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# Analysis of volatile components from *Cortex cinnamomi* with hyphenated chromatography and chemometric resolution

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**Abstract**

In this paper, the combination of hyphenated chromatography and chemometric resolution was investigated as a method to qualitatively and quantitatively determine volatile components in *Cortex cinnamomi* from four main producing areas. With the help of chemometric resolution approaches, whether the chromatographic elution of chemical components is featured by “first-in–first-out” or embedded peaks could be determined. Upon this useful information obtained, the matrix data generated by hyphenated chromatography could be uniquely resolved into pure chromatogram and spectrum of each chemical component involved followed by qualitative and quantitative analysis. The results obtained in this work showed that, 94, 88, 93 and 89 volatile components were separated and 63, 60, 60 and 58 of them qualitatively and quantitatively determined representing about 93.39, 93.62, 92.03 and 92.59% of the total relative content, respectively. The combination of hyphenated chromatography with chemometric resolution could greatly enhance the chromatographic separation and spectral qualitatively determination ability so as to qualitatively and quantitatively detect many more volatile components and improve the analysis accuracy.
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*Keywords:* Hyphenated chromatography; Chemometric resolution; Chromatographic elution; First-in–first-out; Embedded peak; Volatile component; *Cortex cinnamomi*

## 1. Introduction

The therapeutic functions of traditional herbal medicines are closely correlated with their chemical composition [1,2]. However, the great complexity of chemical components involved leads to much difficulty of the qualitative and quantitative determination of traditional herbal medicines. As a result, it is urgently necessary to develop new instruments and related techniques to meet these requirements. In recent years, the combined approaches of hyphenated chromatography such as gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography–diode array detector (HPLC–DAD), HPLC–MS with some chemometric resolution methods like heuristic evolving latent projections (HELP) [3–8], evolving factor analysis (EFA) [9], windows factor analysis (WFA) [10], sub-window factor analysis (SFA) [11,12], orthogonal projection resolution

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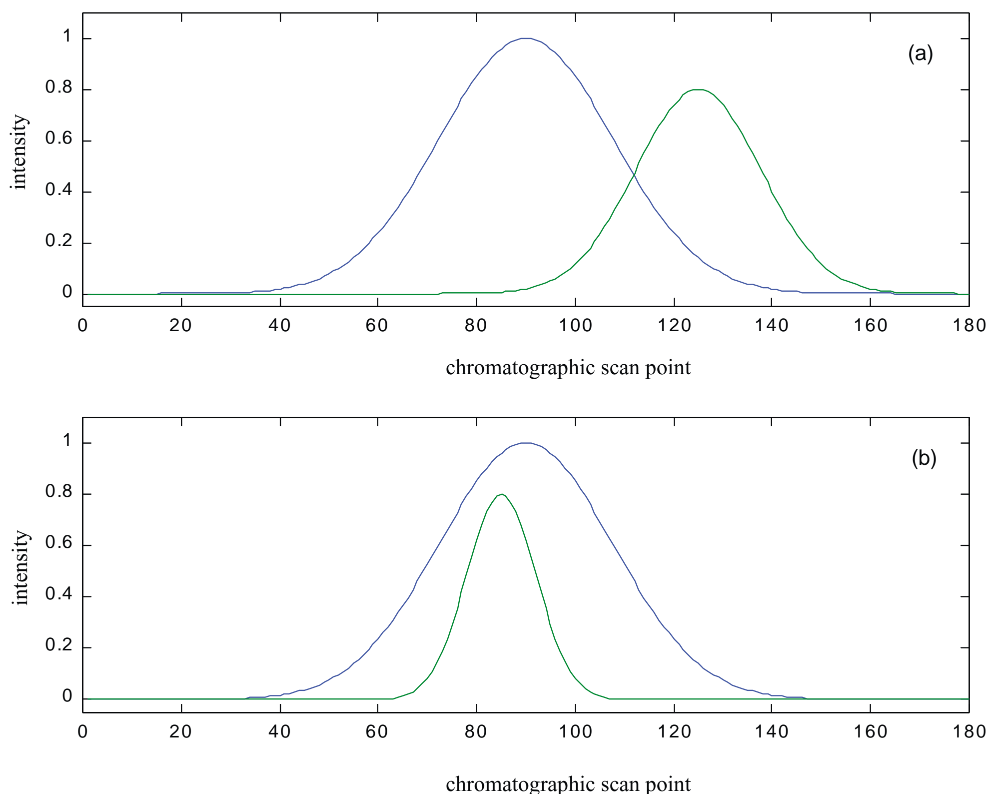


Fig. 1. "First-in–first-out" (a) and embedded (b) chromatographic peaks.

(OPR) [12,13], iterative optimization procedure for resolving embedded peaks (IPREP) [14] and inner chromatogram projection (ICP) [15] have become some kinds of powerful tools widely used to qualitatively and quantitatively analyse several complex systems.

As for the above mentioned combined approaches, the data generated by hyphenated chromatography is a matrix data with the chromatographic and spectral information. In general, these data have several advantages, such as non-negative spectra, non-negative and unimodal chromatographic profiles, and the normal chromatographic peak that elutes first, will disappear first in the retention direction, or say simply "first-in–first-out" (see a simulated data in Fig. 1a). These excellent properties are just so-called dimension advantages proposed by Booksh and Kowalski [16]. The above features could make it possible to resolve the matrix data into pure chromatogram and spectrum of each chemical component involved in a successive way by means of HELP, WFA, SFA and OPR. Then, the qualitative and quantitative determination could be conducted with pure chromatograms and spectra obtained. However, there often exists another special case called embedded peaks in chromatography, in which the assumption of "first-in–first-out" will not be held (see a simulated data in Fig. 1b). Thus, HELP, WFA, SFA and OPR will be surely of no effect [17]. Some other chemometric resolution methods like IPREP, ICP and sequential rank analysis of first-order differentiated matrix of two-way chromatographic data [18] should be employed under this special condition. As a result, it is necessary to determine whether a chromatographic peak cluster investigated is composed of normal or embedded peaks before chemometric resolution.

Volatile components are very important chemical ingredient from traditional herbal medicines such as *Rhizoma atractylodis*, *Pericarpium citri reticulatae*, *Cortex magnoliae officinalis*, *Radix angelicae sinensis*, *Cortex cinnamomi* and others [1]. Their chemical

nature makes them suitable for qualitative and quantitative determination by use of GC–MS, which is one kind of hyphenated chromatographic tools. However, the conventional GC–MS approach, in which the qualitative analysis is simply conducted by means of comparing the mass spectra directly obtained from the total ionic chromatograms (TICs) with those of the reference compounds in MS libraries, can not surely develop a clearer picture of volatile components because of their much complexity. Especially, seriously overlapping chromatographic peaks and existing background along with noise will further result in some wrong qualitative and quantitative results.

In this study, the combination of hyphenated chromatography and chemometric resolution was investigated as a method to determine volatile components from traditional herbal medicines. This combined approach is friendly used to analyse *C. cinnamomi* from four main producing areas of Yulin, Zhaoqing, Yunan (China) and Vietnam here. After the determination of chromatographic elution patterns and then the resolution of the matrix data into pure chromatogram and mass spectrum of each component involved with chemometric approaches, the qualitative identification was conducted according to their retention times and pure mass spectra followed by the quantitative determination with the overall volume integration method [5–8,12,14]. The results showed that much more chemical components could be separated and qualitatively and quantitatively determined correctly.

## 2. Methodology

A data represented by $\boldsymbol{A}_{m\times n}$ from GC–MS is a matrix data. It can be expressed simply as followings:

$$\boldsymbol{A}_{m\times n} = \boldsymbol{C}\boldsymbol{S}^{\mathrm{T}} + \boldsymbol{E} = \sum \boldsymbol{c}_i \boldsymbol{s}_i^{\mathrm{T}} + \boldsymbol{E} \quad (i = 1, 2, \dots, N) \tag{1}$$

where $\boldsymbol{A}_{m\times n}$ denotes an intensity matrix with $N$ components of $m$ chromatographic scan points at $n$ atom mass units. $\boldsymbol{C}$ and $\boldsymbol{S}$ are the pure chromatographic matrix and the pure mass spectral matrix, respectively. $\boldsymbol{E}$ represents the noise. $\boldsymbol{S}^{\mathrm{T}}$ is the transpose of the matrix $\boldsymbol{S}$. In this work, with the help of the local rank analysis technique, the unique resolution of a matrix data into chromatograms ($\boldsymbol{c}_i$) and mass spectra ($\boldsymbol{s}_i$) of the pure chemical constituents involved is carried out with HELP for the normal chromatographic peaks saying "first-in–first-out" chromatograms and with IPREP for embedded peaks. Thus, whether the chromatographic peak cluster under study is normal or embedded peaks should be determined before chemometric resolution.

On the other hand, the drifting baseline due to the background and noise surely presents itself during each chromatographic run. Thus, it is necessary to confirm the background and correct the baseline drift at first. Here, the principle component analysis (PCA) technique is used. First, two regions without any components eluting before and after the peak cluster investigated are selected. Next, PCA is conducted on the sub-matrices corresponding to the two regions, respectively. If only the background exists within the two regions selected, the similarity index (SI) between the first loading vectors of two sub-matrices will approximately to be one. With the first loading vectors obtained, the background spectrum and chromatogram might be determined followed by correcting the baseline drift [3–8].

Fig. 2 shows the simulated two-dimensional chromatograms for Fig. 1a and b. Their evolving latent projection graphs (ELPGs) after correcting the baseline drift are shown in Fig. 3 [3,4]. ELPG is, in practice, a principal component projective curve (PC1 to PC2) from chromatographic or spectral spaces. With the help of PCA, the score matrix might be obtained after all spectra (the rows of $\boldsymbol{A}$ matrix in Eq. (1)) are projected on the loading matrix. Thus, in the ELPG with the score matrix or saying from the spectral space, each line segment should represent one pure component while the curving section denotes the overlapping region containing at least two species. Moreover, the straight lines from various components will separate from each other because of the difference between their spectra. However, as for the embedded peaks, these two straight lines will superpose completely since the two components eluting before and after the overlapping regions are surly the same. Seen from Fig. 3a and b, two straight lines strongly suggest that there are at least two components existing in these simulated systems. However, the peak cluster in Fig. 1b is composed of embedded peaks since the starting and end line segments are overlapping completely in Fig. 3b. On the other hand, the chromatographic elution of chemical components in Fig. 1a

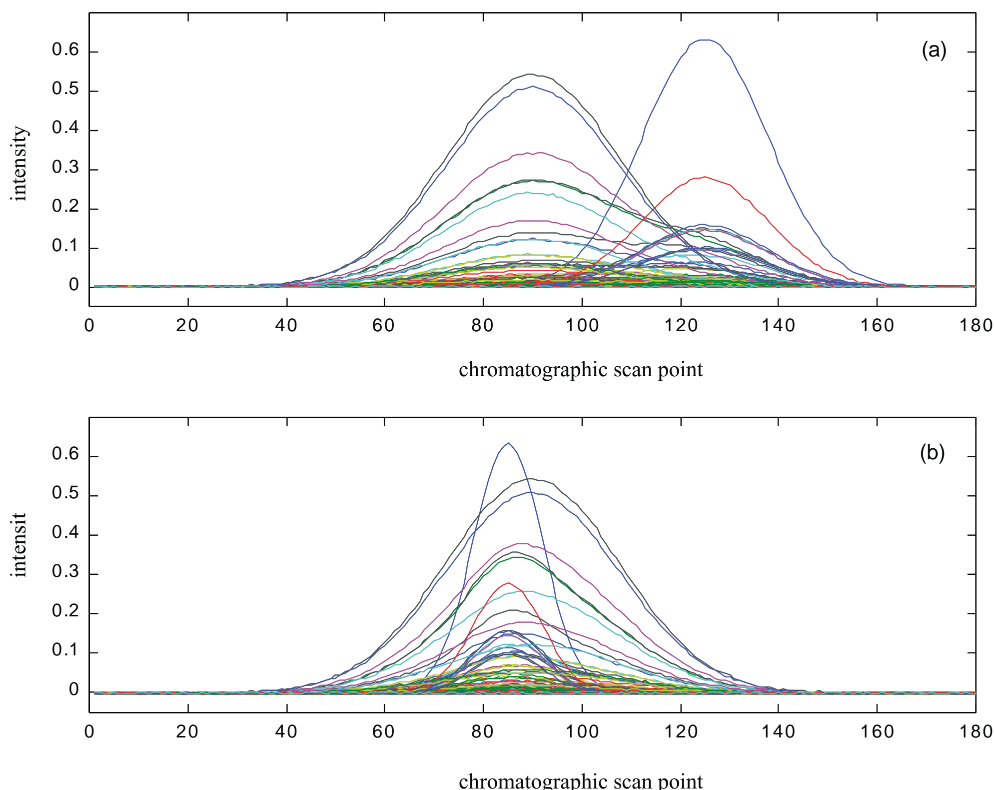


Fig. 2. Two-dimensional chromatograms for Fig. 1a (a) and Fig. 1b (b).

should be "first-in–first-out" because of the separation of two straight lines [3–15].

The chemical information on stepwise eluting and/or embedded peaks can be further identified by a fixed size moving window evolving factor analysis (FSMWEFA) or so-called eigenstructure tracking analysis [3,4]. In the fixed size window method (FSWM) plot (Fig. 4), the noise level is characterized by eigenvalue curves which have similar numerical values and appears together at the bottom. Eigenvalue curves that are higher than the noise level represent the presence of new components. If a system under study contains one single species, only one eigenvalue curve higher than the noise level in its FSWM plot can be obtained. Seen from Fig. 4, two chemical components should exist in these two simulated systems. For I, III, I′ and III′ regions, there is only one pure chemical component eluting, respectively while both II and II′ are overlapping regions containing two components. However, if the pure mass spectra are picked out from these pure regions directly (see Fig. 5), it is interesting that the mass spectra from I′ (Fig. 5c) and III′ (Fig. 5d) are identical completely while those from I (Fig. 5a) and III (Fig. 5b) are different from each other. It might also suggest that I′ and III′ should represent the pure regions of the same component while various components are existing in I and III. As a result, the peak cluster in Fig. 1b is surely featured by embedded peaks. However, the chromatographic peaks in Fig. 1a can hold the assumption of "first-in–first- out". The peak cluster in Fig. 1a and b should be resolved by means of different chemometric resolution approaches, saying HELP, WFA, SFA and OPR for the former while IPREP, ICP and sequential rank analysis of first-order differentiated matrix of two-way chromatographic data for the later one.

After the chemical information on stepwise eluting and/or embedded peaks is determined, various chemometric resolution approaches might be used now. Since the basic principles on HELP, WFA, SFA and OPR

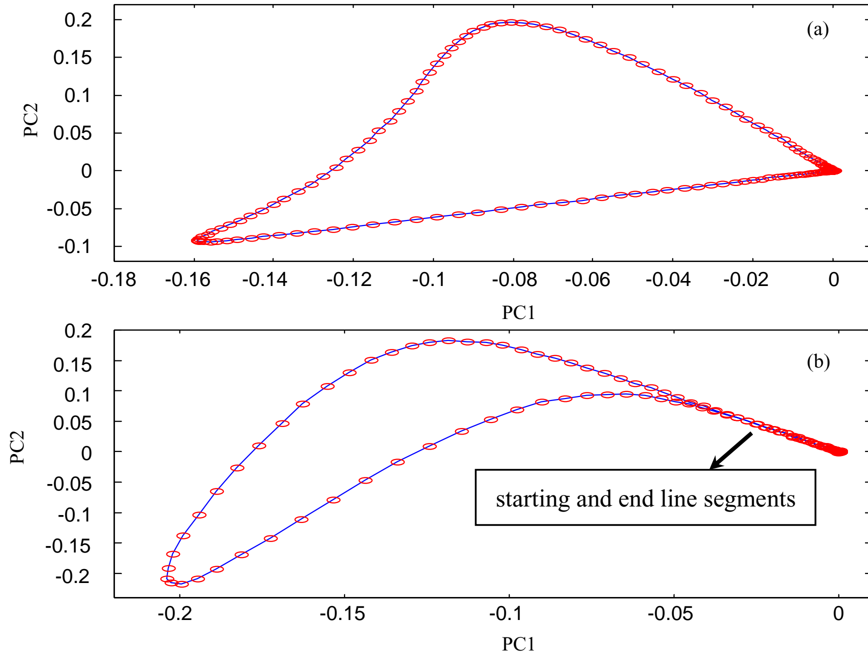


Fig. 3. ELPGs for Fig. 1a (a) and Fig. 1b (b).

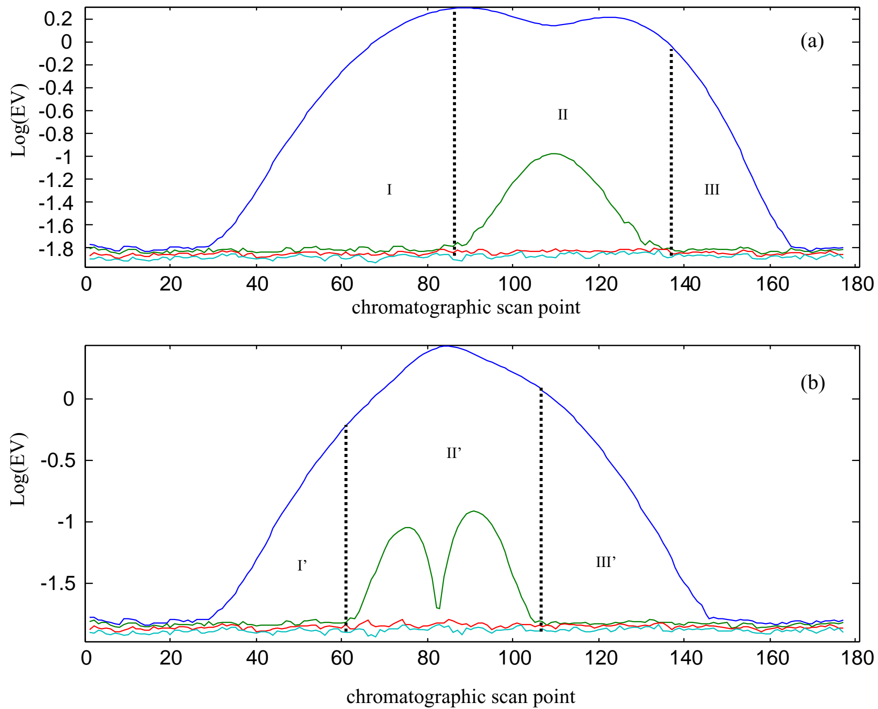


Fig. 4. FSWM plots for Fig. 1a (a) and Fig. 1b (b).

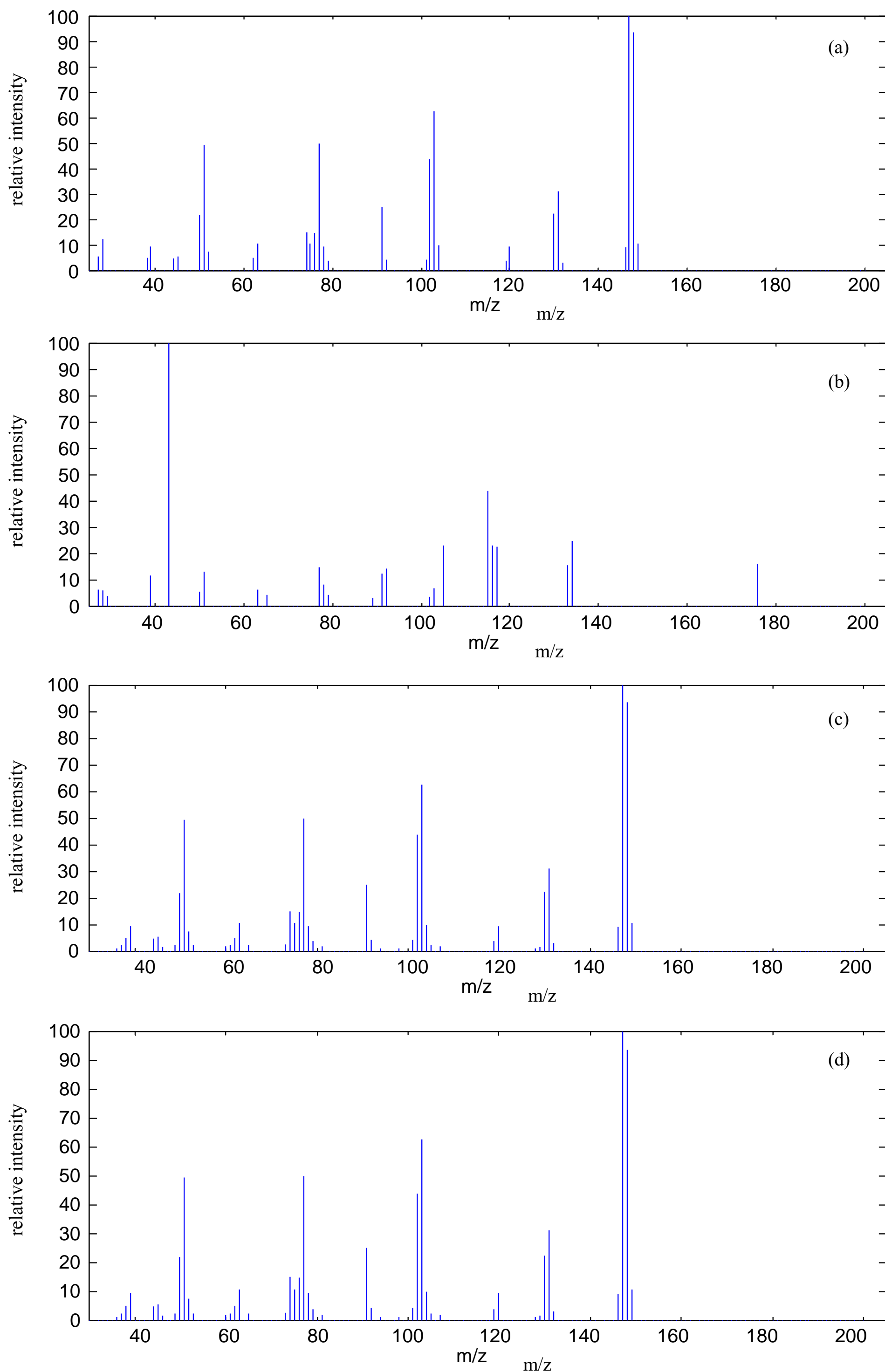


Fig. 5. Mass spectra from the pure regions of I (a), III (b), I′ (c) and III′ (d) in Fig. 4.

for the normal peak clusters while IPREP, ICP and sequential rank analysis of first-order differentiated matrix of two-way chromatographic data for embedded ones have been documented in detail in [3–18], only a brief introduction to HELP and IPREP are restated here.

As for the HELP method, some steps might be described as the following:

(1) Confirm the background and correct a drifting base line.
(2) Determine the number of components in a peak cluster of interest, the selective region and zero-component region of each component by means of the ELPGs and FSWM plots on the basis of the eigenstructure tracking analysis. Here, the selective region is a region with only one pure component existing while the component does not elute in within its zero-component region.
(3) According to the complete selective information and zero-component region obtained above, construct a full rank sub-matrix related to each component in turn and then conduct a unique resolution on the two-dimensional data into pure chromatographic profiles and mass spectra.
(4) In comparison with the original data matrix and the resolved chromatographic profiles and mass spectra, verify the reliability of the resolved result.

The IPREP algorithm is more complex than HELP. The key step of the approach is to locate three points saying $t_0$, $t_1$ and $t_2$ at first and then determine another point $t^*$. If $\boldsymbol{c}_1$, $\boldsymbol{c}_2$, $\boldsymbol{s}_1$ and $\boldsymbol{s}_2$ are pure chromatographic and spectral vectors of the embedding and embedded peaks respectively, then

$$\boldsymbol{A} = \boldsymbol{c}_1\boldsymbol{s}_1^{\mathrm{T}} + \boldsymbol{c}_2\boldsymbol{s}_2^{\mathrm{T}} \qquad (2)$$

$t_0$ is the retention time point at which the intensity of the embedded and embedding peaks reaches the maximal. That is,

$$\boldsymbol{A}(t_0, j_0) = \max(\boldsymbol{A}(t, j)) \qquad (3)$$

Let

$$\boldsymbol{x}^{\mathrm{T}}(t) = \boldsymbol{c}_1(t)\boldsymbol{s}_1^{\mathrm{T}} + \boldsymbol{c}_2(t)\boldsymbol{s}_2^{\mathrm{T}} \quad (t = 1, 2, \ldots, m) \qquad (4)$$

$$\frac{\mathrm{d}\boldsymbol{x}^{\mathrm{T}}(t)}{\mathrm{d}t} = \mathbf{s}_1^{\mathrm{T}}\frac{\mathrm{d}\boldsymbol{c}_1(t)}{\mathrm{d}t} + \boldsymbol{s}_2^{\mathrm{T}}\frac{\mathrm{d}\boldsymbol{c}_2(t)}{\mathrm{d}t} \qquad (5)$$

where $\boldsymbol{x}^{\mathrm{T}}$ represents the row vectors of $\boldsymbol{A}$, $\mathrm{d}\boldsymbol{x}^{\mathrm{T}}(t)/\mathrm{d}t$ is the one order derivate and $m$ is the number of the chromatographic scan points. When the value of $\mathrm{d}\boldsymbol{x}^{\mathrm{T}}(t)/\mathrm{d}t$ becomes non-positive, the corresponding retention time point is $t_1$.

As for $t_2$, a matrix denoted by $\boldsymbol{H}(t)$ should be constructed as the following:

$$\mathbf{H}(t) = \begin{pmatrix} (\mathrm{d}\boldsymbol{x}^{\mathrm{T}}(t)/\mathrm{d}t)_{t=t_1} \\ \boldsymbol{x}^{\mathrm{T}}(t_0) - \boldsymbol{x}^{\mathrm{T}}(t) \end{pmatrix} = \begin{pmatrix} c_1'(t_1) & c_2'(t_1) \\ c_1(t_0) - c_1(t) & c_2(t_0) - c_2(t) \end{pmatrix} \begin{pmatrix} s_1^{\mathrm{T}} \\ s_2^{\mathrm{T}} \end{pmatrix} \quad (t = t_1 + 1, \ldots, m) \qquad (6)$$

with $t_0$ and $t_1$ obtained, $t_2$ is the chromatographic scan point that makes the rank of $\boldsymbol{H}(t)$ decrease from 2 to 1.

$t^*$ is retention time point at which $\boldsymbol{c}_1$ reaches the maximal. That is,

$$\frac{\mathrm{d}c_1(t^*)}{\mathrm{d}t} = 0 \qquad (7)$$

Since $\boldsymbol{c}_1$ is unknown, $t^*$ is determined with $t_1$ and $t_2$ by way of estimating the rank of a matrix denoted by $\boldsymbol{G}(t)$ as following:

$$\boldsymbol{G}(t) = \begin{pmatrix} \mathrm{d}\boldsymbol{x}^{\mathrm{T}}(t)/\mathrm{d}t \\ \boldsymbol{x}^{\mathrm{T}}(t_1) - \boldsymbol{x}^{\mathrm{T}}(t_2) \end{pmatrix} = \begin{pmatrix} c_1'(t) & c_2'(t) \\ c_1(t_1) - c_1(t_2) & c_2(t_1) - c_2(t_2) \end{pmatrix} \begin{pmatrix} s_1^{\mathrm{T}} \\ s_2^{\mathrm{T}} \end{pmatrix} \quad (t = t_1 + 1, \ldots, m) \qquad (8)$$

If $t^\wedge$ makes the rank of matrix $\boldsymbol{G}(t^\wedge)$ drop from 2 to 1, then, one takes $t^\wedge$ as the final estimate of $t^*$. Once $t^*$ is located, $\boldsymbol{c}_1$ could be determined and then $\boldsymbol{s}_2$ is obtained.

To sum up, a simple summarization of the IPREP approach is given as follows:

(1) Confirm the background and correct a drifting baseline.
(2) Locate the selective region of the embedding component by HELP or other chemometric resolution methods and then obtain the pure spectrum of the embedding peak $\boldsymbol{s}_1$.
(3) Estimate the chromatogram $\boldsymbol{c}_2$ of the embedded component by OPR.
(4) Locate $t_0$, $t_1$, $t_2$ and then get the estimate of maximum retention time $t^*$ of the embedding peak by the rank analysis technique.

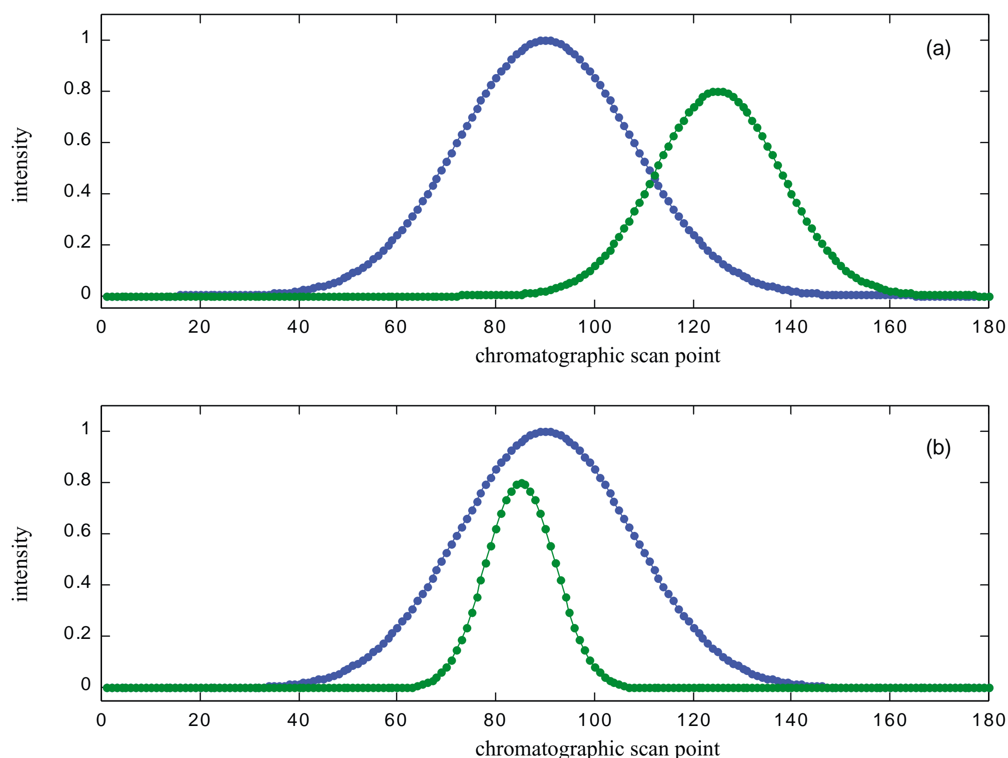


Fig. 6. Resolved chromatograms for Fig. 1a (a) and Fig. 1b (b). The real curves show simulated chromatograms while the dotted lines represent the resolved ones.

(5) With $t^*$ obtained, determine the chromatogram $\boldsymbol{c}_1$ by marker-object projection optimization with $\boldsymbol{s}_1$ (normalized) as the marker object, and finally the pure spectrum $\boldsymbol{s}_2$ of the embedded component by the least-squares method.
(6) In comparison with the original data matrix and the resolved chromatographic profiles and mass spectra, verify the reliability of the resolved result.

With the help of HELP and IPREP, the resolved results could be obtained easily (see Fig. 6. The real curves represent the simulated chromatograms while the dot curves are the resolved profiles). Seen from Fig. 6, the above-mentioned chemometric resolution approaches should be reliable.

Something should be noted here. The IPREP method can only be applied to resolve one kind of embedded peaks, saying the case that the embedded component remains the chromatographic elution after the embedding one reaches the top chromatographic point [14].

## 3. Materials and experimental methods

### 3.1. Materials

Raw materials of *C. cinnamomi* from four main producing areas (Yulin, Zhaoqing, Yunnan (China) and Vietnam) were collected from a pharmaceutical store in Changsha, China.

### 3.2. Extraction of volatile components

About 200 g *C. cinnamomi* was swollen with over 1000 ml of distilled water in a standard extractor which are compulsorily recommended for extracting volatile components from traditional herbal medicines in China and allowed to stand for 30 min. under room temperature. Then, more about 100 ml of distilled water was added. Next, the samples containing volatile components were prepared according to *Chinese Pharmacopoeia* [19].

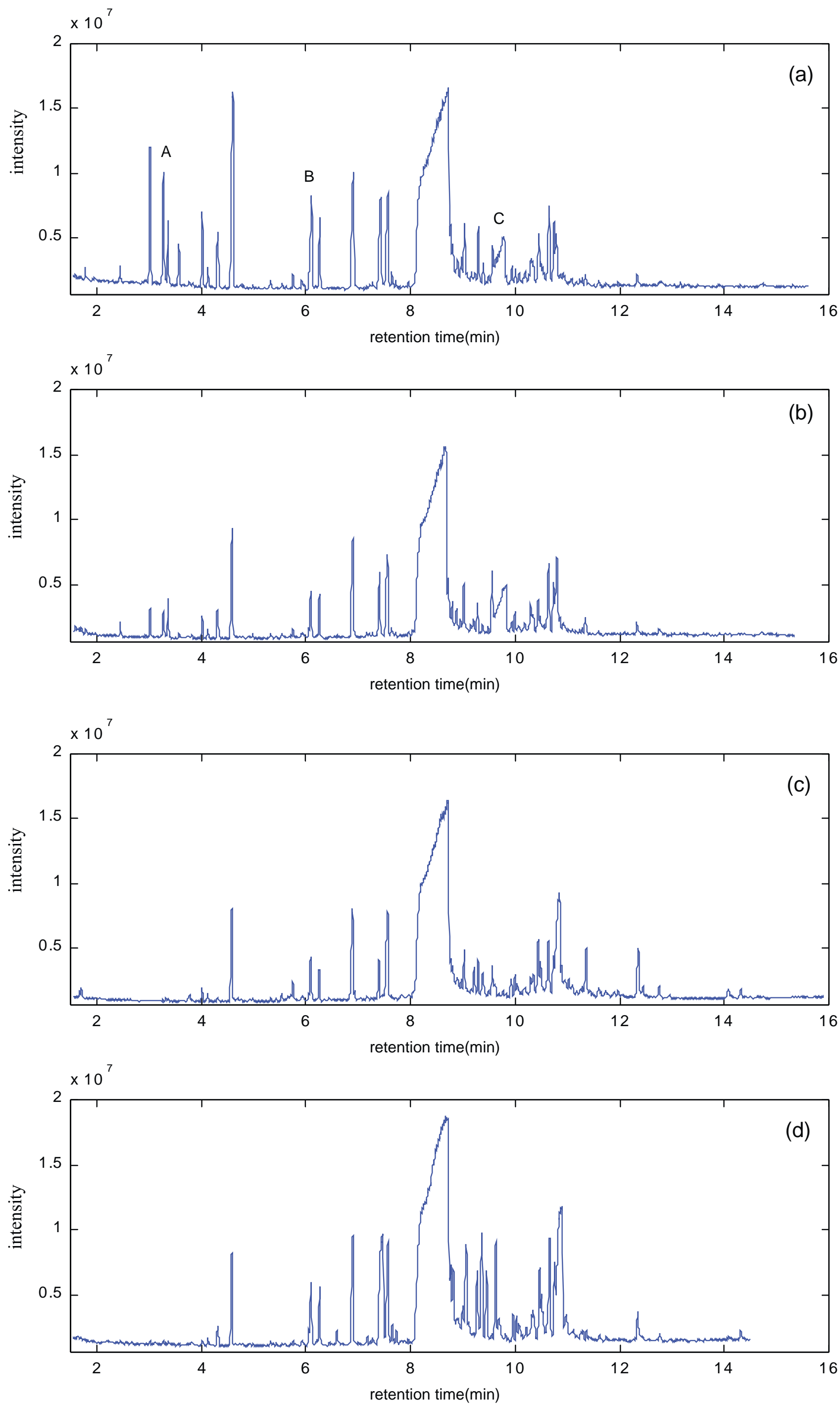


Fig. 7. TICs of volatile components in *C. cinnamomi* from Yulin (a), Zhaoqing (b), Yunnan (c), Vietnam (d).

### 3.3. Instruments

In this work, GC-17A gas chromatography and QP-5000 mass spectrometer from Shimadzu Company were employed.

### 3.4. Detection of volatile components

In the gas chromatographic system, an OV-17 capillary column (30 m × 0.25 mm i.d., manufactured by J&W Scientific, USA) was used. Column temperature was first set at 60 °C, and then programmed from 60 to 270 °C at the rate 15 °C/min. Inlet temperature was kept at 250 °C. Helium carrier gas was used at a constant flow-rate of 1 ml/min. In the mass spectrometer, electron impact ($EI^+$) mass spectra were recorded at 70 eV ionization energy in full scan mode in the 20–350 amu mass range with 0.2 s per scan velocity. The ionization source temperature was set at 230 °C.

### 3.5. Data analysis

Data analyses were performed on a pentium based IBM compatible personal computer. All programs of HELP, SFA, OPR and IPREP were coded in MATLAB 5.1 for windows. The library searches and spectral matching of the resolved pure components were conducted on the national institute of standards and technology (NIST) and Wiley MS libraries containing about 180,000 compounds.

## 4. Results and discussion

### 4.1. Resolution of volatile components from C. cinnamomi with HELP and IPREP

After conducting GC–MS analysis for volatile components, several total ionic chromatograms

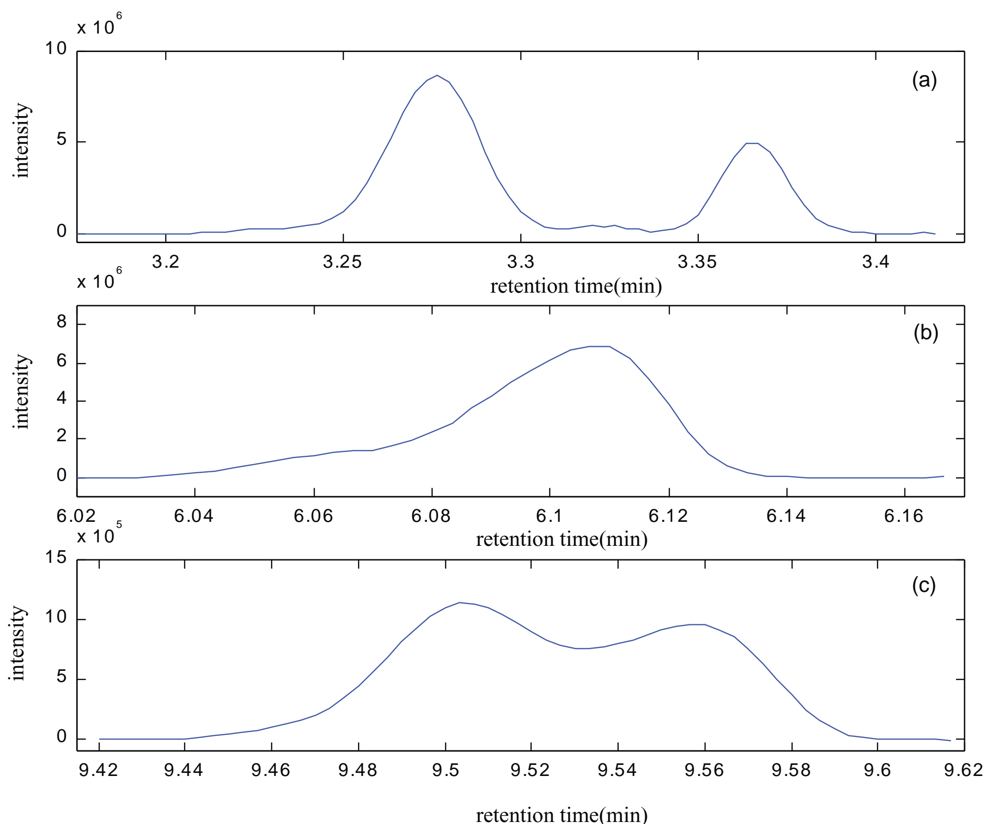


Fig. 8. TICs of the A (a), B (b) and C (c) peak clusters in Fig. 7.

(TICs) might be obtained directly (Fig. 7). Visually seen from these chromatographic profiles, about 32, 30, 26 and 30 chromatographic peaks present themselves in Fig. 7a–d, respectively. However, as pointed out by Davis and Giddings [20], parts of these peaks are possibly overlapping with their adjacent one(s). This situation might be further confirmed by direct searching with the MS databases on account of low similarity indices. On the other hand, the baseline drift could also lead to directly qualitative determination without satisfaction. As a result, the baseline drift should be corrected at first and the overlapping peaks should be resolved into pure chromatograms and mass spectra involved.

Fig. 8 shows the TICs of A (within 3.1–3.5 min), B (within 6.0–6.2 min) and C (within 9.4–9.7 min) peak clusters in Fig. 7a after correcting the baseline drift. Seen from Fig. 8, there are about two, one and two chemical components existing in a, b and c, respectively. In order to determine the chromatographic peak purity, the number of chemical components along with the chromatographic elution in a, b and c, their ELPGs and FSWM plots are shown in Figs. 9 and 10, respectively. Visually seen from Fig. 9a, there might exist two components isolated completely because of two completely separated line segments. Since there are also two straight lines in Fig. 9b, not one but at least two compounds should present themselves in the B peak cluster. It is very interesting to have a look at Fig. 9c. In Fig. 9c, the starting and end line segments are superposed completely. And also, the number of chromatographic eluting points with superposition can be counted (17 points here (Fig. 9c). It strongly suggests that the chromatographic elution of chemical components is featured by embedded peaks for the C peak cluster. Their chemical information could be further confirmed by FSWM profiles in Fig. 10.

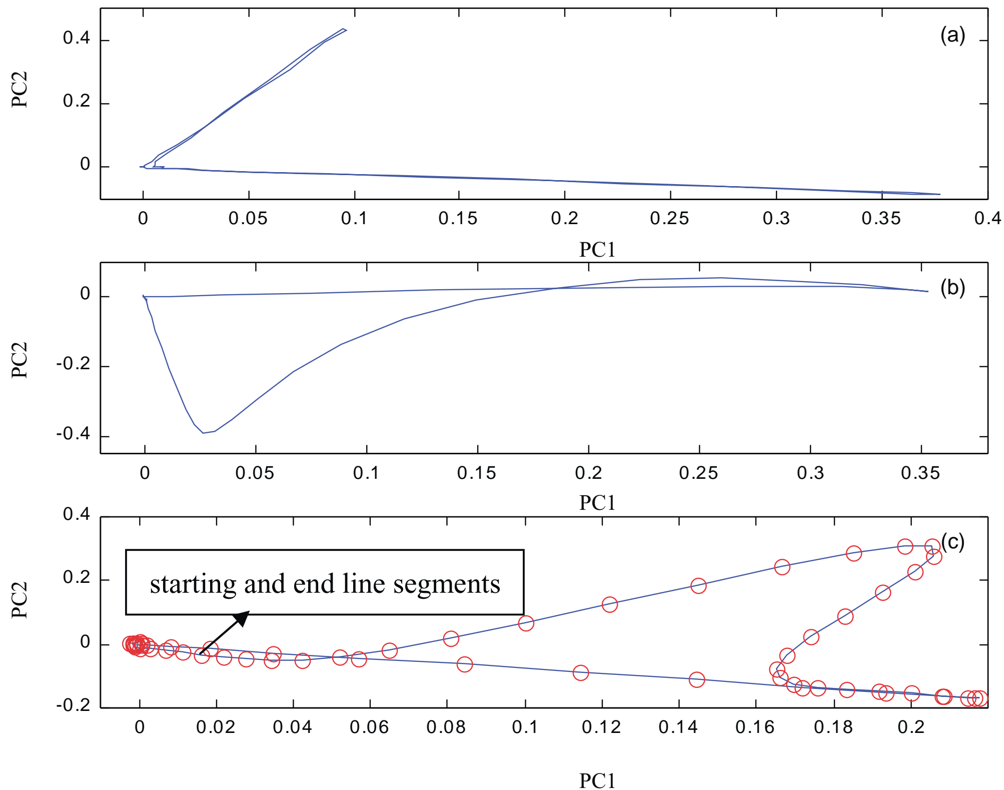


Fig. 9. ELPGs of the A (a), B (b) and C (c) peak clusters.

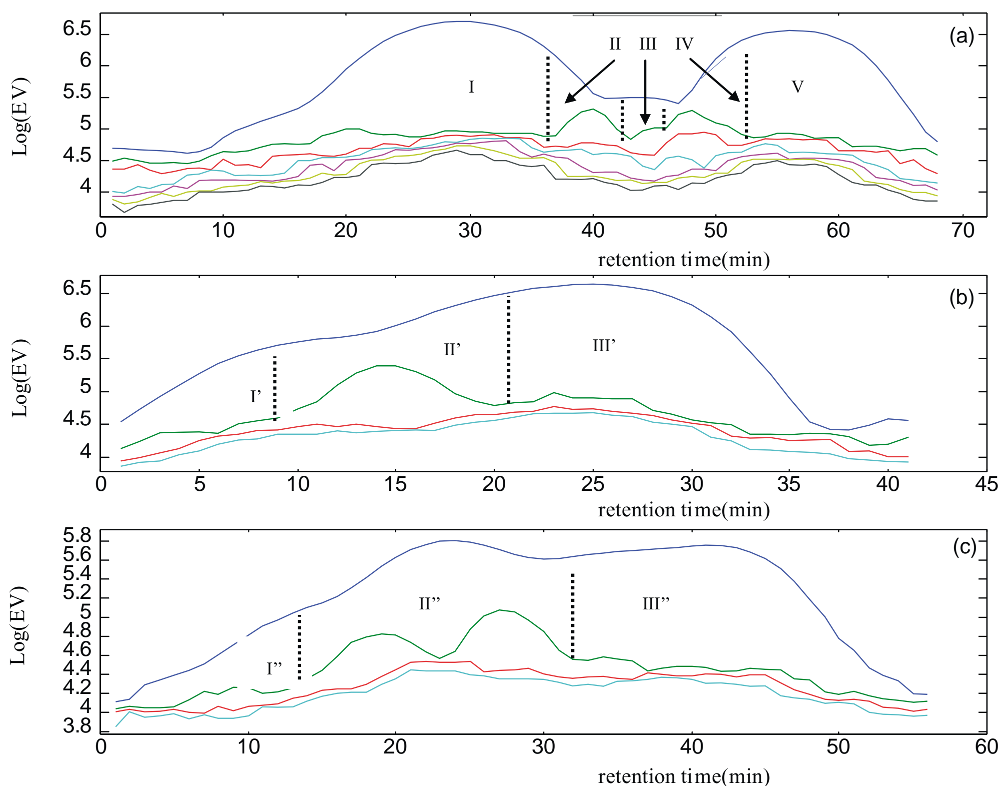


Fig. 10. FSWM plots of the A (a), B (b) and C (c) peak clusters.

Seen from Fig. 10a, all I, III and V are pure regions while II and IV are overlapping ones in which two chemical components elute simultaneously. There is, in practice, another small component eluting in the middle. Thus, not two but three compounds exist in the A peak clusters. I′ and III′ are pure regions while II′ is overlapping one in Fig. 10b. There are two chemical components presenting themselves in the B peak cluster in fact. Moreover, both the A and B peak clusters are with the normal chromatographic elution saying “first-in–first-out”. Seen from Fig. 10c, I″ and III″ are pure regions while II” is overlapping one. However, the completely identical mass spectra obtained from I″ and III″ strongly suggests that I″ and III″ are pure regions containing the same component. As a result, the chromatographic elution pattern in the C peak cluster is characterized by embedded peaks. It is in complete accordance with the determination on the basis of the ELPG in Fig. 9c. Fortunately, this special case in the C peak cluster could be treated by means of the IPREP method [11]. From above, much more chemical components have been detected and the chromatographic elution patterns could be determined by means of chemometric approaches.

Since the number of chemical components together with the chromatographic elution patterns of the A, B and C peak clusters have been determined, HELP and IPREP algorithms could be applied to resolving the matrix data into pure chromatogram and mass spectrum of each component involved now. The resolved chromatograms and mass spectra are shown in Figs. 11 and 12, respectively. In Fig. 12, a, c, e, g, i, k and m are the resolved mass spectra of the components denoted by 1, 2, 3, 4, 5, 6 and 7 in Fig. 11, respectively.

As for other chromatographic peak clusters, the same processes as above could be used. Details

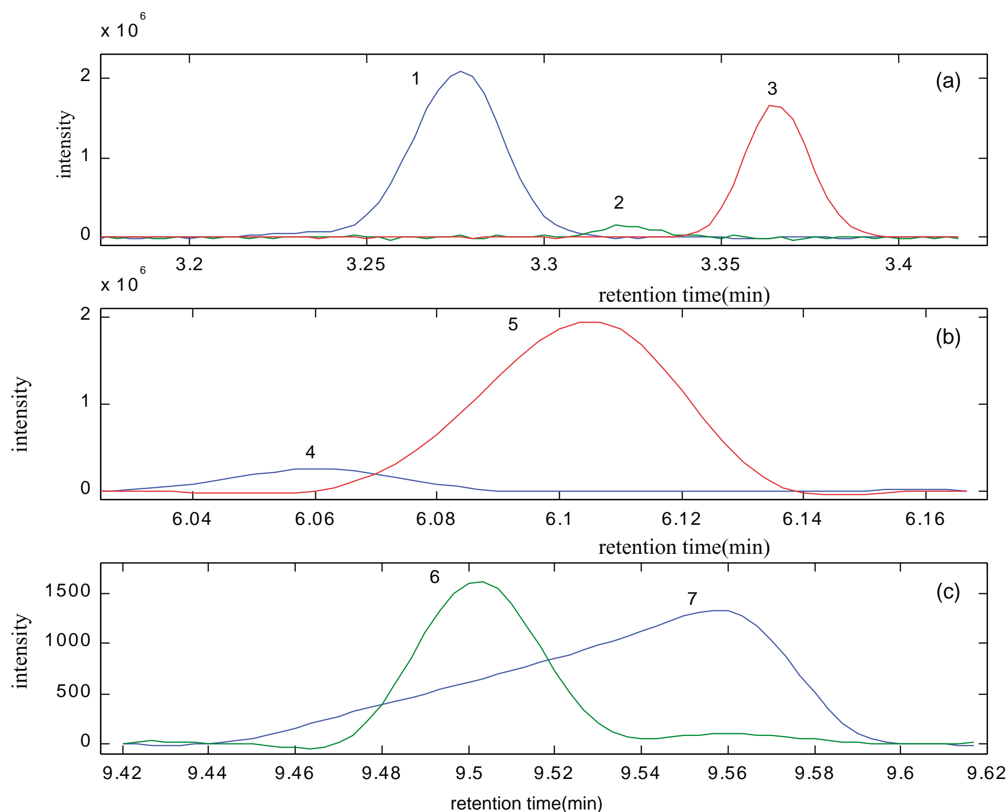


Fig. 11. Resolved chromatograms of the A (a), B (b) and C (c) peak clusters.

about their resolving procedures are not described here.

### 4.2. Qualitative and quantitatively determination of volatile components from C. cinnamomi

From above, the pure chromatogram and mass spectrum of each component involved in all chromatographic peak clusters could be obtained. Thus, with the help of similarity searches in the NIST and Wiley mass libraries along with the retention times obtained, we can conduct qualitative analyses of volatile components now. In comparison with the resolved (Fig. 12a, c, e, g, i, k and m) and standard (Fig. 12b, d, f, h, j, l and n) mass spectra of the No. 1 to 7 components in Fig. 11, they are camphene ($C_{10}H_{16}$), 3-furaldehyde ($C_5H_4O_2$), cardene ($C_8H_8$), 4-terpineol ($C_{10}H_{18}O$), borneol ($C_{10}H_{18}O$), cinnamyl acetate ($C_{11}H_{12}O_2$) and cinnamic acid ($C_9H_8O_2$), respectively. Seen from Fig. 12, it should be reasonable and reliable for the qualitative results obtained by means of HELP and IPREP approaches.

As for the quantitative determination, since the term $\boldsymbol{c}_i\boldsymbol{s}_i^{\mathrm{T}}$ in Eq. (1) of the $i$th component could be obtained on the basis of the above-resolved chromatogram and mass spectrum, the value of $\boldsymbol{c}_i\boldsymbol{s}_i^{\mathrm{T}}$ might be considered as the overall volume integration value [6–8,12,14]. Similar to the general chromatographic quantitative approach in relating the chromatographic peak area to the concentration of the component investigated, $\boldsymbol{c}_i\boldsymbol{s}_i^{\mathrm{T}}$ is directly proportional to the amount of the $i$th component and so its relative content could be quantified. The results in this study show that, there are 94, 88, 93 and 89 volatile components in *C. cinnamomi* from Yulin, Zhaoqing, Yunan and Vietnam separated, 63, 60, 60 and 58 of them qualitatively and quantitatively

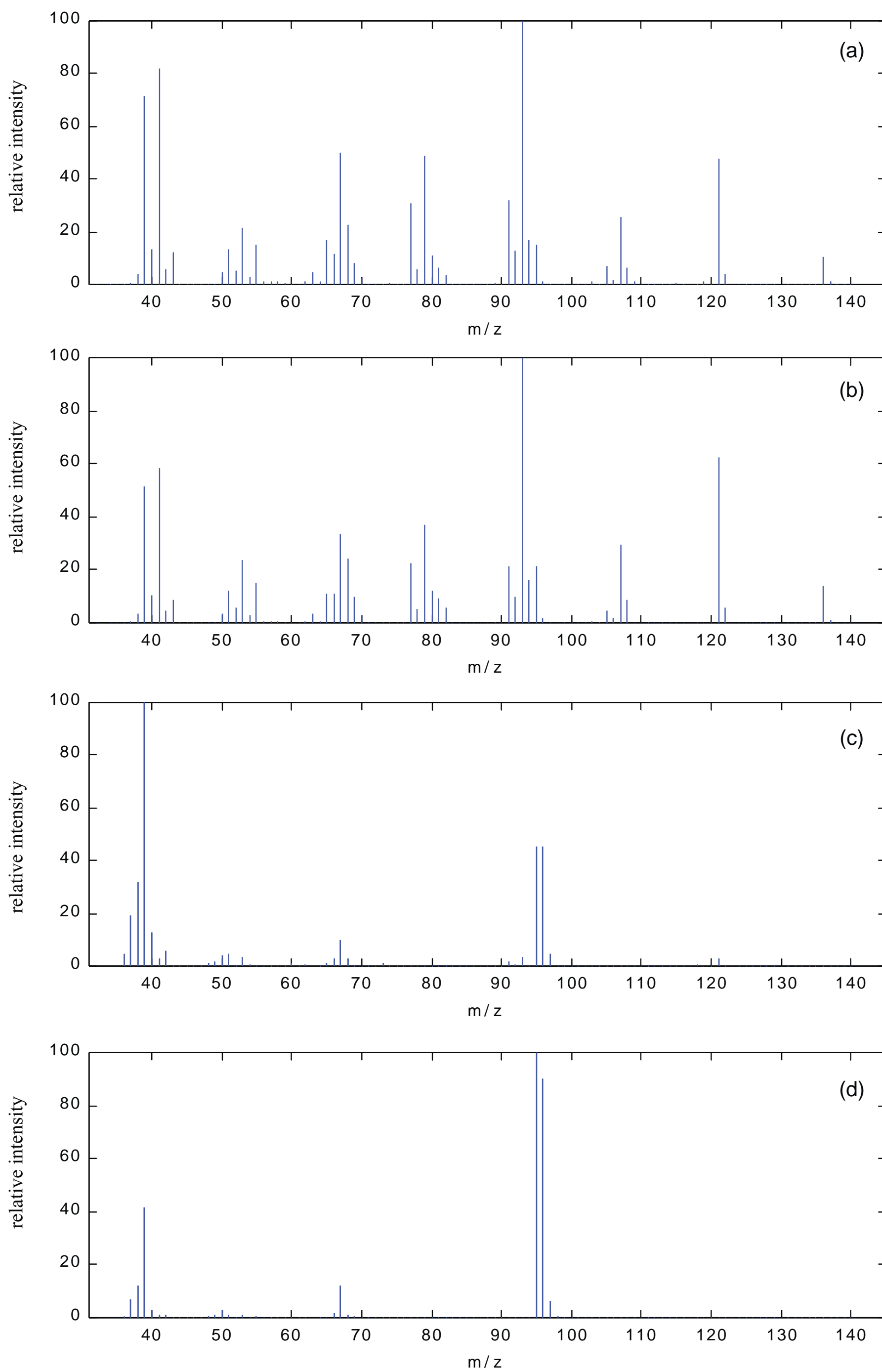


Fig. 12. Resolved and the standard mass spectra of the A (a–f), B (g–j) and C (k–n) peak clusters.

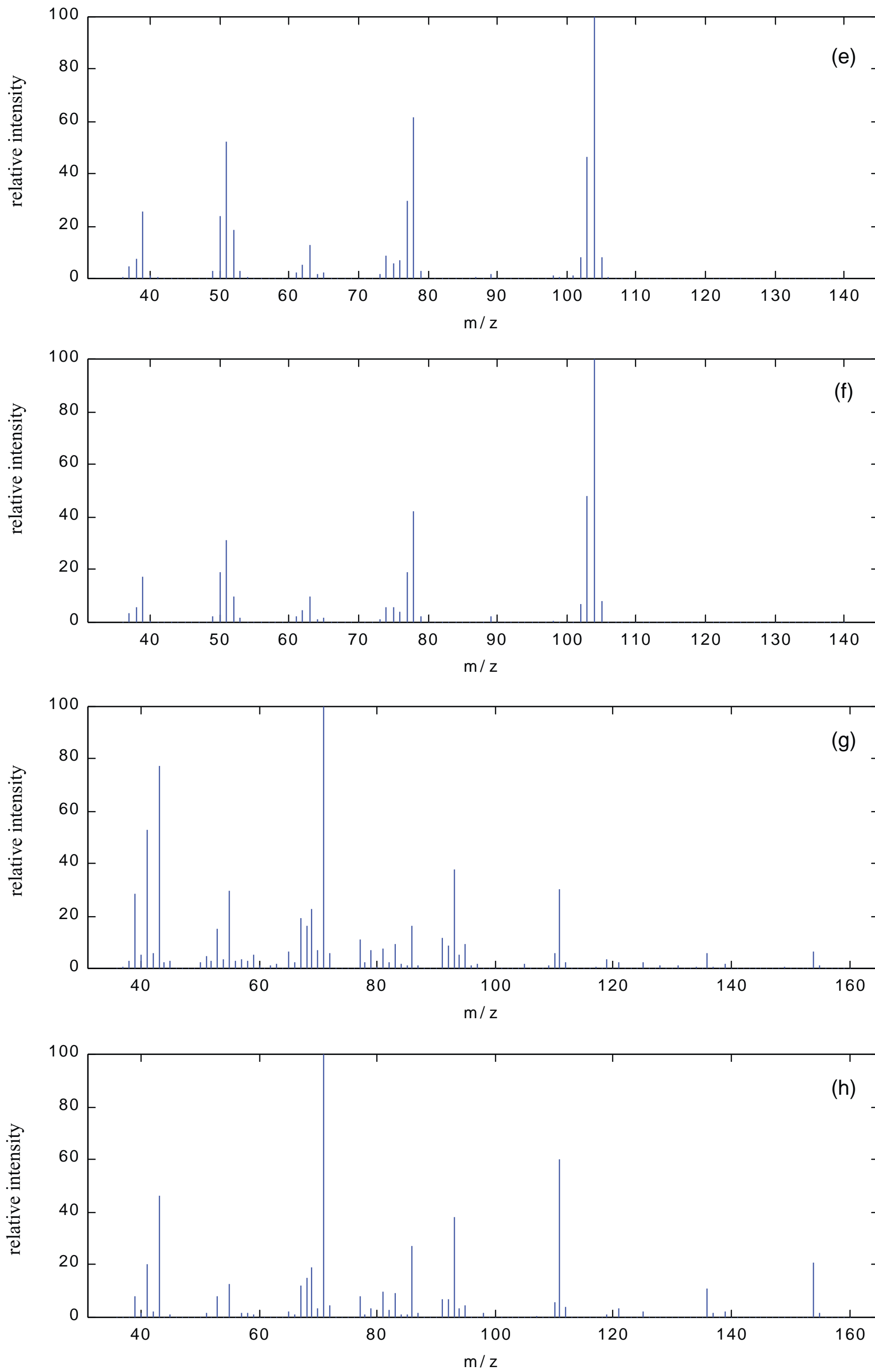


Fig. 12. (*Continued*)

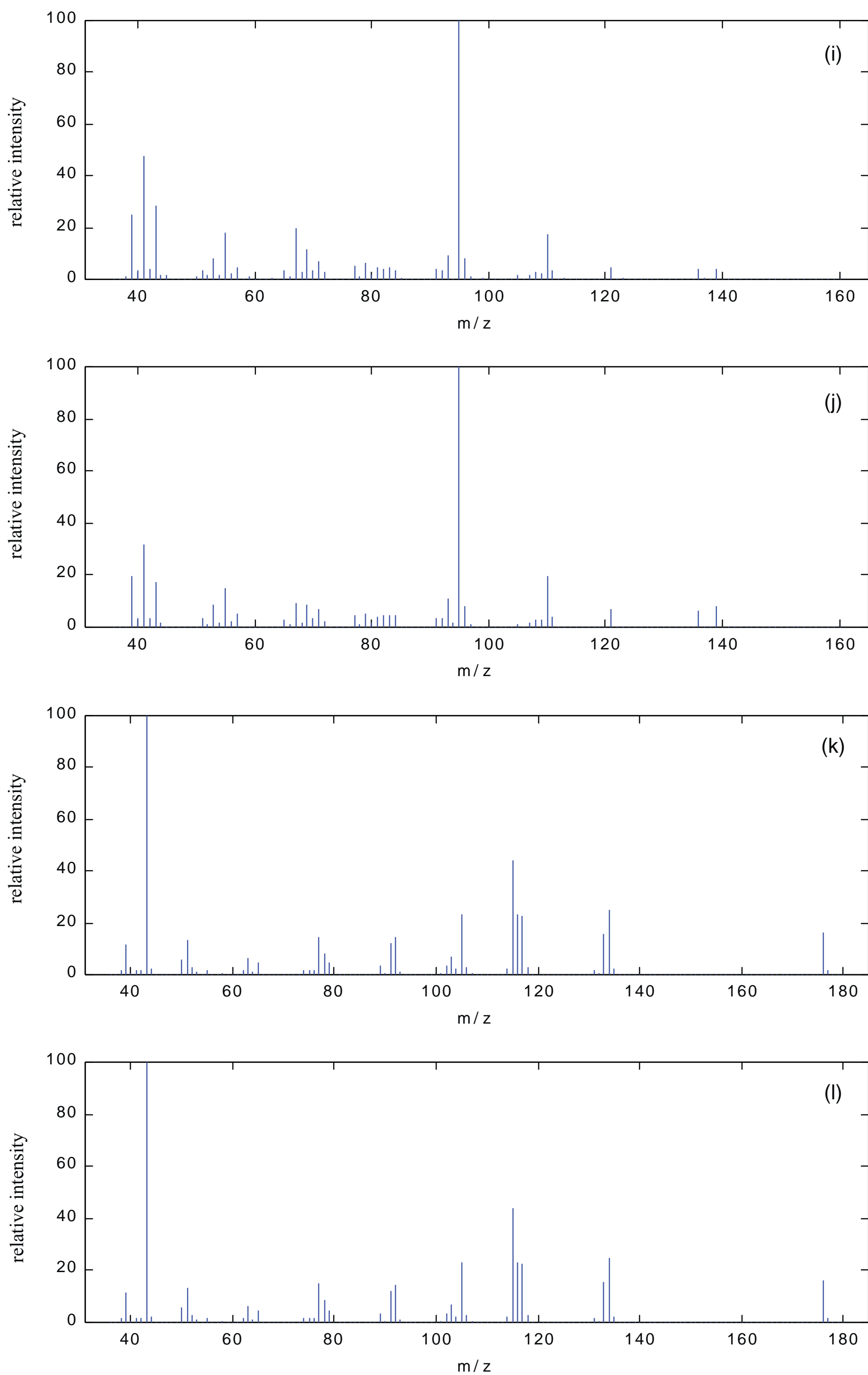


Fig. 12. (*Continued*)

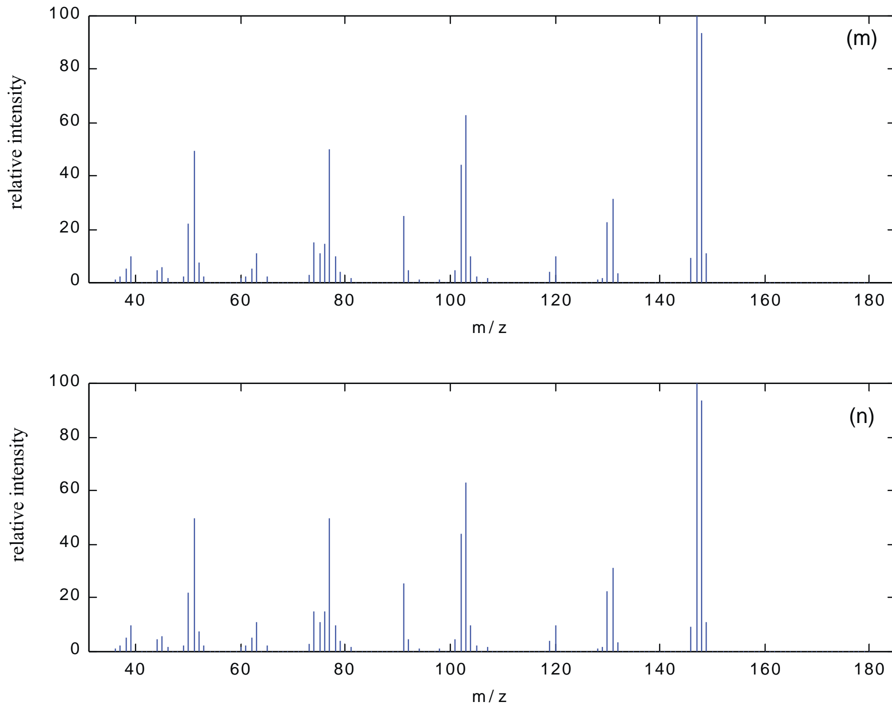


Fig. 12. (*Continued*).

Table 1
Qualitative and quantitative results of volatile components in *C. cinnamomi* from different areas

| Retention time (min) | Name of component | Molecular formula | Relative content (%) | | | |
|---|---|---|---|---|---|---|
| | | | Yulin | Zhaoqing | Yunan | Vietnam |
| 1.79 | Pentanal | $C_5H_{10}O$ | 0.20 | 0.11 | – | – |
| 2.45 | Hexanal | $C_6H_{12}O$ | 0.22 | 0.27 | – | 0.02 |
| 2.92 | β-Pinene | $C_{10}H_{16}$ | 0.06 | 0.02 | – | – |
| 2.95 | *o*-Dimenthylbenzene | $C_8H_{10}$ | 0.03 | 0.03 | – | – |
| 3.02 | *trans*-Ocimene | $C_{10}H_{16}$ | 2.87 | 0.70 | – | 0.06 |
| 3.27 | Camphene | $C_{10}H_{16}$ | 2.40 | 0.75 | 0.01 | 0.04 |
| 3.32 | 3-Furaldehyde | $C_5H_4O_2$ | 0.08 | 0.07 | 0.06 | 0.16 |
| 3.37 | Cardene | $C_8H_8$ | 1.01 | 0.78 | 0.02 | 0.05 |
| 3.57 | β-Pinene | $C_{10}H_{16}$ | 0.81 | 0.12 | – | 0.26 |
| 3.75 | 2-Ethyl-2-hexenal | $C_8H_{14}O_2$ | 0.06 | – | – | – |
| 3.78 | Hexanoic acid | $C_6H_{12}O_2$ | – | 0.06 | 0.20 | 0.21 |
| 3.81 | Ocimene | $C_{10}H_{16}$ | 0.05 | 0.05 | – | 0.03 |
| 4.02 | Limonene | $C_{10}H_{16}$ | 1.25 | 0.49 | 0.25 | 0.07 |
| 4.12 | Methyl heptenone | $C_8H_{14}O$ | 0.30 | 0.19 | 0.13 | 0.10 |
| 4.30 | 4-Ethyl-*o*-xylene | $C_{10}H_{16}$ | 0.45 | 0.43 | 0.04 | 1.25 |
| 4.32 | *p*-Cineole | $C_{10}H_{18}O$ | 0.88 | 0.47 | 0.09 | 0.24 |
| 4.44 | γ-Terpinene | $C_{10}H_{16}$ | – | – | 0.02 | – |
| 4.58 | Benzadehyde | $C_7H_6O$ | 6.33 | 3.46 | 2.37 | 1.59 |
| 4.70 | Heptanoic acid | $C_7H_{14}O_2$ | 0.05 | – | 0.08 | – |
| 4.78 | Terpinolen | $C_{10}H_{16}$ | 0.05 | – | – | – |
| 4.91 | 3,7-Diemthyl-1,6-octadien-3-ol | $C_{10}H_{18}O$ | – | – | 0.04 | – |
| 4.99 | Decananl | $C_{10}H_{20}O$ | 0.04 | 0.08 | 0.04 | 0.02 |
| 5.32 | *endo*-Fenchyl alcohol | $C_{10}H_{18}O$ | 0.16 | 0.11 | 0.05 | 0.07 |

Table 1 (*Continued*)

| Retention time (min) | Name of component | Molecular formula | Relative content (%) | | | |
|---|---|---|---|---|---|---|
| | | | Yulin | Zhaoqing | Yunan | Vietnam |
| 5.37 | Benzyl alcohol | $C_7H_8O$ | – | – | – | 0.01 |
| 5.44 | *p*-Hydroxy-benzaldehyde | $C_7H_6O_2$ | 0.03 | – | 0.07 | 0.04 |
| 5.54 | Benzeneacetaldehyde | $C_8H_8O$ | 0.09 | 0.09 | 0.17 | 0.04 |
| 5.59 | 2,3-Octanedione | $C_8H_{14}O_2$ | 0.04 | – | 0.02 | – |
| 5.69 | Octanoic acid | $C_8H_{16}O_2$ | – | – | 0.22 | – |
| 5.72 | 2-Nonenal | $C_9H_{16}O$ | 0.01 | 0.03 | – | 0.01 |
| 5.76 | Acetophenone | $C_8H_8O$ | 0.22 | 0.16 | 0.34 | 0.07 |
| 5.92 | Camphenehydrate | $C_{10}H_{18}O$ | 0.18 | 0.10 | 0.06 | 0.06 |
| 5.95 | 1-Methyl-4-[1-methylethyl]-2-cyclohexen-1-ol | $C_{10}H_{18}O$ | 0.04 | 0.36 | 0.11 | 0.03 |
| 5.97 | *iso*-Thujol | $C_{10}H_{18}O$ | 0.05 | 0.05 | – | 0.01 |
| 6.05 | 4-Terpineol | $C_{10}H_{18}O$ | 0.28 | 0.21 | 0.16 | 0.25 |
| 6.09 | Borneol | $C_{10}H_{18}O$ | 2.13 | 1.24 | 0.98 | 1.13 |
| 6.12 | 2-Phenyl-1,3-butadiene | $C_{10}H_{10}$ | – | – | 0.03 | – |
| 6.21 | *exo*-Isocamphanone | $C_{10}H_{16}O$ | – | 0.09 | 0.02 | 0.11 |
| 6.26 | α-Terpineol | $C_{10}H_{18}O$ | 1.34 | 0.99 | 0.57 | 0.87 |
| 6.45 | 2-Methylcumarone | $C_9H_8O$ | – | – | – | 0.04 |
| 6.59 | Sabinol | $C_{10}H_{16}O$ | – | – | – | 0.22 |
| 6.84 | 1-Phenyl-1,2-propanedione | $C_9H_8O_2$ | – | – | 0.11 | – |
| 6.90 | Benaenpropanal | $C_9H_{10}O$ | 3.98 | 3.84 | 2.90 | 2.63 |
| 6.94 | *m*-Methylacetophenone | $C_9H_{10}O$ | – | – | 0.15 | – |
| 7.16 | Pulegone | $C_{10}H_{16}O$ | 0.05 | 0.10 | 0.07 | 0.15 |
| 7.21 | Citral | $C_{10}H_{16}O$ | 0.11 | 0.04 | 0.01 | 0.08 |
| 7.27 | Longicyclone | $C_{15}H_{24}$ | 0.10 | 0.08 | 0.26 | 0.13 |
| 7.43 | Capaene | $C_{15}H_{24}$ | 3.15 | 1.89 | 0.91 | 5.06 |
| 7.47 | Benzenepropanol | $C_9H_{12}O$ | – | – | 0.01 | – |
| 7.56 | 3-Phenyl-2-propyn-1-ol | $C_9H_8O$ | 2.80 | 2.91 | 3.19 | 2.32 |
| 7.64 | Elemene | $C_{15}H_{24}$ | 0.23 | 0.22 | 0.04 | 0.24 |
| 7.72 | Isocaryophellene | $C_{15}H_{24}$ | 0.11 | 0.08 | 0.04 | 0.19 |
| 7.95 | Farnesene | $C_{15}H_{24}$ | 0.10 | 0.10 | 0.04 | 0.05 |
| 7.98 | Cedrene | $C_{15}H_{24}$ | – | – | 0.05 | – |
| 8.70 | Cinnamaldehyde | $C_9H_8O$ | 48.36 | 55.43 | 62.05 | 50.18 |
| 8.74 | Benzenepropionic acid | $C_9H_{10}O_2$ | 0.20 | 0.31 | – | – |
| 8.77 | Cinnamyl alcohol | $C_9H_{10}O$ | 0.03 | 0.03 | 0.06 | 0.20 |
| 8.81 | Germacrene | $C_{15}H_{24}$ | 0.23 | 0.41 | 0.17 | 2.23 |
| 8.83 | Isoeugenol | $C_{10}H_{12}O_2$ | – | – | 0.04 | – |
| 8.90 | Nerolidol | $C_{15}H_{26}O$ | 0.19 | 0.23 | 0.12 | 0.19 |
| 9.01 | Sativene | $C_{15}H_{24}$ | 0.28 | – | – | 1.14 |
| 9.04 | Amorphene | $C_{15}H_{24}$ | 1.04 | 1.02 | 0.99 | 1.15 |
| 9.16 | 3-Cyclohexyl-4-peten-2-one | $C_{11}H_{18}O$ | 0.19 | 0.14 | 0.13 | – |
| 9.30 | Cadinene | $C_{15}H_{24}$ | 1.18 | 0.66 | 0.78 | 2.32 |
| 9.41 | Cubebene | $C_{15}H_{24}$ | – | 0.05 | – | 0.81 |
| 9.56 | Cinnamyl acetate | $C_{11}H_{12}O_2$ | 0.26 | 1.78 | 0.24 | 1.76 |
| 9.77 | Cinnamic acid | $C_9H_8O_2$ | 4.04 | 5.90 | 0.43 | 0.47 |
| 10.00 | Palustrol | $C_{15}H_{26}O$ | 0.25 | 0.45 | 0.45 | 0.32 |
| 10.07 | Patchulane | $C_{15}H_{26}$ | 0.15 | 0.06 | 0.05 | 0.13 |
| 10.35 | Spathulenol | $C_{15}H_{24}O$ | 0.22 | 0.35 | 0.41 | 2.12 |
| 10.45 | Decahydro-1,1,4,7-1*H*-cyclouprope a zulene-4-ol | $C_{15}H_{26}O$ | 1.08 | 0.73 | 0.12 | 0.78 |
| 10.49 | Hydroxycinnamic acid | $C_9H_8O_3$ | 0.31 | 0.31 | 0.99 | 0.79 |
| 10.55 | 1,5,5,8-Tetramethyl-12-oxabicyclo 9.1.0. dodeca-3,7-diene | $C_{15}H_{24}O$ | 0.09 | 0.09 | – | – |
| 10.74 | δ-Cadinol | $C_{15}H_{26}O$ | 1.28 | 1.44 | 1.04 | 1.48 |
| 10.78 | *o*-Methoxy-cinnamaldehyde | $C_{10}H_{10}O_2$ | 0.80 | 2.39 | 6.27 | 7.84 |
| 10.80 | Bisabolol | $C_{15}H_{26}O$ | 0.40 | 0.17 | – | – |

Table 1 (*Continued*)

| Retention time (min) | Name of component | Molecular formula | Relative content (%) | | | |
|---|---|---|---|---|---|---|
| | | | Yulin | Zhaoqing | Yunan | Vietnam |
| 10.86 | Eudesmol | $C_{15}H_{26}O$ | 0.09 | 0.24 | – | – |
| 12.33 | Pentadecanoic acid | $C_{15}H_{30}O_2$ | 0.21 | 0.03 | 1.41 | 0.46 |
| 12.76 | Benzyl benzoate | $C_{14}H_{12}O_2$ | 0.09 | 0.18 | 0.33 | 0.13 |
| 14.08 | 9-Hexadecenoic acid | $C_{16}H_{30}O_2$ | – | – | 0.32 | – |
| 14.31 | 7-Tetradecen-1-ol | $C_{14}H_{28}O$ | – | – | 0.33 | 0.19 |

*Note*: "–" means the components not determined.

determined representing about 93.39, 93.62, 92.03 and 92.59% of the total relative content, respectively (see Table 1).

## 5. Conclusions

As volatile components from traditional herbal medicines are very complex, or say a large number of chemical constituents existing and some of them with too small content, several overlapping peak clusters might present themselves in real total ionic chromatograms while conducting GC–MS analysis. Under this situation, it is necessary to detect the chromatographic peak purity, the number of chemical components and to determine whether the chromatographic elution of chemical components involved in the overlapping peak cluster investigated is characterized by the stepwise appearance or embedded peaks. Then, different chemometric resolution approaches should be employed for various chromatographic elution patterns. In this study, the combination of GC–MS with HELP and IPREP methods might be friendly used to the complicated systems such as *C. cinnamomi*. With the help of the combined approach, much more volatile components could be separated and qualitatively and quantitatively determined correctly, suggesting the enhancement of the chromatographic separation and spectral qualitative determination ability. This way will surely show the prosperous prospect for analysts to directly address very difficult problems in analytical chemistry.

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