Comparison of the volatile constituents of *Artemisia capillaris* from different locations by gas chromatography–mass spectrometry and projection method

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Abstract

The volatile chemical constituents of *Artemisia capillaries* (an important traditional Chinese medicine) were determined by gas chromatography–mass spectrometry (GC–MS) and sub-window factor analysis (SFA). Seventy-five components were separated and 43 of them were qualitatively and quantitatively determined, which represented about 89.03% of the total content. This profile was then used to identify and assess the consistency of the herb by using an orthogonal projection method. Four different sources of *A. capillaries* were analyzed and compared with each other. Among the components determined, there were 51 components coexisting in all samples although the relative peak areas of a few showed variations. It is the first time to apply orthogonal projection method to the comparison of different samples, and it reduces the burden of qualitative analysis as well as the subjectivity. The results showed a fair consistency in their GC–MS fingerprint. *A. capillaris* was distinguished from *Artemisia sacrorum* L., a possible substitute in traditional Chinese medicine by comparing the fingerprints with each other.
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Keywords: *Artemisia capillaris*; Sub-window factor analysis; Orthogonal projection method; Common component

1. Introduction

Artemisiae capillaris (capillaris) is the dried sprout of *Artemisia capillaris* Thunb (Compositae), which is a commonly used traditional Chinese medicine, listed in the Chinese Pharmacopoeia and used as a choleretic, anti-inflammatory and diuretic agent for the treatment of epidemic hepatitis [1,2]. Some of the constituents of the essential oil are known to exhibit pharmacological and biological activity [3], and it is used for the therapy of various liver and gall diseases [4]. The composition of the traditional Chinese medicine is very complicated, making it necessary to determine all of the phytochemical constituents of botanical extracts in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance

product quality control. Fingerprint analysis has been introduced and accepted by the WHO as a strategy for the assessment of herbal medicines [5]. The use of fingerprinting in herbs tends to focus on identifying and assessing the stability of the plant [6]. The power of separation by chromatography and qualitative analysis by mass spectrometry furnishes great advantage to the analysis of samples. However, for complicated samples such as traditional Chinese medicines, containing tens or even hundreds of chemical components, it is difficult to achieve complete separation unless rigorous conditions are imposed on the chromatographic separation process. Analysis of the volatile chemical constituents of *Artemisiae capillaris* [7,8], has shown that the essential oil contains monoterpenes, sesquiterpene, alkynyl compounds and other compounds. In these reports, the components of the essential oil are often determined by gas chromatography–mass spectrometry (GC–MS), and the qualitative and quantitative analysis is based on retention index of gas chromatography and mass spectra [9]. However, it is difficult to assess the purity

of chromatographic peaks by general GC, the peak inspected as one component may be mixture of several components. Just as Giddings claimed [10], it is shown that, relative to the maximum peak content or peak capacity for closely spaced peaks, a random chromatogram will never contain more than about 37% of its potential peaks and, worst of all from an analytical point of view, 18% of its potential single-component peaks. The number of observed peaks is not, then, the same as the number of distinct chemical components. The loss of analytical information resulting from this overlap is by itself serious, but the severity of the problem is greatly magnified if we do not have a good estimate of the magnitude of the loss [10]. Sometimes, it would be also difficult to determine the areas of the overlapped peaks. So the results obtained by what has been mentioned above would be questionable. Fortunately chemometric methods such as evolving factor analysis (EFA) [11,12], window factor analysis (WFA) [13,14], heuristic evolving latent projections (HELP) [15,16], sub-window factor analysis (SFA) [17,18], orthogonal projection resolution (OPR) [19] and evolving window orthogonal projection (EWOP) [20] provide tools to such problems [21–24].

The common peak is one of the important indexes for comparing the constituents from different samples, and is often confirmed by retention time and spectrum. It would be a tedious work if there are many constituents in the sample. Furthermore, overlapping peaks and drifts in retention time would decrease the accuracy. Orthogonal projection resolution has been applied to the resolution of overlapping peaks successfully. In this work, orthogonal projection is used for the identification of the common peak for the first time, and it is shown that it can reduce workload and increase the accuracy of the identification of the common peaks.

In this paper, the volatile chemical constituents in *A. capillaris* from four different locations were determined by GC–MS under appropriate conditions, SFA was used as an auxiliary means to the qualitative and quantitative analysis and orthogonal projection method was used for extracting the common peak from different locations. The aim of this study is to develop a characteristic fingerprint of *A. capillaris* for identifying the raw herb. This fingerprint can help distinguish the substitute or adulterant, and further assess the differences of *A. capillaris* grown in various areas of China.

2. Experimental

2.1. Instrumentation and reagents

Chromatography separation was carried out on a Shimadzu GC-17A gas chromatography instrument coupled to a Shimadzu QP5000 mass spectrometer (Compaq-Pro Linear data system, class5k software).

Dry herbs of Gansu and Shandong were purchased from Changsha Jiuzhitang pharmaceutical store market. Similarly, herbs of Hebei and Hunan were purchased from Changsha

Zhiling pharmaceutical store market. All the four samples were identified to be *A. capillaris* by Institute of Materia Medica, Hunan Academy of Traditional Chinese Medicine and Materia Medica.

2.2. Extraction of the essential oil

A. capillaris from Gansu, Shandong, Hebei and Hunan was dried at constant temperature 40 °C for 1 h, and the essential oil was prepared according to the Chinese Pharmacopoeia [25]. *A. capillaris* powder (100 g) and distilled water (500 mL) were placed into extract apparatus and subjected to hydro-distillation for 8 h at 100 °C. The obtained buff essential oil was dried over anhydrous sodium sulfate and stored at 4 °C for subsequent experiments. The yields of the four samples were 0.28%, 0.23%, 0.24% and 0.23% (v/w).

2.3. Analytical condition

The separation was performed on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film OV-101. The column was maintained at 50 °C after injection, then programmed at 8 °C min^{-1} to 250 °C, which was maintained for 5 min. Split injection was conducted with a split ratio of 1:10 and helium was used as carrier gas of 0.2 mL min^{-1} flow-rate, the volume of injection was 0.1 μL. The mass spectrometer was 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/scan. The inlet, ionization source temperature were 280 °C and 230 °C, respectively.

2.4. Data analysis

Data analysis was performed on a Pentium III 850 (Intel) personal computer; all programs were coded in Matlab 5.3 for windows. Resolved spectra were identified by matching against the standard mass spectral database of national institute of standards and technology (NIST147), which contains 147,000 compounds.

3. Results and discussion

3.1. Qualitative and quantitative analyses

The total ion chromatogram (TIC) of essential oil in *A. capillaris* from Gansu is shown in Fig. 1. The large number of peaks in the plot shows that it is indeed a complicated system. A majority of the peaks are baseline separated. But some of them, which seem to be a single peak, have different mass spectrum at different position, are actually an overlapping peak, Furthermore, some peaks are apparently overlapped. The chromatography segment (24.45–24.9 min) named peak cluster A in Fig. 1 is such an example. The TIC of peak cluster A is shown in Fig. 2. It seems that there are two components in the segment, but the diverse spectra at
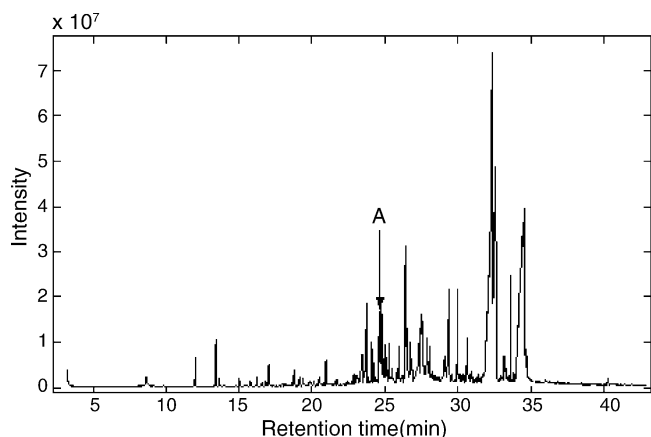
Fig. 1. The total ion chromatogram (TIC) obtained from the essential oil from *Artemisia capillaris* of Gansu.

different parts of the cluster indicates there would be more than three components or there must be severe noise. Routine direct search can definitely not reach the satisfactory result. Thus, sub-window factor analysis is used to extract the pure spectra and pure chromatogram. Background, baseline shift and severe heteroscedastic noise in the raw dimensional data is pretreated by corresponding method [26–28]. According to the rank estimation method [29,30], there are five components in this peak cluster. And they are marked as components 1–5 according to elution sequence. Then the pure spectrum of each component can be extracted by SFA, which could resolve the pure spectra by analyzing the correlation of two subwindows without previous resolution of their concentration profiles. There are two advantages to use SFA here. Firstly, extracting pure spectra by SFA is free from restriction from the selective region. Secondly, SFA is a self-verified method, so the reliability of the pure spectrum can be guaranteed. The elaborated knowledge of SFA can be seen in ref. [17]. By similarity searches in the mass library, each component in this cluster can be identified. Components 1, 3 and 4 in this cluster can be identified. These three components
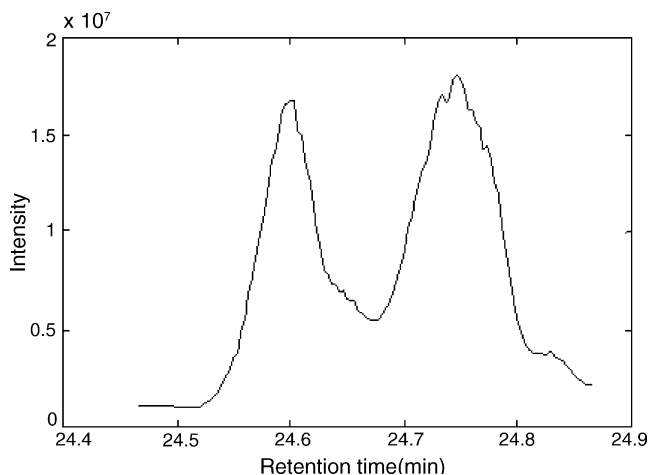

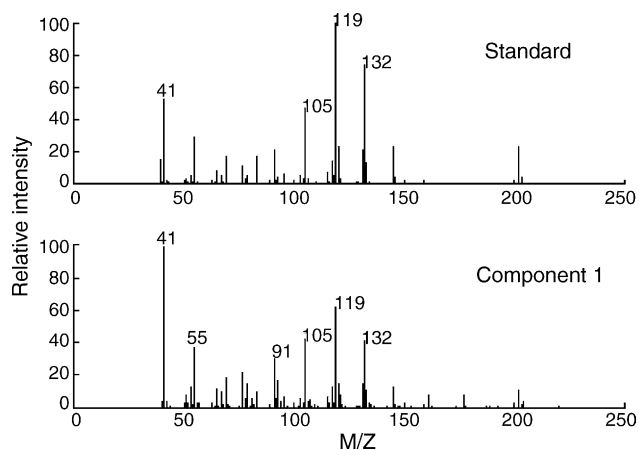Fig. 3. Standard mass spectrum of curcumene (C15H22) and resolved mass spectrum of component 1 by SFA.

are curcumene, germacrene D and cetene with the similarity of matching result 0.952, 0.962, 0.958, respectively. Components 2 and 5 cannot be identified because of the limitation of the mass library at hand. With the help of the pure mass spectra of the components obtained, the accuracy and reliability of the results are increased greatly. The resolved mass spectra together with the standard spectrum of each component from the library are also given in Figs. 3–5, respectively. After the pure spectra have been acquired, the pure chromatogram can be obtained by solving a least squares equation. The chromatogram resolved of cluster A is shown in Fig. 6.

In the same way, the components in other peak clusters can be resolved. The qualitative results are shown in Table 1. Seventy-five constitutes are resolved, among them 43 components are identified. Unfortunately, 32 components remain unidentified, because of the low signal-to-noise ratio or the absence of the compound from the mass spectra database, and some of the researched components may be questionable. With the pure chromatographic curve obtained for each component, quantitative analysis is carried out by the total
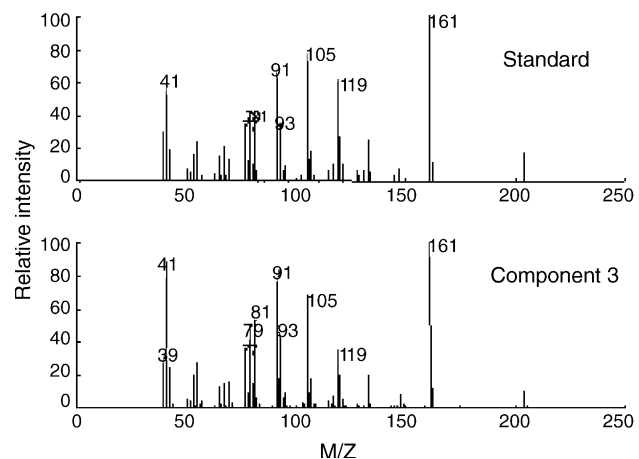

Fig. 2. The total ion chromatogram of peak cluster A.

Fig. 4. Standard mass spectrum of germacrene D (C15H24) and resolved mass spectrum of component 3 by SFA.
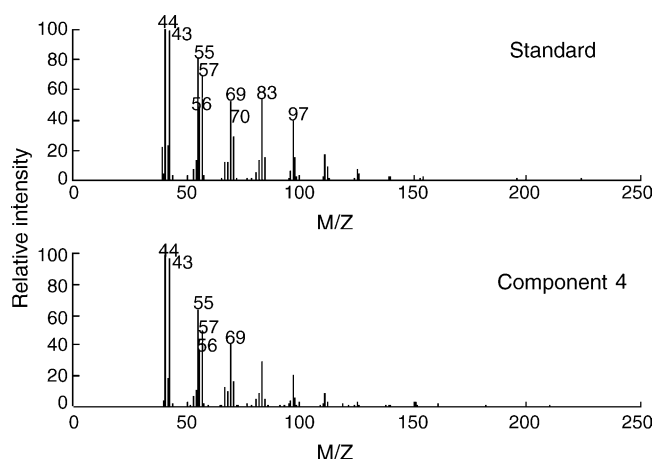
Fig. 5. Standard mass spectrum of cetene ($C_{16}H_{32}$) and resolved mass spectrum of component 4 by SFA.

volume integration [31]. The final quantitative results are listed in Table 1. The components which have been qualitative analyzed account for 89.03% of the total volume.

3.2. Identification of the common components by orthogonal projection resolution

The other raw herb samples from three different sources in China have been investigated under the same experimental conditions. The total ion chromatograms are shown in Fig. 7, where Symbols 1–4 represent Hebei, Gansu, Shandong and Hunan, respectively. As mentioned previously, there are total 75 constituents resolved in the sample of Ganshu, and it is interesting to know whether these components are present in other three samples.

Generally, one may analyze each component by similarity search in MS library and relevant resolution method, but it would be an arduous and trivial work. In the mean time, to those components without high similarity, there would be much subjective assumption to the analytical results. Here,
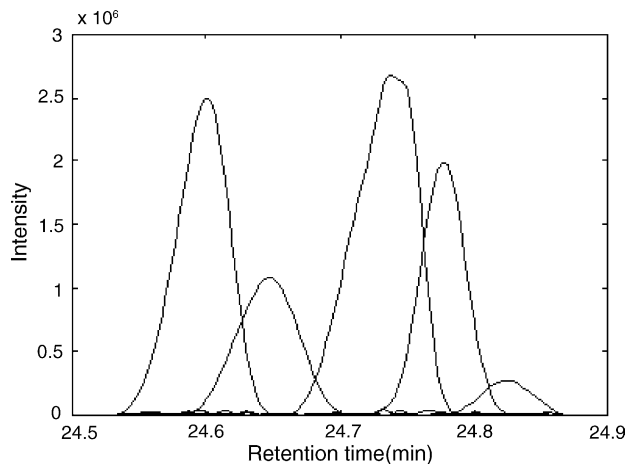

Fig. 6. Resolved chromatogram of peak cluster A containing five components.

orthogonal projection method is adopted to identify each component directly instead of resolving each sample data one by one.

The orthogonal projection matrix $\mathbf{P}_i$ on to the complementary subspace $\mathbf{X}_i^T$ is defined as:

$$\mathbf{P}_i = \mathbf{I} - \mathbf{X}_i^T(\mathbf{X}_i^T)^+ \tag{1}$$

where the superscript + denotes the Moore-Penrose pseudoinverse and $\mathbf{I}$ designates the identity matrix. $\mathbf{X}_i^T$ represents different sub-matrices, which are a series of fixed size window matrices moving along the chromatographic direction.

Assume that the subspace spanned by the mixture spectra in $\mathbf{X}_i^T$ is $\mathbf{M}$. The residue vector $\mathbf{r}_i$ is given by:

$$\mathbf{r}_i = \mathbf{P}_i\mathbf{v}_a \tag{2}$$

where $\mathbf{v}_a$ denotes the spectrum of certain component that resolved by the SFA, and $\mathbf{r}_i$ is the projection of $\mathbf{v}_a$ on the orthogonal complementary subspace of $\mathbf{M}$.

Therefore, one has the length of the residue vector:

$$re_i = ||\mathbf{r}_i||^2 \quad (i = 1, 2, \ldots, m - w + 1) \tag{3}$$

where $||\mathbf{r}_i||$ designates the Euclidean norm of the vector, m is the number of measured chromatographic points and w is the size of window.

Plotting the valve of re_i versus the index i, one obtains a graph, here we call it spectrum projection graph, which can tell us whether the component is present or absent, and where the component elutes. Suppose the submatrix $\mathbf{X}_i$ contains component a, then the spectrum of component a is in the subspace $\mathbf{M}$ spanned by the mixture spectra in $\mathbf{X}_i^T$, hence the length of the residue vector will be close to zero. Otherwise, if the component a is not in the sub-matrix, then re_i will have a relatively large valve.

Peak cluster A is still adopted to illustrate the procedure. X_1 and X_2 are the determined data matrix of Gansu and Hunan. There are five components in it, and each pure spectrum has been extracted. Because the retention time drift is not severe, the submatrix X of X_2 can be selected from 24.0 to 25.1 min. And then the pure spectrum v_1 and v_2 of components 1 and 2 are orthogonal projected to X. The spectrum projection graph is shown in Fig. 8. There is a range in which the length of the residue vector is close to zero to component 1, whereas to component 2, there is not. One can determine component 1 existing in sample of Gansu and Hunan, and component 2 does not exist in sample of Hunan. For those components without high similarity in MS library, if only they are common components, there must be high similarity between their spectra. This would avoid subjective assumption effectively. The other extracted spectrum is just disposed by the same procedure. There are 51 common components existing in the four samples. However, because of the lower signal-to-noise ratio, some of the components may be fail to be detected.

Table 1
Qualitative and quantitative results of volatile constituents in *Artemisia capillaris herba*. from four different sources

Component[a]	Ganshu		Hunan		Hebei		Shandong	
	Retention time (min)	Content (%)	Retention time (min)	Content (%)	Retention time (min)	Content (%)	Retention time (min)	Content (%)
2-Ethyl-furan	–	–	3.569	2.07	3.569	0.56	3.568	0.85
Phenol	–	–	3.962	1.93	3.958	0.75	3.944	0.97
Hexanal	–	–	5.698	0.39	5.682	0.84	5.660	1.10
α-Pinene	12.008	0.6	12.083	0.55	12.062	0.72	12.088	0.63
β-Pinene	13.399	1.1	13.453	1.24	13.443	0.76	13.435	0.66
5-Hexenyl-oxirane	13.623	0.16	13.708	2.31	13.697	2.72	13.685	5.81
2-Pentyl-furan	–	–	13.894	0.12	13.893	0.18	13.882	0.38
Sabinene	14.952	0.17	15.045	0.37	14.945	0.63	15.002	0.62
Limonene	14.989	0.17	15.085	0.41	14.995	0.66	15.053	0.65
2,6,6-Trimethyl-,[.+/−.]-bicyclo[3.1.1]-hept-2-ene	15.790	0.12	–	–	–	–	15.863	1.27
8-Nonen-2-one	–	–	16.254	1.95	16.248	2.32	16.238	1.88
6-*cis*-Nonenal	16.530	0.08	16.594	0.39	16.590	0.64	16.580	0.64
Decanal	16.773	0.1	–	–	–	–	16.830	0.58
β-Linalool	17.060	0.48	17.089	3.31	17.069	2.49	17.073	5.79
4-Isopropenyl-1-methylcyclohexanol	18.774	0.37	–	–	–	–	18.817	4.82
p-Menth-1-en-8-ol	19.130	0.22	–	–	–	–	–	–
2-Methyl-decane	19.325	0.12	–	–	–	–	–	–
2,6-Nonadienol	20.471	0.19	20.500	0.18	20.506	0.15	20.498	0.28
Bornyl acetate	20.906	0.49	–	–	–	–	20.959	4.74
p-Propenyl-anisole	–	–	–	–	20.946	0.61	–	–
Copaene	22.873	0.33	22.971	0.53	22.954	0.34	22.957	0.48
α-Gurjunene	–	–	23.100	0.55	23.102	0.41	23.104	1.32
β-Ylangene	–	–	23.205	0.84	23.193	0.46	23.205	0.42
[+]Sativen	23.415	1.22	–	–	23.570	0.45	23.419	0.49
α-Bergamotene	23.610	0.35	–	–	23.664	0.47	–	–
4,11,11-Trimethyl-8-methylene-[1R-(1R@,4Z@,9S@)]-bicyclo[9.2.0]undec-4-ene	23.702	1.91	23.780	10.06	23.773	5.66	23.759	2.04
α-Caryophyllene	24.267	0.41	24.351	0.46	24.348	0.52	24.337	0.34
Curcumene	24.592	1.21	24.655	4.86	24.652	4.14	24.649	2.33
Germacrene D	24.741	1.76	24.797	5.11	24.799	4.55	24.777	0.67
Cetene	24.787	0.96	–	–	–	–	24.845	0.48
4,8-Dimethyl-tridecane	24.942	0.17	24.948	0.13	24.954	0.12	24.949	0.18
α-Farnesene	24.962	0.61	25.041	1.26	25.050	1.13	25.027	0.76
β-Farnesene	25.070	0.43	25.154	0.28	25.156	0.37	25.142	0.93
Cedrene	25.228	0.41	25.302	0.39	25.287	0.58	25.297	0.67
Isoledene	25.330	0.81	25.403	1.24	25.398	1.41	25.389	0.89
Nerolidol	25.948	0.72	–	–	–	–	–	–
trans-Z-α-Bisabolene epoxide	26.393	4.39	26.437	25.33	26.446	27.06	26.435	21.57
cis-Z-α-Bisabolene epoxide	26.877	0.53	26.836	1.15	26.851	2.66	26.845	2.73
τ-Muurolol	27.294	1.28	27.369	2.18	27.361	3.97	27.370	2.76
α-Cadinol	27.467	1.31	27.565	1.88	27.566	3.09	27.546	2.91
Caryophyllene oxide	27.836	1.06	27.890	1.28	27.893	1.21	27.881	1.54
Isoaromadendrene epoxide	28.016	0.65	28.064	1.17	28.080	1.31	28.051	0.67
2,15-Hexacanedione	29.984	1.78	30.050	2.40	30.061	2.28	30.045	3.49
Pentadecylic acid	30.627	1.28	–	–	–	–	–	–
n-Hexadecanoic acid	32.089	26.29	32.066	4.50	32.082	6.01	32.061	4.03
Falcarinol	32.601	11.76	32.507	5.38	32.507	5.81	32.483	0.65
Cyclopentaneundecanoic acid	33.163	0.52	–	–	–	–	33.208	0.54
9,12,15-Octadecatrienal	33.327	0.40	–	–	–	–	33.282	0.72
Phytol	33.600	2.73	–	–	–	–	33.638	0.80
9,12,15-Octadecatrienal	34.491	19.38	34.402	1.74	34.417	1.16	–	–
Total content		89.01		87.81		89.08		85.23

[a] Compounds identification based on mass spectral library searching alone are tentative.
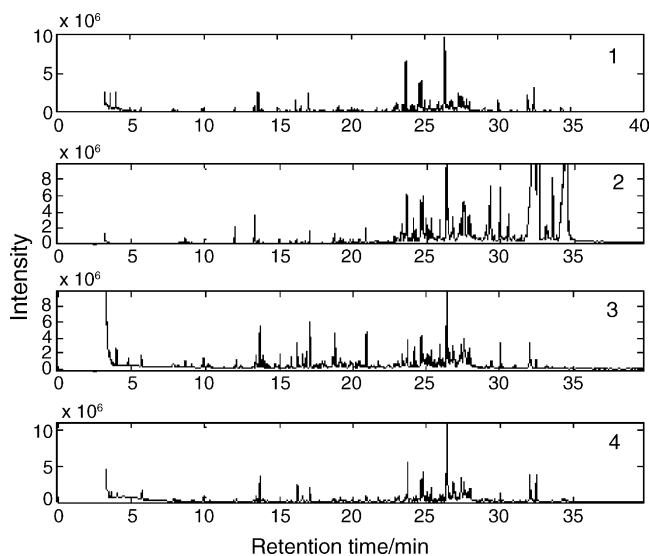
Fig. 7. The total ion chromatogram (TIC) obtained from the essential oil from *Artemisia capillaris* of four different sources: (1) Hebei, (2) Gansu, (3) Shandong and (4) Hunan.

3.3. Consistency assessment of samples from various locations

The primary application of fingerprinting analysis may be consisting in assessing the consistency of raw herbs from
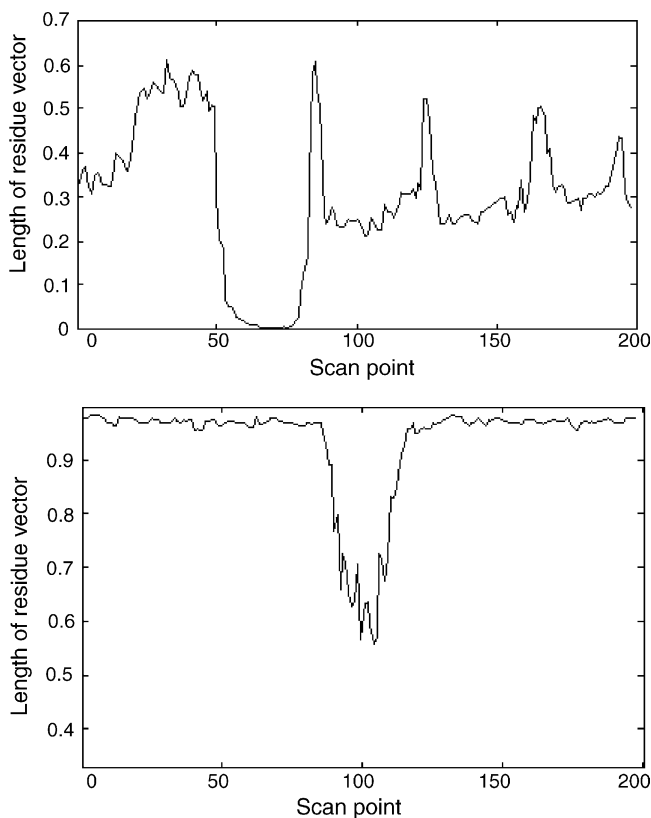

Fig. 8. spectrum projection graphs with a window size of four for components 1 and 2.

different locations. In this study, raw herb samples from four sources in China were investigated. To those constituents not to be common components in the other three samples, the pure spectra are extracted as described in Section 3.1. The qualitative and quantitative results are also listed in Table 1. Among the determined components, there are 51 common components existing in all samples though the relative peak areas of a few showed variations to some extent. Pattern in common of the four samples is constructed by the use of similarity assessment soft. Then each chromatogram is compared with the common pattern, and total similarity of the chromatogram is 0.8447, 0.7528, 0.7806 and 0.7233, respectively. Such result is not enough to prove that they are congeneric herbs. In fact, in the plot, we can find there are two largest variations in the peaks between the retention time of 31–32 min and 34–35 min. But the spectra are the same which indicates that they are the same components only that the content is different from each other, especially to that of Gansu. According to known references, they are not the active components in *A. capillaries*. If the weighs of the two peaks in the chromatograms is decreased to the same level of the other three samples, we can reconstruct common pattern of the four chromatogram, then each chromatogram is compared with the common pattern again, and total similarity of the chromatogram is 0.9012, 0.9118, 0.9342 and 0.8980, respectively. The similarity increases greatly, with a limited number of samples, it is justly to be believed that *A. capillaris* from the four sources exhibited a fair consistency in the constituents. A larger set of samples, however, is certainly needed for a reliable conclusion.

Although great homology is exhibited on the whole, there is still some diversity in content and constitute among the four *A. capillaries* of difference producing areas. It can be seen in Table 1, for example, *n*-hexadecanoic acid, [Z]-[−]-1,9-heptadecadiene-4,6-diyne-3-ol-falcarinol and 9,12,15-octadecatrienal are the high concentration constituents in *Artemisia capillaris* of Gansu, the content of β-farnesene in that of Hunan is higher, *trans*-Z-α-bisabolene epoxide is the high concentration constituent in that of Hunan, shandong and Hebei. In the whole, those to be not the common components are lower content components. The difference among different sources reflect the discrepancy of curative effect, it can also illustrate the importance of quality control.

3.4. Differentiation of raw herbs

The developed GC–MS fingerprint can be used to distinguish possible substitutes or adulterants from *A. capillaris*. *Artemisia sacrorum* L., to be subgen Sect. Abrotanum Bess., is served as *A. capillaris* in the district of Hilongjiang province. The individual herb purchased from Jiuzhitang drug market, is prepared by the same procedure as described above to determine their volatile constituents. The chromatogram of *A. sacrorum* L. is quite different from the profiles of *A. capillaris* in the number of peaks and retention time. Moreover, the volatile constituents are apparently

different. Bicyclic monoterpene constituents are the main components in *A. sacrorum* L., including camphor, 3-carene, cineole, 4-methyl-(methylethyl)-bicyclo[3,1,0]hexan-3-ol, etc.; in *A. capillaris,* the main components are caryophyllene, α-caryophyllene, *n*-hexadecanoic acid, β-farnesene, etc. It is apparently from the fingerprint analysis that *A. sacrorum* L. is not *Artemisia capillaris* from content and composition. The developed GC–MS fingerprinting method can be used to distinguish possible substitutes or adulterants from *A. capillaries*.

4. Conclusion

GC–MS combined with correlative chemometric methods were proposed for the fingerprint analysis of *A. capillaris*, *which* comprehensively reveal the quality and quantity of chemical constituents of traditional medicines for effective evaluation of sameness or differences of analytical samples. Fifty-one common components representing the characteristics of this herbs constituents and higher similarity in chromatogram showed consistence among the samples from four different locations.

Acknowledgement

References

[1] H.Y. Hsu, Y.P. Chen, S.J. Sheu, C.H. Hsu, C.C. Chen, H.C. Chang, Chinese Materia Medica—A Concise Guide, Modern Drug Press, Taipei, 1985, p. 209.

[2] W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine, Springer-Verlag, New York, 1992, p. 179.

[3] H.Z. Zheng, Z.H. Dong, Modern Study of Traditional Chinese Medicine, vol. 4, Xue Yuan Press, Beijing, 1999, p. 3092.

[4] A.Y. Zhou, Chin. J. Pract. Med. 3 (2003) 835.

[5] World Health Organization, Guidelines for the Assessment of Herbal Medicines, Munich, WHO, Geneva, 28 June 1991.

[6] J.L. Natalie, P. Peter, Drug Inform. J. 32 (1998) 497.

[7] S.-B. Yang, J.-R. Guang, Chin. Herbal Med. 27 (1996) 269.

[8] J. Wang, L.-J. Zhao, L.-L. Yang, J.-M. Han, Wilding Resource China (6) (1996) 52 (in Chinese).

[9] L. Zhang, H.-J. Fang, J. Med. Anal. 14 (1994) 52 (in Chinese).

[10] J.C. Giddings, Anal. Chem. 55 (1983) 418.

[11] M. Maeder, Anal. Chem. 59 (1987) 527.

[12] M. Maeder, A. Zilian, Chemom. Intell. Lab. Syst. 3 (1988) 205.

[13] E.R. Malinowski, J. Chemom. 6 (1992) 29.

[14] W. Den, E.R. Malinowski, J. Chemom. 7 (1993) 89.

[15] O.M. Kvalheim, Y.Z. Liang, Anal. Chem. 64 (1992) 936.

[16] Y.Z. Liang, O.M. Kvalheim, H.R. Keller, D.L. Massart, P. Kiechle, F. Erni, Anal. Chem. 64 (1992) 946.

[17] R. Manne, H.L. Shen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 171.

[18] H.L. Shen, R. Manne, Q.S. Xu, D.Z. Chen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 323.

[19] F. Cuesta Sanchez, S.C. Rutan, N.D. Gil Garcia, D.L. Massart, Chemom. Intell. Lab. Syst. 36 (1997) 153.

[20] C.J. Xu, J.H. Jiang, Y.Z. Liang, Analyst 124 (1999) 1471.

[21] F. Gong, Y.Z. Liang, H. Cui, F.T. Chau, B.P.T. Chau, J. Chromatogr. A 909 (2001) 237.

[22] X.N. Li, H. Cui, Y.Q. Song, Y.Z. Liang, Acta Pharm. 36 (2001) 215.

[23] C.J. Xu, Y.Z. Liang, Y.Q. Song, J.S. Li, Fresenius J. Anal. Chem. 371 (2001) 331.

[24] F.Q. Guo, Y.Z. Liang, C.J. Xu, L.F. Huang, J. Chromatogr. A 1016 (2003) 99.

[25] Chinese Pharmacopoeia Committee, Chinese Pharmacopoeia, Publishing House of People's Health, 2000, Appendix 64.

[26] O.M. Kvalheim, Y.Z. liang, Anal. Chem. 64 (1992) 936.

[27] H.R. Keller, D.L. Massart, Anal. Chem. Acta 263 (1992) 21.

[28] X.N. Li, Y.Z. Liang, F.T. Chau, Chemom. Intell. Lab. Syst. 63 (2002) 139.

[29] E.R. Malinowski, Factor Analysis, Wiley/Interscience, New York, 1991.

[30] H.L. Shen, Y.Z. Liang, O.M. Kvalheim, R. Manne, Chemom. Intell. Lab. Syst. 51 (2000) 49.

[31] F. Gong, Y.G. Peng, H. Cui, Y.Z. Liang, A.K.M. Leung, F.T. Chou, Chem. J. Chin. Univ. 20 (1999) 199.