	1.76	1.37	–	0.45	1.21

[a] RRT: relative retention time.
[b] Original value of the representative standard chemical fingerprint (RSCF).
[c] Relative peak area (RPA) = peak area/homologous peak area from RSCF.
[d] Mean of three experiments.
[e] RPA of RSCF.

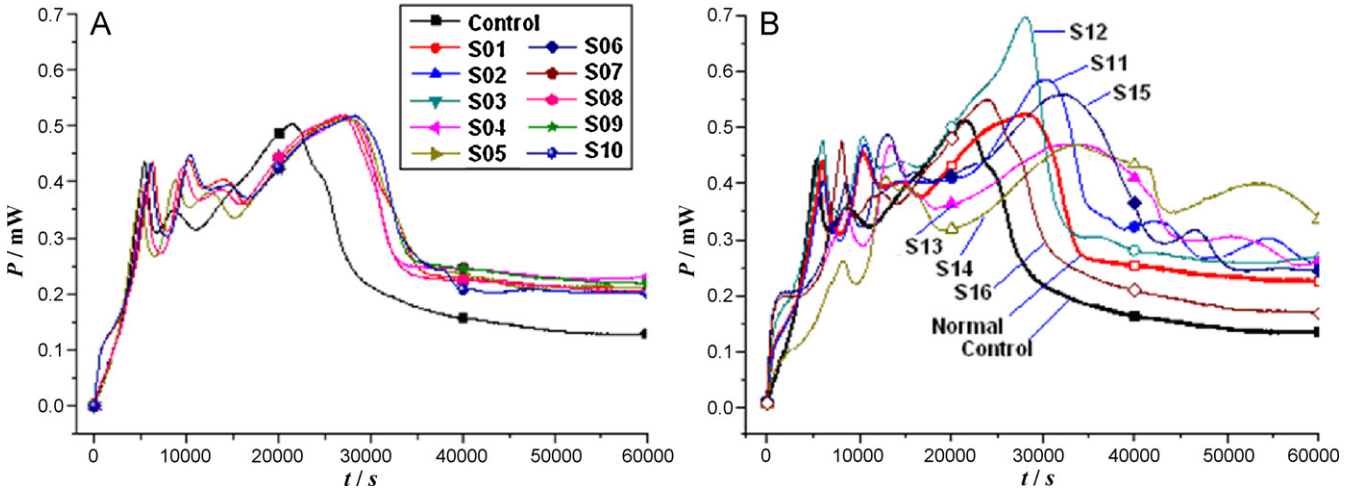

Fig. 5. Biological fingerprints of YZHI. BF was the thermogenesis curve of *S. aureus* as influenced by YZHI obtained using 3114 TAM air isothermal calorimeter at 37 °C. (A) Control, thermogenesis curve of *S. aureus* without YZHI; S01–S10, qualified samples; (B) control, thermogenesis curve of *S. aureus* without YZHI; normal, reference standard generated by simulating the mean BF of 10 batches of qualified samples; S11, non-sterilized samples; S12, high temperature-accelerated samples; S13, light-accelerated samples; S14, samples exposed to the environment; S15, insufficiently sterilized samples; S16, over-sterilized samples.

Table 3
Quantitative parameters of *S. aur*eus growth affected by YZHI[a] ($n = 3$).[b]

Sample	k_1/h^{-1}	k_2/h^{-1}	T_m^1/s	P_m^1/mW	T_m^2/s	P_m^2/mW	Q_t (J)	Similarity
S01	1.556	0.201	6320	0.435	27280	0.518	21.892	0.996
S02	1.517	0.207	5920	0.437	26740	0.518	21.141	0.996
S03	1.583	0.206	6060	0.433	28000	0.518	22.381	0.997
S04	1.580	0.194	5980	0.439	27700	0.513	21.957	0.992
S05	1.580	0.194	5760	0.428	27560	0.512	22.350	0.982
S06	1.636	0.209	5810	0.412	27490	0.518	21.759	0.997
S07	1.560	0.174	6120	0.435	27960	0.516	22.434	0.998
S08	1.637	0.204	6030	0.429	27710	0.518	21.237	0.999
S09	1.509	0.160	6010	0.431	28020	0.515	21.965	0.992
S10	1.555	0.167	5960	0.433	28130	0.517	21.698	0.989
RSBF[c]	1.571	0.191	5997	0.431	27659	0.516	21.881	1.000
S11	1.026	0.178	6200	0.398	30200	0.576	25.185	0.973
S12	0.706	0.136	8480	0.394	32340	0.551	26.285	0.972
S13	0.672	0.107	8360	0.347	32460	0.463	25.656	0.962
S14	0.916	0.150	8120	0.254	33880	0.462	26.153	0.929
S15	0.996	0.174	6140	0.470	28160	0.688	25.660	0.978
S16	1.494	0.183	7120	0.467	24880	0.542	19.752	0.982

[a] YZHI: Yinzhihuang injection.
[b] Mean of three experiments.
[c] RSBF: representative standard biological fingerprint.

showed that samples could be divided into two clusters: samples S01–S10 and S16 were in cluster I; samples S11–S15 in cluster II. Most samples in cluster I were normal, and the squared Euclidean distance was <1.5, which indicated good homogeneity of these samples. In cluster II, samples could be divided into two classes. The first was S11 and S15 close to cluster I; then S12, S13 and S14 were clustered far from the other samples. S16 (over-sterilized samples) were clustered with normal samples at ~2. This indicated that S16 was difficult to distinguish from normal samples, and the prevalence of mis-classification was 6.25% (one error in classification out of 16 samples), which was lower than that for CF. Nevertheless, BF could not be used to distinguish samples as significantly as CF. This is because the furthest cluster distance of BF was only 6, whereas the distance of CF was 14. It was therefore necessary to combine BF with CF for the QC of herbal injections.

3.3. Results of the combination of CF with BF

To assure the quality of YZHI from chemical and biological aspects, CF and BF were combined. The ability to distinguish between samples was evaluated by HCA (Fig. S3 in Supporting Information). Compared with the HCA result of CF and BF respectively, a great elevation of discrimination capability was shown (Fig. S3). Samples could be divided into two clusters: cluster I for normal samples and cluster II for abnormal samples. Samples S11, S15 and S16, which were misjudged by CF or BF, were correctly distinguished from normal samples. Furthermore, the distance between abnormal samples was S11 < S15 < S16 < S13 < S12 < S14, which was much closer to the real situation. This could be very helpful in predicting the potentially hazardous nature of certain components of herbal injections. If developed further, CF could be used to elucidate the general difference between chemical constituents in samples. Similarly, BF could be used to calibrate/complement/magnify the differences in the biological properties of samples. Compared with the Ministry of Authorized Standards (which determined only the content of baicalin for the QC of YZHI), the combination of BF and CF could elicit more comprehensive QC information for YZHI. It could be adopted as an effective complementary method for the QC of other herbal injections.

4. Conclusions

In the present study, to monitor the product consistency and stability of YZHI from chemical and biological aspects, CF and BF from HPLC and TAM were combined. Both methods detected the fluctuation in quality precisely and sensitively. The former emphasized fluctuation of the quality of chemical constituents, while the latter emphasized fluctuation of the quality of biological products.

The results of similarity analyses of CF showed that normal samples had very similar fingerprints. CF changed considerably if samples were treated at high temperatures and in a lot of light, and if they were exposed to the environment. Most abnormal samples could be identified by CF or BF. However, S11 (non-sterilized samples) and S15 (insufficiently sterilized samples) could not be detected by CF. S16 (over-sterilized samples) could not be detected by BF.

When we combined CF with BF, all samples were classified correctly. This combination could give more detailed and complete information of the conditions of samples. Hence, using such a combination, it will become easier to predict the safety of products. Many ADEs caused by QC problems could therefore be avoided earlier.

This work introduced a novel approach to the QC and prediction of severity of adverse reactions of herbal injections. It would be equally applicable to the analyses of other types of herbal injections of TCMs. HPLC and TAM fingerprints provided a powerful and meaningful tool in complementing the existing methods of evaluation of the QC of herbal injections.

Acknowledgements

The project was supported by the Special Major Foundation for National New Drug Innovation of China (nos. 2009ZX09502-003, 2009ZX09308-005), the Beijing Natural Science Foundation (no. 7112128), National Natural Science Foundation of China (nos. 30873385 and 30701109), Open Research Fund of the State Key Laboratory Breeding Base of Systematic Research Development and Utilization of Chinese Medicine Resources.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.05.006.

References

[1] T. Xue, R. Roy, Studying traditional Chinese medicine, Science 300 (2003) 740–742.

[2] K.M. Ji, J.J. Chen, M. Li, Z.G. Liu, L.X. Xia, C.B. Wang, Z.K. Zhan, X.L. Wu, Comments on serious anaphylaxis caused by nine Chinese herbal injections used to treat common colds and upper respiratory tract infections, Regul. Toxicol. Pharm. 55 (2009) 134–138.
[3] K. Chan, L.K.S. Yin, S.S. Zhao, Harmonization of monographic standards is needed to ensure the quality of Chinese medicinal materials, Chin. Med. 4 (2009) 18, doi:10.1186/1749-8546-4-18.
[4] Z.D. Zeng, F.T. Chau, H.Y. Chan, C.Y. Cheung, T.Y. Lau, S.Y. Wei, D.K. Mok, C.O. Chan, Y.Z. Liang, Recent advances in the compound-oriented and pattern-oriented approaches to the quality control of herbal medicines, Chin. Med. 3 (2008) 9, doi:10.1186/1749-8546-3-9.
[5] H.J. Chen, X. Li, J.W. Chen, S. Guo, B.C. Cai, Simultaneous determination of eleven bioactive compounds in *Saururus chinensis* from different harvesting seasons by HPLC-DAD, J. Pharm. Biomed. Anal. 51 (2010) 1142–1146.
[6] Y. Xie, Z.H. Jiang, H. Zhou, X. Cai, Y.F. Wong, Z.Q. Liu, Z.X. Bian, H.X. Xu, L. Liu, Combinative method using HPLC quantitative and qualitative analyses for quality consistency assessment of a herbal medicinal preparation, J. Pharm. Biomed. Anal. 43 (2007) 204–212.
[7] S.K. Yan, G.A. Luo, Y.M. Wang, Y.Y. Cheng, Simultaneous determination of nine components in Qingkailing injection by HPLC/ELSD/DAD and its application to the quality control, J. Pharm. Biomed. Anal. 40 (2006) 889–895.
[8] M.L. Qi, X.X. Ge, M.M. Liang, R.N. Fu, Flash gas chromatography for analysis of volatile compounds from *Houttuynia cordata* Thunb., Anal. Chim. Acta 527 (2004) 69–72.
[9] M. Ganzera, Quality control of herbal medicines by capillary electrophoresis: potential, requirements and applications, Electrophoresis 29 (2008) 3489–3503.
[10] Z.D. Zeng, Y.Z. Liang, F.T. Chua, S. Chen, M.K.W. Daniela, C.O. Chan, Mass spectral profiling: an effective tool for quality control of herbal medicines, Anal. Chim. Acta 604 (2007) 89–98.
[11] X.H. Fan, Y.Y. Cheng, Z.L. Ye, R.C. Lin, Z.Z. Qian, Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines, Anal. Chim. Acta 555 (2006) 217–224.
[12] M.D. Huang, H. Becker-Ross, S. Florek, M. Okrussa, B. Welz, S. Morés, Determination of iodine via the spectrum of barium mono-iodide using high-resolution continuum source molecular absorption spectrometry in a graphite furnace, Spectrochim. Acta B 64 (2009) 697–701.
[13] Z.P. Zeng, J.G. Jiang, Analysis of the adverse reactions induced by natural product-derived drugs, Br. J. Pharmacol. 159 (2010) 1374–1391.
[14] Y.B. Zhou, M. Victor, H. Matthew, C. Larry, An overview of adverse drug reaction monitoring in China, Int. J. Pharm. Med. 20 (2006) 79–85.
[15] J. Hu, H.C. Shang, J. Li, L. Zhang, J.H. Zhang, W.K. Zheng, T.Q. Li, B.L. Zhang, Y.Q. Li, Adverse drug reactions linked to Ciwujia injection: a systematic review of 521 cases, J. Evid. Base Med. 3 (2010) 37–43.
[16] Y. Jiang, B. David, P.F. Tu, Y. Barbin, Recent analytical approaches in quality control of traditional Chinese medicines – a review, Anal. Chim. Acta 657 (2010) 9–18.
[17] S.P. Boyle, P.J. Doolan, C.E. Andrewsa, R.G. Reida, Evaluation of quality control strategies in Scutellaria herbal medicines, J. Pharm. Biomed. Anal. (2010), doi:10.1016/j.jpba.2010.11.019.
[18] E.H. Liu, L.W. Qi, K. Li, C. Chu, P. Li, Recent advances in quality control of traditional Chinese medicines, Comb. Chem. High T. Scr. 13 (2010) 869–884.
[19] R. Tilton, A.A. Paiva, J.Q. Guan, R. Marathe, Z.L. Jiang, W.V. Eyndhoven, J. Bjoraker, Z. Prusoff, H.L. Wang, S.H. Liu, Y.C. Cheng, A comprehensive platform for quality control of botanical drugs (PhytomicsQC): a case study of Huangqin Tang (HQT) and PHY906, Chin. Med. 5 (2010) 30, doi:10.1186/1749-8546-5-30.
[20] Y.L. Zhao, D. Yan, J.B. Wang, P. Zhang, X.H. Xiao, Anti-fungal effect of berberine on *Candida albicans* by microcalorimetry with correspondence analysis, J. Therm. Anal. Calorim. 102 (2010) 49–55.
[21] O. Braissant, D. Wirz, B. Göpfert, A.U. Daniels, Use of isothermal microcalorimetry to monitor microbial activities, FEMS Microbiol. Lett. 303 (2010) 1–8.
[22] R. Li, F.L. Jiang, Q. Xiao, J.H. Li, X.R. Liu, Q.L. Yu, Y. Liu, C. Zeng, Microcalorimetric, spectroscopic and microscopic investigation on the toxic effects of CdTe quantum dots on *Halobacterium halobium* R1, Nanotechnology 21 (2010) 75–102.
[23] W.J. Kong, Y.L. Zhao, X.H. Xiao, Z.L. Li, Y.S. Ren, Action of palmatine on *Tetrahymena thermophila* BF5 growth investigated by microcalorimetry, J. Hazard. Mater. 168 (2010) 609–613.
[24] T.X. Wu, H.C. Shang, Z.X. Bian, J.H. Zhang, T.Q. Li, Y.P. Li, B.L. Zhang, Recommendations for reporting adverse drug reactions and adverse events of traditional Chinese medicine, J. Evid. Base Med. 3 (2010) 11–17.
[25] L. Fan, G. Wang, L.S. Wang, Y. Chen, W. Zhang, Y.F. Huang, R.X. Huang, D.L. Hu, D. Wang, H.H. Zhou, Herbal medicine Yin Zhi Huang induces CYP3A4-mediated sulfoxidation and CYP2C19-dependent hydroxylation of omeprazole, Acta Pharmacol. Sin. 28 (2007) 1685–1692.
[26] J.H. Zhang, H.C. Shang, W.K. Zheng, J. Hu, H.J. Xu, H. Wang, L. Zhang, M. Ren, B.L. Zhang, Systematic review on the compatibility of Shuanghuanglian injection combined with western medical injections, J. Evid. Base Med. 3 (2010) 27–36.
[27] B.Q. Li, X. Dong, G.Q. Yang, S.H. Fang, J.Y. Gao, J.X. Zhang, F.M. Gu, X.M. Miao, H. Zhao, Role of chlorogenic acid in the toxicity induced by Chinese herbal injections, Drug Chem. Toxicol. 33 (2010) 415–420.
[28] S.F. Zhang, D. Yan, H.Y. Tang, M. Yang, Y.S. Wang, X.H. Xiao, The toxic effect of solubilizing excipients on *Tetrahymena thermophila* BF5 growth investigated by microcalorimetry, Chin. Sci. Bull. 55 (2010) 1870–1876.