Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1667–1672
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

An approach to develop binary chromatographic fingerprints of the total alkaloids from *Caulophyllum robustum* by high performance liquid chromatography/diode array detector and gas chromatography/mass spectrometry

Yiping Li, Zhen Hu, Langchong He *

Faculty of Pharmacy, School of Medicine, Xi'an Jiaotong University, No. 205 Zhuque Road, Xi'an 710061, PR China

Received 24 October 2006; received in revised form 20 December 2006; accepted 26 December 2006
Available online 7 January 2007

Abstract

An approach was proposed to develop binary chromatographic fingerprints by means of high performance liquid chromatography/diode array detector (HPLC/DAD) and gas chromatography/mass spectrometry (GC/MS). HPLC fingerprint and GC/MS fingerprint that, respectively, represent chemical characteristics of aporphinoid and quinolizidine alkaloids of the total alkaloids from *Caulophyllum robustum* were developed, which were used to construct binary chromatographic fingerprints of the total alkaloids. Moreover, the authentication and validation of the binary fingerprints were performed. Then, a data-level information fusion method was employed to capture the chemical information encoded in two chromatographic fingerprints. Finally, based on the fusion results, the quality of 10 batches of the total alkaloids samples was further evaluated by similarity measure and cluster analysis method. In comparison with conventional fingerprint, the binary chromatographic fingerprints which represent the characteristics of more constitutions can comprehensively and properly reveal the quality characteristics of the total alkaloids. The binary chromatographic fingerprints can reach more objective conclusions in the practice of quality control of the total alkaloids. In conclusion, the binary chromatographic fingerprints are suitable for quality control of the total alkaloids. The presented approach is a powerful and meaningful tool to comprehensively conduct the quality control of traditional Chinese medicine (TCM).

Keywords: Binary chromatographic fingerprints; Total alkaloids; High performance liquid chromatography; Gas chromatography/mass spectrometry

1. Introduction

Traditional Chinese medicine (TCM) has a long therapeutic history over thousands of years, and currently it is still attracting considerable attention worldwide because of its low toxicity and good therapeutical performance [1–3]. Therefore, quality control of herbal medicines is an important concern for both the health authorities and the public [1,4]. The fingerprint of herbal medicines, especially the chromatographic fingerprint, has gained rising attentions in recent years and been internationally accepted as a feasible means for the quality control of herbal medicines [5–11]. Lately, Chinese manufacturers are also required by Chinese State Food and Drug Administration (SFDA) to standardize injections made from TCM and their raw materials using chromatographic fingerprinting [12].

Several chromatographic techniques such as high performance liquid chromatography (HPLC) [13–19], gas chromatography (GC) [20], thin layer chromatography (TLC) [21], capillary electrophoresis (CE) [22], can be applied for fingerprinting. It is well known that TCM is a complex mixture containing hundreds of chemically different constituents which are usually responsible for the therapeutic effects [23]. Therefore, it is almost impossible to develop appropriate analytical method to represent all chemical characteristics of constituents in a chromatogram. Under these circumstances, it is meaningful to develop novel fingerprint based on various chromatographic approaches and additional information for the quality control of TCM. Among chromatographic fingerprint methods, "multiple chromatographic fingerprints" is an effective method [24], and it has been recommended by Food and Drug Adminis-

* Corresponding author. Tel.: +86 29 82655145; fax: +86 29 82655145.
E-mail address: helc@mail.xjtu.edu.cn (L. He).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.12.028

tration (FDA) and SFDA [6,12]. Multiple chromatographic fingerprints is just a combined chromatographic fingerprints, which consist of more than one fingerprint and represent the whole characteristics of chemical constitutions of the complex medicine by using different separation principles or test conditions.

In this study, the raw materials total alkaloids from *Caulophyllum robustum* was investigated to develop binary chromatographic fingerprinting for quality control by high performance liquid chromatography and gas chromatography/mass spectrometry. In our previous research, it was demonstrated that the total alkaloids from *Caulophyllum robustum* could significantly inhibit proliferation of ECV304 cells in MTT test [25]. Now the total alkaloids from *Caulophyllum robustum* are investigated to a new drug for anti-tumor as an angiogenetic inhibitor. Currently, the total content of alkaloids was determined by spectrophotometric method with total alkaloid content set at >50% in the raw materials. Apparently, this standard is not sufficient for quality control. The total alkaloids from *Caulophyllum robustum* principally contain two kinds of alkaloids, quinolizidine and aporphinoid alkaloids [26]. In this study, HPLC and GC/MS fingerprints that, respectively, represent chemical characteristics of aporphinoid alkaloids and quinolizidine alkaloids in raw materials were developed, which constructed binary chromatographic fingerprints of the total alkaloids from *Caulophyllum robustum*. Furthermore, the binary chromatographic fingerprints were applied to evaluate the quality of various samples. The proposed methods are more likely to be accepted as an effective tool in the quality control of other complex herbal medicines.

2. Experimental

2.1. Regents and materials

Standard chemicals *N*-methylcytisine, lupanine, argentamin, anagyrine, α-isolupanine, magnoflorine and taspine were purchased from the National Institute of China for the Control of Pharmaceutical and Biological Products and Sigma (Sigma Inc., USA). Methanol and acetonitrile were of HPLC grade (Sigma Inc., USA). Ultrapure water was prepared with Milli-Q water purification system (Millipore, France). Other chemicals were of analytical-reagent grade.

Totally 10 samples of the total alkaloids from *Caulophyllum robustum* (marked as 1–10) were collected from two Chinese medicine manufacturers. Samples 1–5 were from manufacturer A, and samples 6–10 were from manufacturer B. The names of the manufacturers had been removed in order to preserve confidentially.

2.2. Sample preparation

The total alkaloids from *Caulophyllum robustum* were prepared in Chinese medicine manufacturers as follow. Powdered *Caulophyllum robustum* was refluxed with 95% ethanol three times. Following evaporation of the ethanol in vacuo, the aqueous residue was extracted with 1% hydrochloric acid three times overnight while being stirred. The combined acidic fraction was passed through positive ion exchange resin column (Lanxiao, China) and was washed with 4% ammonia water-ethanol until the eluate was tested negative for alkaloids in the Dragendorff test. The total alkaloids were obtained following the concentration of the eluate in vacuo.

0.10 g total alkaloids was dissolved in 10 mL methanol with a supersonic process for 15 min, and then filtered through a 0.45 μm Econofilter (Agilent, USA) prior to injection into the HPLC and GC/MS system.

2.3. Chromatographic analysis

2.3.1. HPLC analysis

The HPLC analysis was performed using a Shimadzu 2010 high performance liquid chromatograph (Shimadzu Inc., Japan) equipped with a SPM-10Avp diode array detector (Shimadzu Inc., Japan), an intelligent quaternary pump, and a Shimadzu Class vp 6.0 software. The column used was a Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent, USA) coupled with Agilent C_{18} guard column (7.5 mm × 4.6 mm, 5 μm). The mobile phase was solvent A (H_3PO_4:H_2O = 0.01:100) and solvent B (CH_3OH:CH_3CN = 3:1) in the gradient mode as follows: 10% B at 0–5 min, 10–30% B at 5–20 min, 30–65% B at 20–70 min. The flow-rate was 1.0 mL/min. The sample injection volume was 10 μL. The column compartment was kept at the temperature of 30 °C, and DAD detection was performed in the range of 200–594 nm at 1 nm/step.

2.3.2. GC/MS analysis

The GC/MS analysis was performed with Shimadzu GC-17A gas chromatography instrument (Shimadzu Inc., Japan) coupled to a Shimadzu QP2010 mass spectrometer (Shimadzu Inc., Japan), NIST mass spectrometer database (Shimadzu Inc., Japan), and a GC/MS solution workstation. The column used was a DB-5MS (30 m × 0.25 mm) capillary column coated with 0.25 μm film 5% phenyl methyl siloxane (Agilent, USA). The column temperature was maintained at 100 °C for 2 min after injection, then programmed at 10 °C/min to 200 °C, then 5 °C/min to 280 °C and maintained for 6 min. Split injection was conducted with a split ratio of 1:10 and helium was used as carrier gas of 2.0 mL/min flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 40–400 amu, the ionization energy was 70 eV and the scan rate was 0.2 s per scan. The inlet and ionization source temperature were 280 and 230 °C, respectively.

2.4. Method validation

The validation of the analytical method was carried out with sample solutions. The instrument/injection precision (repeatability) was obtained by analyzing the variations of relative retention time and relative peak area of six injections. The intra-day and inter-day precisions of the method were evaluated using multiple preparations of the same sample. Five replicate samples were prepared and analyzed in a single day and on three different days.

2.5. Similarity of binary chromatographic fingerprints

It is well established that the samples with similar chromatographic fingerprint likely have similar properties. Therefore, the consistency of herbal medicines can be tested through comparing the similarity between the chromatographic fingerprints of samples and the reference/standard fingerprints. However, the reported methods which were employed to quantitatively measure the similarity were based on a single chromatogram [13,14,22]. Therefore, the information contained in the binary fingerprints should be combined.

In this study, a data fusion-based method [24,27] was employed to evaluate the similarity of binary chromatographic fingerprints. To accurately capture the information encoded in a chromatogram, a chromatographic fingerprint was usually mathematically represented by a vector of response value of signal, area or height of the peak, etc. Thus, to binary chromatographic fingerprints, assume that vector X and vector Y represent fingerprint of HPLC and fingerprint of GC/MS, respectively,

$$X = [X_1, \ldots, X_i, \ldots, X_m]^{\mathrm{T}}, \qquad Y = [Y_1, \ldots, Y_j, \ldots, Y_n]^{\mathrm{T}}$$

where X_i denotes absolute area of the ith peak of fingerprint of HPLC, Y_j denotes absolute area of the jth peak of fingerprint of GC/MS, and the superscript T indicates the transpose of matrix. To minimize the variability in different chromatographic methods, fingerprint of HPLC and fingerprint of GC/MS were normalized using the following equation:

$$X_i' = \frac{X_i}{\sum_{i=1}^{n} X_i} \tag{1}$$

where X_i' is the normalized peak area, and X_i is the absolute area of peak i in the fingerprint of HPLC or fingerprint of GC/MS.

The new fused vector Z' representing integral chemical characteristics of binary fingerprints is formed by $Z' = [\mathrm{X}', \theta\mathrm{Y}']$, where θ is the combined coefficient. The combined coefficient (θ) is weight coefficient of two vectors combined. Consequently, the similarity between binary chromatographic fingerprints can be easily determined by comparing their fusion vector using commonly used similarity measures, e.g. cosine, correlation coefficient, etc. [28].

2.6. Data analysis

Data analysis was performed on a Pentium IV 1.7G processor. Similarity evaluation system for chromatographic fingerprint of TCM (Chinese Pharmacopoeia Committee, 2004A) was used to the similarity evaluation and SPSS 12.0 for windows (SPSS Inc., USA) was employed to statistical analysis.

3. Results and discussion

3.1. HPLC and GC/MS analysis of the total alkaloids

The resulting chromatograms are shown in Figs. 1 and 2. On the basis of the comparison of the recorded UV spectra and mass spectra with reference to standard compound or literatures [26,29,30] and a MS library (NIST), the main peaks

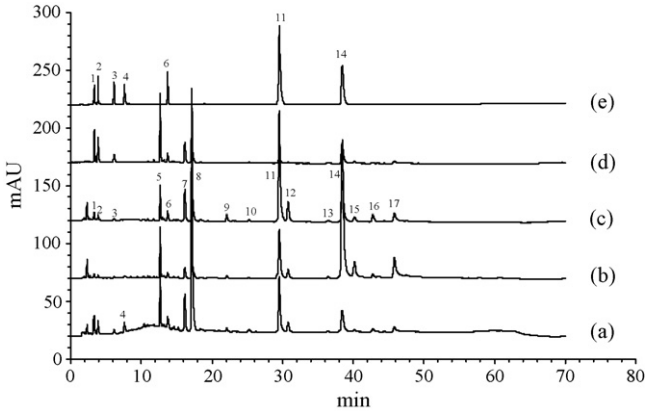

Fig. 1. HPLC chromatogram of the total alkaloids from *Caulophyllum robustum*: (a) 220 nm; (b) 245 nm; (c) 267 nm; (d) 306 nm; (e) standard chemicals at 267 nm; numbers 1–17 stand for peaks at 267 nm, 1. *N*-methylcytisine 2. argentamin 3. lupanine 4. α-isolupanine 6. anagyrine 11. magnoflorine 14. taspine.

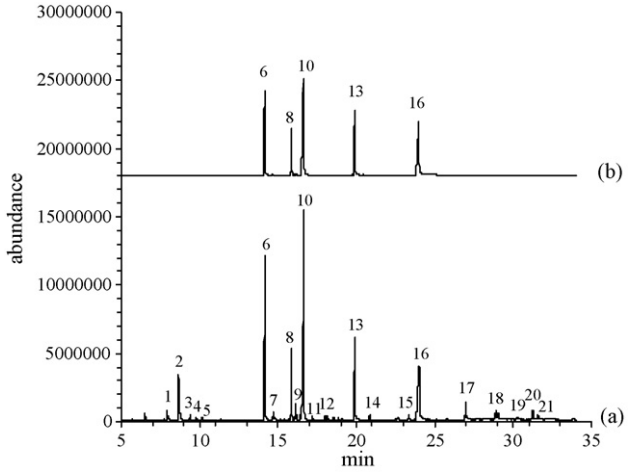

Fig. 2. GC/MS total ion chromatogram of the total alkaloids from *Caulophyllum robustum*: (a) the total alkaloids; (b) standard chemicals; numbers 1–21 stand for peaks, 6. *N*-methylcytisine 7. cytosine 8. α-isolupanine 9. 5,6-dehydro-α-isolupanine 10. lupanine 13. anagyrine 14. boldine 16. argentamin.

of two chromatograms were identified in Tables 1 and 2. The total alkaloids from *Caulophyllum robustum* principally contain two kinds of alkaloids, quinolizidine alkaloids, such as *N*-methylcytisine and lupanine, and aporphinoid alkaloids, such as magnoflorine and taspine.

In order to obtain a large amount of detectable peaks in the HPLC chromatogram, the spectra of all peaks in the chromatogram of the total alkaloids were investigated with

Table 1
Identification of constituents of HPLC analysis of the total alkaloids

Peak no.	Retention time	Identity	UV-λ_{max}
1	3.34	*N*-Methylcytisine	306, 230
2	3.87	Argentamin	302, 230
3	6.03	Lupanine	220
4	7.76	α-Isolupanine	220
6	13.74	Anagyrine	310, 233
11	29.51	Magnoflorine	267, 300, 220
14	39.76	Taspine	245, 348, 332

Table 2
Identification of constituents of GC/MS analysis of the total alkaloids

Peak no.	Retention time	Identity	[M+]	Similarity (%)
6	14.17	*N*-Methylcytisine	204	93
7	14.72	Cytisine	190	90
8	15.86	α-Isolupanine	248	90
9	16.11	5,6-Dehydro-α-isolupanine	246	89
10	16.67	Lupanine	248	95
13	19.89	Anagyrine	244	93
14	20.86	Boldine	326	90
16	23.99	Argentamin	260	91

diode array detector. The chromatograms of the total alkaloids were also obtained at wavelengths of 220, 245, 267 and 306 nm (shown in Fig. 1), which respectively were maximal UV absorption wavelengths of the principle components of the total alkaloid, such as lupanine, taspine, magnoflorine and *N*-methylcytisine. As shown in Fig. 1, due to low concentration or weak UV absorption of quinolizidine alkaloids, quinolizidine and aporphinoid alkaloids cannot be well represented in a HPLC chromatogram. Therefore, 267 nm was selected as detection wavelength so that the chromatogram contained the maximum amount of detectable peaks and aporphinoid alkaloids can be well represented.

Because of low boil point of quinolizidine alkaloids, GC was employed to analyze quinolizidine alkaloids. As shown in Fig. 2, quinolizidine alkaloids can be well represented. Therefore, HPLC chromatogram and GC chromatogram collectively represent the main chemical characteristic components of the total alkaloids.

3.2. Validation of binary chromatographic fingerprints

The analytical methods of binary fingerprinting have been validated based on the relative retention time and the relative peak area of each peak. In HPLC and GC/MS chromatogram, taspine and lupanine indicated high and stable content, respectively, therefore they were chosen as the reference substance. All peaks' relative retention time and relative peak area were obtained with reference to these two substances. The precisions were represented by the relative standard deviation (R.S.D.).

The instrument/injection precision (repeatability) of HPLC was below 0.38% ($n = 6$) for relative retention times and within 0.20–1.37% ($n = 6$) for relative peak areas. The intra-day precisions of HPLC were 0.21–0.41% ($n = 5$) for relative retention times and 1.16–2.23% ($n = 5$) for relative peak areas, while the inter-day precisions were 0.27–0.40% ($n = 5$) for relative retention times and 1.69–3.54% ($n = 5$) for relative peak areas.

The injection precision of GC/MS was in the range of 0.02–0.33% ($n = 6$) for relative retention times and 0.15–0.34% ($n = 6$) for relative peak areas. The intra-day precisions of GC/MS were 0.03–0.35% ($n = 5$) for relative retention times and 1.19–2.37% ($n = 5$) for relative peak areas, while the inter-day precisions were within the range of 0.02–0.35% ($n = 5$) for relative retention times and 1.39–3.18% ($n = 5$) for relative peak areas, respectively.

The results indicated that the proposed method is precise and repeatable. The proposed binary fingerprinting, therefore, is acceptable.

3.3. Binary chromatographic fingerprints of the total alkaloids

According to the definition of fingerprints of TCM, a chromatographic fingerprint is in practice a chromatographic pattern of some common kinds of pharmacologically active and chemically characteristic components in the TCM. This chromatographic profile should feature the fundamental attributions of "integrity" and "fuzziness", in other words, "sameness" and "differences". The chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully.

With HPLC and GC/MS methods, 10 batches of the total alkaloids samples from two different factories in China were analyzed in the optimum conditions, respectively. Peaks existed in all chromatograms of 10 samples were assigned as "common peaks", indicating the sameness among various samples. The HPLC chromatograms from 10 samples consisted of 14 common peaks within 70 min, shown in Fig. 3A. The GC/MS chromatograms from 10 samples consisted of 14 common peaks within 35 min, shown in Fig. 3B. R.S.D. values of 14 common peaks in HPLC chromatograms among 10 batches of samples were less than 1.68% for relative retention time and less than 3.87% for relative peak area, while R.S.D. values of 14 common peaks in GC/MS chromatograms among 10 batches of samples were less than 0.41% for relative retention time and less than 4.25% for relative peak area, which means the common peaks were in good correspondence in all samples. Besides the common peaks, there were about 20–25 non-common peaks in each HPLC chromatogram and about 25–30 non-common peaks in each GC/MS chromatogram, which represents the fuzziness among 10 batches of samples. The amount of the non-common peak areas is about 4.7%, less than the national standard of 10% [12].

So, the peak profile of the 14 components analyzed by HPLC and the 14 components analyzed by GC/MS made up the binary fingerprints of the total alkaloids. The average HPLC and GC/MS chromatograms from 10 samples were regarded as sub-fingerprints of the standardized characteristic binary chromatographic fingerprints of the total alkaloids as suggested by SFDA [12].

It is well known that, many of the constituents of TCM, whether active or inactive, exert synergistic activity and hence the fingerprint should ideally include the most of the possible constituents of TCM. In the sense, the developed binary chromatographic fingerprints show superiority to conventional single HPLC or GC/MS fingerprint in the quality control.

3.4. Quality control for the total alkaloids using binary chromatographic fingerprints

In this study, 10 samples of the total alkaloids from two different manufacturers in China were investigated (samples 1–5

Table 3
Similarity values of 10 batches of the total alkaloids samples

Method	Sample number									
	1	2	3	4	5	6	7	8	9	10
Binary chromatographic fingerprints	0.961	0.881	0.876	0.962	0.887	0.965	0.865	0.965	0.963	0.965
HPLC fingerprint	0.988	0.941	0.936	0.984	0.953	0.985	0.927	0.985	0.982	0.984

were from manufacturer A, and samples 6–10 were from manufacturer B).

The fingerprints of HPLC and of GC/MS were represented by a vector of 14- and 14-dimensions, respectively, as described in Section 2.5. In addition, according to the quantitative results of quinolizidine and aporphinoid alkaloids, we can conclude that contents of aporphinoid alkaloids represented in HPLC were nearly 10 times that of quinolizidine alkaloids represented in GC/MS. As a consequence, the combined coefficient (θ) is 0.1. Thus, using the data fusion method described in Section 2.5, all the binary chromatographic fingerprints of 10 samples were represented by fusion vectors. Subsequently, the similarity of fingerprints based on the standard fingerprints was quantitatively determined using the angel cosine measure. The results of similarity were shown in Table 3. In order to compare with the binary chromatographic fingerprints, the similarity of conventional chromatographic fingerprints only by HPLC was also quantitatively determined using the angel cosine measure.

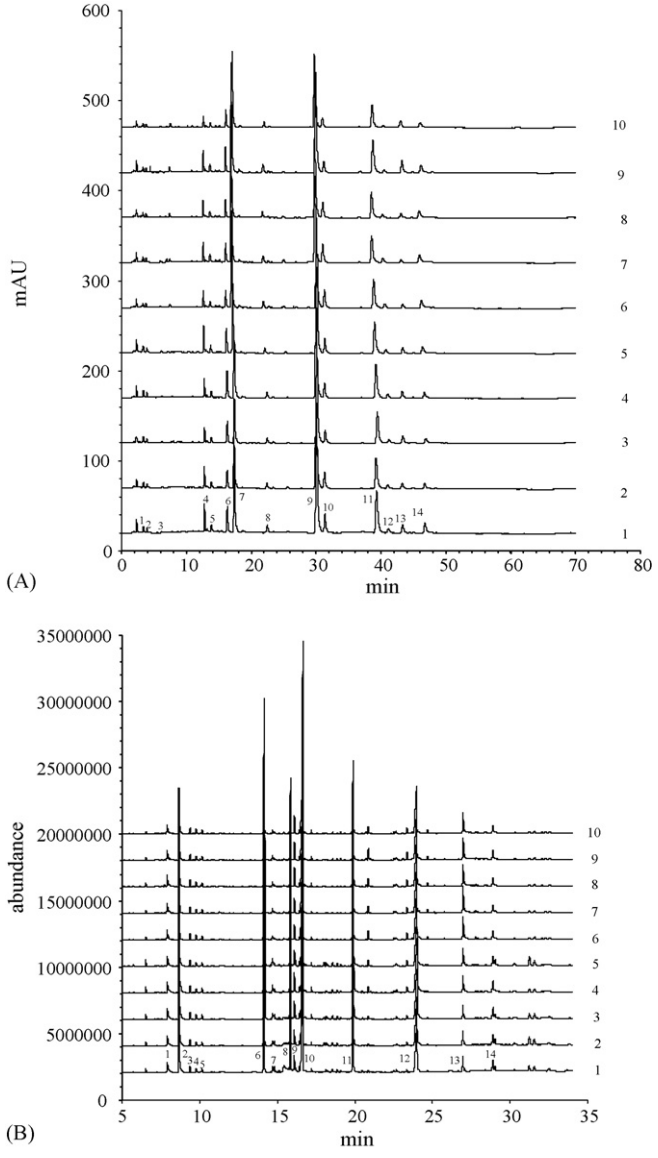

Fig. 3. Chromatogram of 10 batches of the total alkaloids from *Caulophyllum robustum*: (A) HPLC chromatogram: numbers 1–14 stand for the common peaks; (B) GC/MS total ion chromatogram: numbers 1–14 stand for the common peaks; numbers 1–10 stand for the different samples: 1–5 from manufacturer A; 6–10 from manufacturer B.

As shown in Table 3, similarity values of single fingerprint and binary fingerprint have different patterns. Similarity values of 10 samples based on the single fingerprint are more than 0.95, except for samples 2, 3, 7, but all more than 0.90. Using the binary chromatographic fingerprints, similarity values of samples 2, 3, 5, 7 are less than 0.90. Therefore, if 0.90 is set as an appropriate threshold, it is easy to find that samples 2, 3, 5, 7 are unacceptable based on the binary chromatographic fingerprint, but all 10 samples are acceptable according to the result of single chromatographic fingerprint. Therefore, we can more objectively discriminate normal samples from the frauds using the binary chromatographic fingerprints than using single chromatographic fingerprint. As a consequence, it was demonstrated that the presented binary chromatographic fingerprinting is more powerful and capable of identifying the consistency of samples than the single fingerprint method only by HPLC for quality control.

In this study, hierarchical cluster analysis based on peaks characteristics from the binary fingerprints profiles of the tested 10 samples and the standard binary fingerprints was applied, and the nearest neighbor and cosine, which is a pattern similarity measure, were selected as measurement for hierarchical cluster analysis. Seen from Fig. 4, samples are divided into four main clusters, and the samples from manufacturer A (1–5) are different from the samples from manufacturer B (6–10). Samples 6, 8, 9, 10 (from manufacturer B) are clustered together with the standard fingerprints firstly, which suggests that the products from manufacturer B are superior to those from manufacturer A. Samples 2, 3, 5, 7 are different from other samples, which are unacceptable samples for quality control. As a consequence, the presented binary chromatographic fingerprinting is more likely to reveal the further qualitative characteristics of the total alkaloids from *Caulophyllum robustum*, so that it has much more classification and discrimination power in quality control.

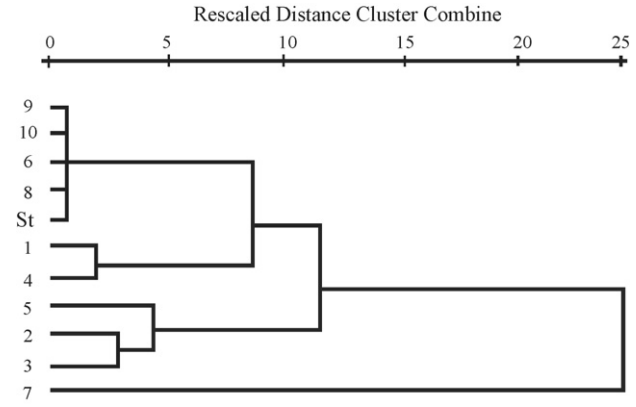

Fig. 4. Dendrograms resulting from single linkage between samples hierarchical cluster analysis: 1–5 from manufacturer A; 6–10 from manufacturer B; St: the standard binary chromatographic fingerprints.

4. Conclusion

In general, binary chromatographic fingerprinting, which consists of fingerprint of HPLC/DAD and GC/MS, is proposed as a strategy for quality control of the total alkaloids from *Caulophyllum robustum* instead of single chromatographic fingerprinting. The binary chromatographic fingerprints hence can comprehensively and properly reveal the quality characteristics of the total alkaloids, and logically it can reach more objective conclusions in the practice of quality control of the total alkaloids. The presented approach is equally applicable to other TCM. Suffice it to say that binary chromatographic fingerprints would provide a powerful and meaningful tool to comprehensively conduct the quality control of TCM products.

Acknowledgements

This work was financially supported by Science and Technology Project of Shannxi Province (No. 2005K10-G1).

References

[1] D. Normile, Science 299 (2003) 188–190.
[2] S.J. Lin, H.H. Tseng, K.C. Wen, T.T. Suen, J. Chromatogr. A 730 (1996) 17–23.
[3] S. Bent, R. Ko, Am. J. Med. 116 (2004) 478–485.
[4] D.M. Eisenberg, R.B. Davis, S.L. Ettner, S. Appel, S. Wilkey, M. vanRompay, R.C. Kessler, J. Am. Med. Assoc. 280 (1998) 1569–1575.
[5] WHO, WHO Traditional Medicine Strategy 2002–2005, World Health Organizaion, Geneva, 2002.
[6] FDA, Guidance for Industry Botanical Drug Products Center for Drug Evaluation and Research (CDER), Food and Drug Administration, 2000.
[7] WHO, General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, World Health Organization, Geneva, 2000.
[8] Final Proposals for Revision of the Note for Guidance on Quality of Herbal Remedies, EMEA, 1999.
[9] J.D. Philipsom, British Herbal Medicine Association, Forword, 1996.
[10] J.B. Calixto, Braz. J. Med. Biol. Res. 33 (2000) 179–189.
[11] L. Kelly, International Symposium on Quality of Traditional Chinese Medicine with Chromatographic Fingerprint, Guangzhou, 2001, pp. 1–4.
[12] Drug Administration Bureau of China, Requirements for Studying Fingerprint of Traditional Chinese Medicine Injections (Draft), 2000.
[13] Y.H. Cao, L.C. Wang, X.J. Yu, J.N. Ye, J. Pharm. Biomed. Anal. 41 (2006) 845–856.
[14] X.H. Fan, Y. Wang, Y.Y. Cheng, J. Pharm. Biomed. Anal. 40 (2006) 591–597.
[15] M. Goppel, G. Franz, Planta Med. 70 (2004) 432–436.
[16] X. Zhang, Z. Cui, D. Wang, H.Y. Zhou, J. Asian Nat. Prod. Res. 5 (2003) 171–182.
[17] Y.Y. Cheng, M.J. Chen, W.D. Tong, J. Chem. Info. Comput. Sci. 43 (2003) 1068–1076.
[18] A. Hasler, O. Sitcher, B. Meier, J. Chromatogr. 605 (1992) 41–48.
[19] P. Pietta, P. Mauri, A. Bruno, A. Rava, E. Manera, P. Ceva, J. Chromatogr. 553 (1991) 223–231.
[20] M. Yuan, Z. Zeng, L.F. Song, T. Yang, X.X. Liu, C. Cao, H.P. Zeng, Chin. J. Anal. Chem. 31 (2003) 455–458.
[21] P.S. Xie, Y.Z. Yan, J. Planar Chromatogr. 1 (1988) 29–34.
[22] L.C. Wang, Y.H. Cao, X.P. Xing, J.N. Ye, Chromatographia 62 (2005) 283–288.
[23] T. Xue, R. Roy, Science 300 (2003) 740–741.
[24] X.H. Fan, Y.Y. Cheng, Z.L. Ye, R.C. Lin, Z.Z. Qian, Anal. Chim. Acta 555 (2006) 217–224.
[25] Y.P. Li, L.C. He, Acad. J. Xi'an Jiaotong Univ. 17 (2005) 185–187.
[26] M. Tomita, T. Takahashi, Yakugaku Zasshi 78 (1958) 680–681.
[27] C.M.R. Ginn, P. Willett, J. Bradshaw, Perspect. Drug Discov. Des. 20 (2000) 1–16.
[28] Y.Y. Cheng, M.J. Chen, Y.J. Wu, Acta Chim. Sin. 60 (2002) 2017–2021.
[29] E.J. Kennelly, T.J. Flynn, E.P. Mazzola, J.A. Roach, T.G. McCloud, D.E. Danford, J.M. Betz, J. Nat. Pro. 62 (1999) 1385–1389.
[30] K. Asres, A. Tei, M. Wink, Biochem. Syst. Ecol. 25 (1997) 305–308.