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Analyzing of the volatile chemical constituents in *Artemisia capillaris herba* by GC–MS and correlative chemometric resolution methods

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Abstract

The volatile chemical constituents in traditional Chinese medicine (TCM), *Artemisia capillaris herba*, were determined by gas chromatography–mass spectrometry (GC–MS). The acquired two-dimensional data were resolved by correlative chemometric resolution methods. The noise in the raw data is pretreated by roughness penalty smoothing method. With the data denoised, heteroscedastic noise and signal-to-noise ratio were decreased apparently, which was favorable to the determination of component number. The selective range can be extracted from rankmap obtained by fixed size moving window evolving factor analysis (FSMWEFA) conveniently. The overlapped chromatographic peaks were resolved into pure chromatograms and pure spectra with evolving window orthogonal projection (EWOP). The purity of the resolved pure spectra were improved furthermore with the heteroscedastic noise decreased through roughness penalty smoothing method, although the basic structure of the raw data changes little. Qualitative analysis was performed by similarity search in NIST147 and Willey library. Pure chromatograms are in favor of quantitative analysis, which was obtained by total volume integration. Forty-two of seventy-five separated constituents in essential oil, accounting for 89.03% of the total content, were identified. The result proves the combined approaches to be powerful for the analysis of complex herbal samples.

Keywords: GC–MS; Roughness penalty smoothing; EWOP; Correlative chemometric resolution methods; *Artemisia capillaris herba*; Essential oil

1. Introduction

Artemisia capillaris herba is the dry sprout of *Artemisia capillaris Thunb* distributing in the northeast area of China broadly. As one of famous traditional Chinese medicine (TCM), it is listed officially in the Chinese pharmacopoeia and used as a choleretic, anti-inflammatory and diuretic agent in the treatment of epidemic hepatitis [1]. Some biologically active compounds such as capillarisin, chlorogenic acid, scopoletin and caffeic acid in the *A. capillaris herba* extract have been determined by capillary electrophoretic methods [2] and affinity chromatography [3].

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The essential oil is also one of the main pharmacological active individuals [4]. Some papers have reported about the analysis of the volatile chemical constituents in *A. capillaris herba* [5,6]. Those constituents up to 0.1% have been partly quantitatively analyzed. There are monoterpene, sesquterpene, alkynyl compounds, and other compounds in the essential oil. In these reports, the components in the essential oil are often determined by GC/MS, and the qualitative and quantitative analysis is based on retention index of gas chromatography and mass spectra [7]. However, the purity of chromatograms would be difficult to be identified by general GC or GC/MS. Moreover, the constituent inspected as one component may be mixture of several components. 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.

However, modern hyphenated chromatographic instruments and correlative rapidly developed chemometric resolution techniques are favorable turn to this kind of problems. Because chromatographic profile is provided with the characteristic of coming in first would go out first, some evolving approaches such as evolving factor analysis (EFA) [8,9], window factor analysis (WFA) [10,11], heuristic evolving latent projections (HELP) [12,13], sub-window factor analysis (SFA) [14,15], and evolving window orthogonal projection (EWOP) [16] have progressed rapidly.

The accurate chemical rank is the very beginning of most chemometric resolution procedures for the two-way data. An incorrect estimation of that will give a misguidance for further qualitative and quantitative analysis. Due to the complicated instrument background, serious heteroscedastic noise and poor signal-to-noise ratio in the signals of some components, especially, the heteroscedastic noise is an even more serious problem in the data obtained from GC/MS [17]. It might induce extraneous components in principle component analysis and result in over determined estimation of the chemical rank of the two-way data from hyphenated instruments [17–19]. It is necessary to pretreat the raw data before resolving the two-dimensional data. Many smoothing and denoising methods were proposed, such as roughness penalty smoothing method [20], wavelet denoising technique [21,22] and convolution smoothing approach based on least squares developed by Savitzky and Golay [23–26].

In this paper, the volatile chemical constituents in *A. capillaris Thunb*, is determined by GC/MS under appropriate condition, which is similar to the determination of *Notoptergium incium* in the previous paper [27]. Because of the heteroscedastic noise and lower signal-to-noise ratio at some retention time regions, it seems to be necessary to pretreat the raw data. In this work, after background and baseline shift correction, the gained two-dimensional data is pretreated by roughness penalty smoothing method. Then the overlapping peaks are resolved with EWOP. With pure chromatographic curve and mass spectrum obtained, quantitative and qualitative analysis will be done with more accuracy and reliability.

2. Theory

Suppose $\boldsymbol{A}$ is a measured absorbance matrix, $\boldsymbol{C}$ is the concentration profile, and $\boldsymbol{S}$ is the spectrum. According to Lambert–Beer Law or the similar, the matrix can be represented by the product of two matrices:

$$\boldsymbol{A}_{m\times n} = CS^{\mathrm{T}} + \boldsymbol{E} \tag{1}$$

where the superscript T denotes the matrix or vector transposition, $\boldsymbol{E}$ is the array of the measurement noise.

The data treatment used here can be described by the following five steps:

(1) Firstly, the measured matrix $\boldsymbol{A}$ is divided into different submatrices corresponding to baseline separated peaks and peak clusters.
(2) Secondly, to avoid the pitfalls of background, baseline shift and noise in measured data, it is necessary to detect the background, and correct background and baseline shift, then the derived data is processed by roughness penalty smoothing method.

The basic idea of roughness penalty smoothing method is adding roughness penalty item on the basis of least square estimation, the core equation of the roughness penalty method for function curve estimation is given as:

$$S[f(w)] = \sum_{i=1}^{n}(x_i - f_i)^2 + \alpha\int_{w_1}^{w_n}[f^n(w)]^2\,\mathrm{d}w \tag{2}$$

where α is the penalty parameter, and the effect of penalty item to target function is depending on the value of α. The detailed description is referred to ref. [20].

(3) Estimating the elution sequence of each component by fixed size moving window evolving factor analysis (FSMWEFA) [28].
(4) Pure spectra can be extracted from selective range of components by FSMWEFA, and the chromatographic profiles can be obtained by EWOP. The detailed theory can be seen in ref. [16]
(5) Qualitative analysis is performed by similarity searches in the NIST 147 mass spectral library. Quantitative results are obtained by calculating the volume of total two-way response.

3. Experimental

3.1. Reagents

Individual herb from Gansu province was purchased from Changsha Jiuzhitang pharmaceutical store market. And it was identified to be *A. capillaris herba* by a researcher from Institute of Materia Medica, Hunan Academy of Traditional Chinese Medicine and Materia Medica.

3.2. Extraction of the essential oil

Herb of *A. capillaris herba* was dried at constant temperature 40 °C for 1 h. The essential oil was prepared according to the Chinese pharmacopoeia [29]. 100 gram *A. capillaris* powder was put into extract apparatus and subjected to hydro-distillation for 8 h, the obtained buff essential oil was dried over anhydrous sodium sulfate and stored at 4 °C for subsequent experiments. The yield of the sample was 0.28% (v/w).

3.3. Analytical condition

GC–MS was performed with Shimadzu GC-17A gas chromatography instrument coupled to a Shimadzu QP5000 mass spectrometer (Compaq-Pro Linear data system, class5k software). Compounds were separated on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film OV-101. The column temperature was maintained at 50 °C for 6 min after injection, then programmed at 8 °C min^{-1} to 250 °C, then 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 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 0.2s per scan. The inlet, ionization source temperature were 280 °C and 230 °C, respectively.

3.4. 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) and Willey library.

4. Results and discussion

4.1. Separation of the essential oil

The total ion chromatogram (TIC) of essential oil of *A. capillaris herba*. 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, some of them seem to be single peak with different mass spectrum at different position, actually it is an involving peak, other peaks are apparently overlapped, peak cluster A in Fig. 1 is such an example. The TIC of peak cluster A is shown in Fig. 2, it seems in the plot that it is a two components peak, moreover, the ‘two components’ has not gotten to completely baseline separation. If it is searched directly, the spectrum of the former changes a lot at different positions, in the meantime, the similarity is relatively lower to assure the accuracy, and the searched compound is something containing nitrogen and sulfur which is not likely to be compounds in the essential oil. The matching degree is too low to guarantee high accuracy and reliability. Furthermore, quantitative analysis becomes difficult because of the overlapped peaks. It is necessary to resolve such overlapped peaks.

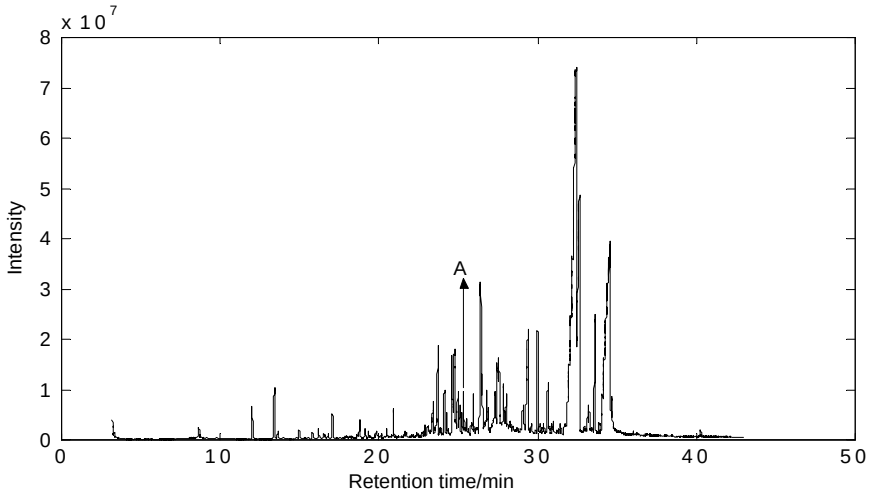

Fig. 1. The total ion chromatograms (TIC) obtained from the volatile oil from *A. capillaris*.

According to the baseline separated chromatographic peaks or peak cluster on the TIC, original chromatogram is divided into 47 segments, consequently, the determined two-dimensional data is divided into 47 sub-matrixes. As an overlapped peak, peak cluster A within 24.85–25.2 min (or between 6500 and 6590 scan points) is taken as an example to illustrate the data analysis process. The raw chromatograms are shown in Fig. 2(A). The spinous shape shown in Fig. 2(A) indicates lower signal-to-noise ratio and heteroscedastic noise in the data.

4.2. Smoothing methods applied to dealing with heteroscedastic noise in GC/MS

Background can be estimated by eigenvalue decomposition to the data of zero component region before and after chromatographic peak, and then it can be deducted through least squares [30].

Then the rankmap [28] obtained by FSMWEFA of the peak cluster A is shown in Fig. 3. The map tells the local rank in the elution sequence. If the local rank is one, that is, there is one eigenvalue is significantly

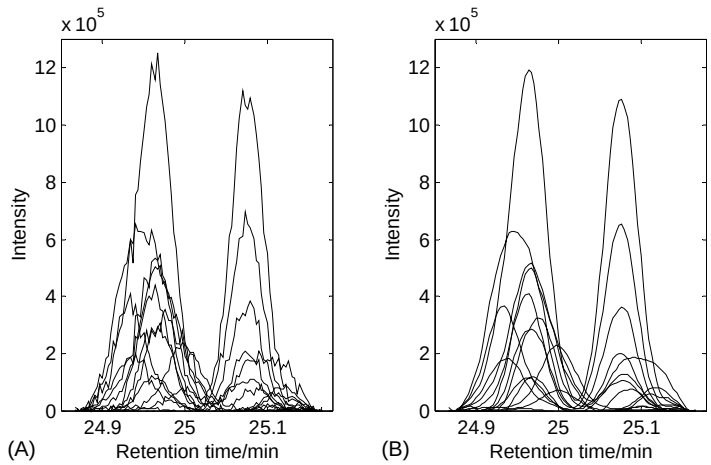

Fig. 2. The total ion chromatogram of peak cluster A. (A) Data without smoothing; (B) Data after smoothing.

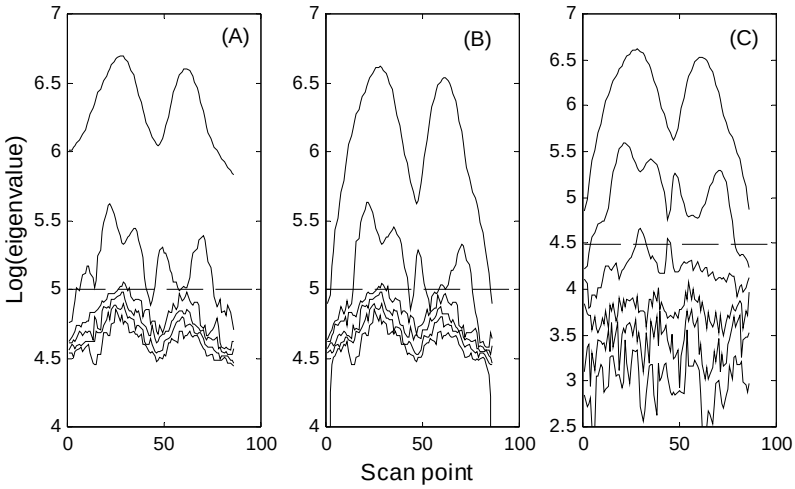

Fig. 3. Rank map obtained by FSMWEFA (window size = 6) for cluster A. (A) Result of the raw data, (B) result of the data after correcting background and baseline shift, and (C) result of the smoothed data by roughness penalty processing.

greater than noise level, there is one component. And if the local rank is two, there are two components co-eluting. Fig. 3(A) shows the rankmap result of the raw data with window size of 6. Line 1 and 2 denoting the first and second eigenvalue can provide the information of the components, the third to sixth lines embody the noise, the noise level can be set as the dash line, and its value is about 5 in the plot. Apparently, it is a kind of typical heteroscedastic noise, the greater the signal, the bigger the noise. Fig. 3(B) is the rankmap of the peak cluster A after background and baseline shift corrected. Comparing Fig. 3(A) and (B), we can see that the difference between them are mainly come from the region of scan point 1 to scan point 10. In this region, Fig. 3(A) seems to tell two components, while there is only one component in the same region shown in Fig. 3(B). In fact, it is the result of background. It can be seen from Fig. 3(B) that there seems to be five components in the peak cluster A, but Fig. 3(A) tells the wrong story. Notice that the lower signal-to-noise ratio and heteroscedastic noise are still existence in both cases. Then, the data is smoothed by rough penalty smoothing method to improve the detection ability of the GC–MS data, as indicated in ref. [31]. Unlike polynomial regression, the roughness penalty approach is a kind of nonparametric regression, which aims to overcome white noise in hyphenated chromatographic data. The chromatograms after smoothing are shown in Fig. 2(B). It can be seen that the data has been reasonably smoothed without apparent distortion. Its rankmap is shown in Fig. 3(C). In Fig. 3(C), not only the first and second eigenvalue provide the component information, but also the third eigenvalue can tell the refined structure of the component elution information, the start and end point of each component. For example, to component three, the elution range is from scan point 30–70, whereas, it cannot be obtained from Fig. 3(B). The more important is that the noise level has also been decreased to 4.5. The signal-to-noise level is enhanced significantly. Furthermore, the eigenvalve representing the noise level has been on the same level in Fig. 3(C). All these indicate that heteroscedastic noise in the data has been removed and the signal-to-noise ratio is increased with the help of smoothing treatment. The roughness penalty method cannot only enhance the detection ability but also improve quality of resolved chromatographic profiles and spectra significantly for the noisy GC/MS data without losing qualitative and quantitative information. From this optimized FSMWEFA, we can accurately estimate that there are five components in the system.

4.3. Resolution of the overlapping peak by EWOP

In peak cluster A, the components is named as 1, 2, 3, 4, and 5 according to elution sequence. The selective range of components can be obtained conveniently

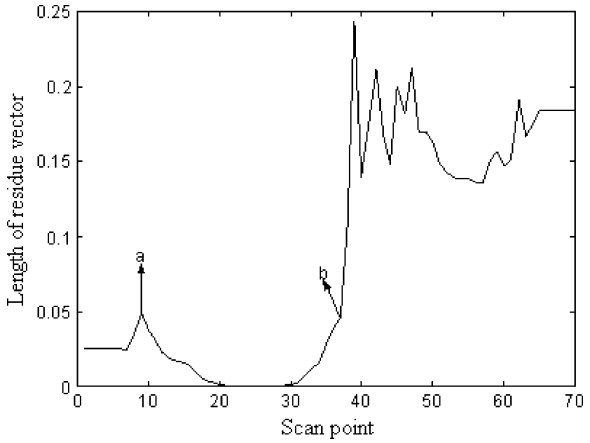

Fig. 4. Zero-concentration graph with a window size of 4 for component 2.

from FSMWEFA in Fig. 3(C), then the pure spectrum can be derived directly. The pure spectrum can also abstracted by sub-window factor analysis. Then the pure spectrum obtained of component is projected on a series of moving windows, the elution region of the component can be perceived by visual inspection. The elaborated theory of EWOP can be seen in ref. [15]. The zero-concentration graph (ZCG) of component two in peak cluster A obtained in accordance with EWOP theory is shown in Fig. 4. From ZCG the zero-concentration region of the component of interest can be identified immediately. The theory part can be referred to ref. [15] in detail. The y-coordinate of the curve in ZCG expresses the length of the residue vector. In the point of theory, where the length of residue vector is close to zero, that is to say where the segment of the y-coordinate of the curve is close to zero indicates the elution region of component. However, in practice, the position of inflexion is usually adapter to locate the start and end point of zero concentration range. Here, in the plot, zero concentration range is identified to be the scan point from a to b in the plot. Then the chromatographic profile can be obtained by local orthogonal projection.

A new matrix $\boldsymbol{X}_1$ is derived after one component is stripped:

$$\boldsymbol{X}_1 = \boldsymbol{X} - CS^{\mathrm{T}} \tag{3}$$

The spectra and chromatographic profiles of components 2, 3, 4, and 5 can be obtained in the same way. The chromatogram resolved of cluster A is shown in Fig. 5. In evolving window orthogonal projection, projection matrix is built with moving window, hereby the developed zero concentration graph will be convenient to confirm zero concentration region. With the help of ZCG, chromatographic profile can be obtained accurately. This will supply a prompt and convenient way to the component stripping furthermore.

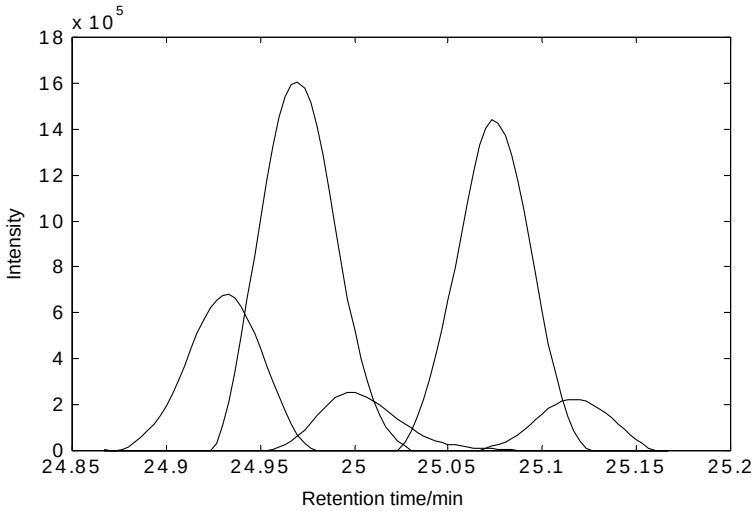

Fig. 5. Resolved chromatogram of peak cluster A containing five components.

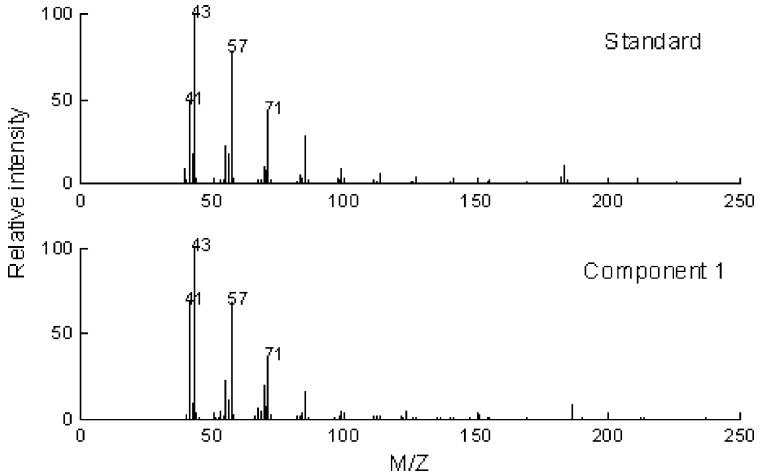

Fig. 6. Standard mass spectrum of 4,8-dimethyl-tridecane($C_{15}H_{32}$) and resolved mass spectrum of component 1 by EWOP.

By similarity searches in the NIST147 and Willey mass library, component 1, 2, and 4 in this cluster can be identified. These three components are 4,8-dimethyl-tridecane(C15H32), alpha-Farnesene (C15H24) and beta-farnesene (C15H24) with the similarity of matching result 0.952, 0.962, and 0.958, respectively. Component 3 cannot be identified because of the limitation of the mass library. By the use of pure mass spectra of the components, 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. 6–8.

The components in other peak clusters are resolved similarly. The qualitative results are shown in Table 1. Seventy-five constitutes are resolved, and 42 components are identified. Unfortunately, 33 components remain unidentified, because of the low signal-to-noise

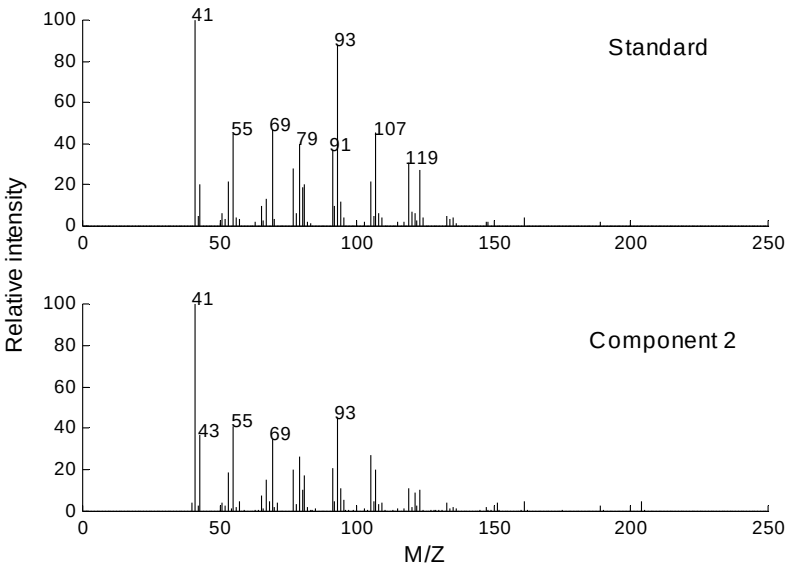

Fig. 7. Standard mass spectrum of alpha-farnesene ($C_{15}H_{24}$) and resolved mass spectrum of component 2 by EWOP.

Table 1
Qualitative and quantitative results of volatile constituents in *A. capillaris herba*

Retention time (min)	Compound	Molecular formula	Relative content (%)
8.636	3-Methylpentanol	$C_6H_{14}O$	0.32
12.008	Alpha-pinene	$C_{10}H_{16}$	0.6
13.399	Beta-pinene	$C_{10}H_{16}$	1.13
13.623	5-Hexenyl-oxirane	$C_8H_{14}O$	0.16
14.952	Sabinene	$C_{10}H_{16}$	0.17
14.989	Limonene	$C_{10}H_{16}$	0.17
15.790	2,6,6-Trimethyl-,[+/−]-bicyclo[3.1.1]-hept-2-ene	$C_{10}H_{16}$	0.12
16.530	6-*cis*-Nonenal	$C_9H_{16}O$	0.08
16.773	Decanal	$C_{10}H_{20}O$	0.1
17.060	Beta-linalool	$C_{10}H_{18}O$	0.48
18.774	*Z*-beta-terpineol	$C_{10}H_{18}O$	0.37
19.130	[-]-Alpha-terpineol	$C_{10}H_{18}O$	0.22
19.325	2-Methyl-decane	$C_{11}H_{24}$	0.12
20.912	Bornyl acetate	$C_{12}H_{20}O_2$	0.48
22.873	Copaene	$C_{15}H_{24}$	0.20
23.415	[+]Sativen	$C_{15}H_{24}$	1.22
23.610	Alpha-bergamotene	$C_{15}H_{24}$	0.35
23.705	4,11,11-Trimethyl-8-methylene-[1R-(1R@,4*Z*@,9S@)]-bicyclo[9.2.0]undec-4-ene	$C_{15}H_{24}$	1.91
24.267	Alpha-caryophyllene	$C_{15}H_{24}$	0.41
24.592	Curcumene	$C_{15}H_{22}$	1.21
24.741	Germacrene D	$C_{15}H_{24}$	1.76
24.787	Cetene	$C_{16}H_{32}$	0.96
24.942	4,8-Dimethyl-tridecane	$C_{15}H_{32}$	0.19
24.962	Alpha-farnesene	$C_{15}H_{24}$	0.61
25.073	Beta-farnesene	$C_{15}H_{24}$	0.43
25.228	Cedrene	$C_{15}H_{24}$	0.41
25.333	Isoledene	$C_{15}H_{24}$	0.81
25.948	Nerolidol	$C_{15}H_{26}O$	0.72
26.393	*trans-Z*-Alpha-bisabolene epoxide	$C_{15}H_{24}O$	4.39
26.877	*cis-Z*-Alpha-bisabolene epoxide	$C_{15}H_{24}O$	0.53
27.294	Tau-Muurolol	$C_{15}H_{26}O$	1.28
27.467	Alpha-cadinol	$C_{15}H_{26}O$	1.31
27.836	Caryophyllene oxide	$C_{15}H_{24}O$	1.06
28.016	Isoaromadendrene epoxide	$C_{15}H_{24}O$	0.65
29.984	2,15-Hexacanedione	$C_{16}H_{30}O_2$	1.78
30.627	Pentadecylic acid	$C_{15}H_{30}O_2$	1.28
32.089	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	26.29
32.601	[*Z*]-[-]-1,9-heptadecadiene-4,6-diyne-3-ol-Falcarinol	$C_{17}H_{24}O$	11.76
33.163	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	0.52
33.327	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	0.40
33.600	Phytol	$C_{20}H_{40}O$	2.73
34.491	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	19.38

ratio or the absence of the compound from the mass spectra database, and some of the researched components maybe questionable.

4.4. Quantitative analysis

With the pure chromatographic curve obtained for each component, quantitative analysis is carried out by the total volume integration [32]. The total amount of each component is then proportional to the overall volume of its two-way response. The advantage of this quantitative method over general peak-area integration is that all mass spectral absorbing points are taken into consideration, it also avoid the disadvantage that general peak-area integration is carried out with peak area while overlapping peaks are approximately treated by

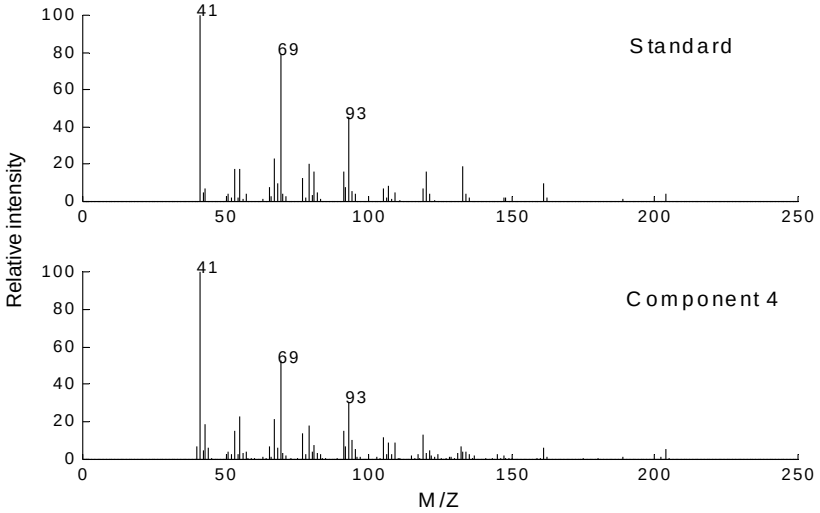

Fig. 8. Standard mass spectrum of beta-farnesene ($C_{15}H_{24}$) and resolved mass spectrum of component 4 by EWOP.

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

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References

[1] W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine, Springer Verlag, New York, 1992.
[2] S.Y. Sheu, C.L. Chieh, W.C. Weng, J. Chromatogr. A 911 (2001) 285–293.
[3] H.L. Wang, H.F. Zou, J.Y. Ni, L. Kong, S. Gao, B.C. Guo, J. Chromatogr. A 870 (2000) 501–510.
[4] H.Z. Zheng, Z.H. Dong, J. She, Modern Study of Traditional Chinese Medicine, Xue Yuan Press, Beijing, 1999, p. 3092.
[5] S.B. Yang, J.R. Guang, Chin. Herb. Med. 27 (1996) 269–272.
[6] J. Wang, L.J. Zhao, L.L. Yang, J.M. Han, Wild. Res. China 6 (1996) 52–60.
[7] J.P. Luo, Y.P. Liu, Y.F. Feng, X.L. Guo, Acta Pharm. Sinica 38 (2003) 307–310.
[8] M. Maeder, Anal. Chem. 59 (1987) 527–530.
[9] M. Maeder, A. Zilian, Chemom. Intell. Lab. Syst. 3 (1988) 205–213.
[10] E.R. Malinowski, J. Chemom. 6 (1992) 29–40.
[11] E.R. Malinowski, J. Chemom. 10 (1996) 273–279.
[12] O.M. Kvalheim, Y.Z. Liang, Anal. Chem. 64 (1992) 936–945.
[13] Y.Z. Liang, O.M. Kvalheim, H.R. Keller, D.L. Massart, P. Kiechle, F. Erni, Anal. Chem. 64 (1992) 946–953.
[14] R. Manne, H.L. Shen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 171–176.
[15] H.L. Shen, R. Manne, Q.S. Xu, D.Z. Chen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 323–328.
[16] C.J. Xu, J.H. Jiang, Y.Z. Liang, Analyst 124 (1999) 1471–1476.
[17] O.M. Kvalheim, F. Brakstad, Y.Z. Liang, Anal. Chem. 66 (1994) 43–51.
[18] H.R. Keller, D.L. Massart, Y.Z. Liang, O.M. Kvalheim, Anal. Chim. Acta 263 (1992) 29–36.
[19] C. Ritter, J. Gilliard, J. Cumps, B. Tilquin, Anal. Chim. Acta 318 (1996) 125–136.
[20] Y.Z. Liang, A.K.M. Leung, F.T. Chau, J. Chemom. 13 (1999) 511–524.
[21] L.M. Shao, B. Tang, X.G. Shao, Chin. J. Anal. Chem. 25 (1997) 15–18.
[22] X.G. Shao, W.S. Cai, Anal. Lett. 32 (1999) 743–760.
[23] A. Savitzky, M.J.E. Golay, Anal. Chem. 36 (1964) 1627–1639.
[24] P.A. Gorry, Anal. Chem. 62 (1990) 570–573.
[25] J. Steinier, Y. Termonia, J. Deltour, Anal. Chem. 44 (1972) 1906–1909.
[26] A. Khan, Anal. Chem. 59 (1987) 654–657.
[27] F.Q. Guo, Y.Z. Liang, C.J. Xu, L.F. Huang, J. Chromatogr. A 1016 (2003) 99–110.

[28] H.R. Keller, D.L. Massart, Anal. Chim. Acta 246 (1991) 379–390.
[29] Chinese Pharmacopoeia Committee, Chinese Pharmacopoeia, Publishing House of People's Health, Appendix 64, 2000.
[30] O.M. Kvalheim, Y.Z. Liang, Anal. Chem. 64 (1992) 936–945.
[31] X.N. Li, Y.Z. Liang, F.T. Chau, Chemom. Intell. Lab. Syst. 63 (2002) 139–153.
[32] F. Gong, Y.G. Peng, H. Cui, Y.Z. Liang, A.K.M. Leung, F.T. Chou, Chem. J. Chin. Univ. 20 (1999) 199–203.