Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1016 (2003) 99–110
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of the volatile chemical constituents of *Notoptergium incium* by gas chromatography–mass spectrometry and iterative or non-iterative chemometrics resolution methods

Fang-Qiu Guo, Yi-Zeng Liang*, Cheng-Jian Xu, Lan-Fang Huang

College of Chemistry and Chemical Engineering, Research Center for Modernization of Chinese Herbal Medicine, Central South University, Changsha 410083, PR China

Received 18 March 2003; received in revised form 14 July 2003; accepted 23 July 2003

Abstract

The qualitative and quantitative determination of the chemical constitutes in traditional Chinese medicine (TCM) is an important task, which builds the foundation of the theory of pharmacological activity. The hyphenated chromatography instruments combined with the related chemometric methods provide powerful tools for the resolution of such complex systems. The familiar chemometrics methods can be roughly divided into two different kinds, the iterative one such as orthogonal projection approach (OPA) and non-iterative one representing by evolving window orthogonal projection (EWOP). One can use different kinds of methods according to overlapping condition, and then the measured data matrix can be resolved into pure concentration profiles and mass spectra of the chemical components with relative high efficiency and acceptable accuracy. One kind of TCM, named *Notoptergium incium* (NI) was analyzed by gas chromatography–mass spectrometry (GC–MS) and resolved by above chemometric approach. Experiment results show the efficiency and convenience of the proposed approach. 65 of the 98 separated constituents in essential oil, accounting for 92.13%, were identified by mass spectroscopy (MS).

Keywords: Evolving window orthogonal projection; Orthogonal projection approach; Essential oils; Chemometrics; *Notoptergium incium*

1. Introduction

Historically, especially in Asian areas, traditional Chinese medicines (TCMs) have played an important role in clinical therapy. Because of their high pharmacological activity, low toxicity and rare complication [1], more and more interests have been re-attracted in recent years. However, building the theory on pharmacological activity of TCMs is not an easy job. To our knowledge, there are mainly two reasons, firstly, the TCMs are indeed very complicated systems, for example, even a kind of TCM for treating a common cold contains hundreds of chemical components. Analyzing such complex systems is indeed a challenge to analytical researchers. Secondly, in contrast to western medicine, the fundamental pharmacological activity of TCM is synergetic effect, which is also difficult to deal with. Therefore, building a database of

* Corresponding author. Tel.: +86-731-8822841; fax: +86-731-8825637.
E-mail address: yzliang@public.cs.hn.cn (Y.-Z. Liang).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0021-9673(03)01327-X

composition of different TCMs is necessary and important.

Chromatography is a powerful tool for analyzing complicated system as TCM. In classical chromatographic analysis, the identification and quantitative analysis of components in the system can be achieved by optimizing the separation condition repeatedly and if all the standards of analysts are available. However, even a TCM for treating the common cold contains hundreds of chemical components, it is very difficult to analyze such complex systems unless baseline separation conditions are achieved, which is usually an arduous job [2]. Fortunately, recent developed hyphenated chromatographic instruments and fast-growing chemometrics resolution methods provide powerful tools to accomplish this job. Current approaches available in chemometrics can be roughly divided into iterative or non-iterative one [3]. Examples of iterative resolution methods are iterative transformation factor analysis (ITTFA) [4], orthogonal projection approach (OPA) [5], and elementary matrix transformation (IMEMT) [6]. The corresponding extra information needed for the iterative methods is the non-negativity and unimodality constraints. These methods are often automatic and easy to use, but sometimes have convergent problems.

The second group of the resolution methods can be regarded as evolving methods. The examples are evolving factor analysis (EFA) [7], window factor analysis (WFA) [8], heuristic evolving latent projections (HELP) [9,10], subwindow factor analysis [11], and evolving window orthogonal projection (EWOP) [12]. The feature of this kind of resolution methods is their using the informative "windows". These methods have been applied to solve many real problems successfully [13–15]. These local-rank methods are efficient, but usually require an experienced user.

Extracting the desired chemical information from huge amounts of data when evolving method is employed usually takes experienced users a long working hours. However, the resorts to iterative method are also not always successful, especially in some strongly overlapped, or collinearity condition. In this paper, we combine the advantage of two groups of methods. An overlap index (OVI) [16] is adopted here to evaluate the separation quality of different peak cluster in two-dimensional hyphenated chromatography. In general, when separation performances is good, an iterative method OPA is employed to resolving the mixture system, and when separation performances is not good, and here OPA is sometimes not suitable, an evolving method EWOP is manipulated to analyzing sub-system in *Notoptergium incium* (NI).

NI collected in Chinese pharmacopoeia is the dry root and rootstock of *Notoptergium incium* ting ex H.T. Chang and *N. forbessii* Boiss. It is a traditional Chinese medicinal herb, which has been used as a diaphoretic, an anti-febrile and an anodyne [17,18]. There are lots of volatile constituents, which have the function of analgecize and inflammation resistance in NI.

2. Theory

When a set of samples is measured, the data can always be collected in a matrix $\boldsymbol{X}$, with every row representing an object (spectrum of a sample) and every column a variable (chromatogram at some wavelength, wave-number or *m*/*e* unit, or generally, a concentration profile). According to the Lambert–Beer Law or the similar, the measured matrix $\boldsymbol{X}$ can be expressed as a product of two matrices:

$$\boldsymbol{X} = CS^{\mathrm{T}} + \boldsymbol{E} \tag{1}$$

Here, $\boldsymbol{C}$ and $\boldsymbol{S}$ are pure concentration profiles and spectra respectively, the superscript T denotes the matrix or vector transposition, and $\boldsymbol{E}$ is the array of measuring noise. One can identify the chemical constituents from measured matrix $\boldsymbol{X}$. The chemometric resolution approach used here can be described by the following five steps:

1. Pretreatment of the original matrix. Firstly, the measured matrix $\boldsymbol{X}$ is divided into different submatrices corresponding to baseline separated peaks and peak clusters. Secondly, to avoid the pitfalls of background and baseline shift in measured data, it is necessary to detect the background, and correct background and baseline shift.
2. Evaluate the separation quality of each peak clusters, and overlap index ($\mathrm{OVI}_{\mathrm{unknown}}$) can be expressed by the following equation:

$$\mathrm{OVI}_{\mathrm{unknown}} = -\log\det\left(\frac{(\boldsymbol{A}_i\boldsymbol{A}_i^{\mathrm{T}})}{n}\right). \tag{2}$$

Here, $\boldsymbol{A}_i$ is the normalized key spectral matrix [16], det(·) the determinant of a matrix and n denotes the number of chemical components in the peaks clusters, which can be obtained by factor analysis methods [19,20].

3. Two kinds of methods, iterative one, OPA or non-iterative one EWOP are used here to solve the analytical systems, respectively.

   OPA is a stepwise approach based on an orthogonalization approach developed by Sanchez et al. [5]. There are four steps of the method.

   (i) Initially, the dissimilarity of each spectrum with respect to the mean spectrum is calculated.
   (ii) The concentration profiles are then determined by least squares.
   (iii) New estimates for the individual pure spectra are determined by least-squares.
   (iv) The sum of squares of the residuals, SSR, is calculated, steps 2–4 are repeated until the relative differ in the SSR between two successive iteratives is lower than a pre-defined threshold, typically set to 0.1%. The extensive details about OPA can be seen in [5].

   EWOP is an evolving method proposed by Xu et al. [12] and the procedure of this method can be arrived at the following steps.

   (i) With evolving factor analysis the selective regions for each component in the cluster are determined (where there is only one singular value) and the corresponding component spectra are extracted by PCA for each of the selective regions.
   (ii) The spectra within a small moving window within the cluster are linear combinations of one or more component spectra.
   (iii) For one component at a time, the extracted normalized spectrum is projected to be orthogonal component in relation to the components within the window. This corresponds to the net analyte signal for the selected component in relation to the components within the window. The length of the projection (the "net analyte spectrum") is a scalar that in a way shows how much of the extracted spectrum that is left after projection.
   (iv) When this scalar (in the range of 0–1) is plotted versus the position of moving window, one obtains the zero concentration graph (ZCG). As long as the component is present within the window, the orthogonal projection has zero length and the scalar value is likewise zero. Other regions (windows with significant non-zero values) defines the regions where the component is absent, but to be a "zero component region" for that component it must contain at least one other co-elution component as found from step (i).
   (v) Next, the extracted spectrum for the first eluting component is projected to be orthogonal to the spectra within its zero component region, and the measured spectra in the elution region for the component (zero value in the ZCG) are correlated to this projection. The result is a measure the concentration profile obtained from the net analyte signal that is unaffected by the co-eluting components. But, the profile has to be rescaled by dividing with the measured data matrix can be modeled as the outer product of the concentration profile and the extracted spectrum.
   (vi) The contribution from the extracted component is subtracted from the data matrix and the procedure is repeated from (v) until the concentration profiles of all components within the cluster are extracted.

4. Verify the reliability of the resolution results of two kinds of methods by comparison of two sets of spectra.

   Suppose the pure spectra obtained by OPA and EWOP is $\boldsymbol{s}_{\mathrm{OPA},i}$ and $\boldsymbol{s}_{\mathrm{EWOP},i}$, respectively, and then the similarity of spectra can be obtained by calculating:

$$\mathrm{SI} = \sum_{i=1}^{n} \frac{\boldsymbol{s}_{\mathrm{OPA},i}^{\mathrm{T}} \boldsymbol{s}_{\mathrm{EWOP},i}}{n} \quad i = 1, \ldots, n. \tag{3}$$

   Here, n is component number in the system.

5. Qualitative analysis is performed by similarity searches in the NIST mass spectral library. Quantitative results are obtained by calculating the volume of total two-way response.

3. Experimental

3.1. Reagents

Individual herb was purchased from Changsha Zhiling pharmaceutical store market, and it was identified to be the dry root and rootstock of *Notoptergium incium* ting ex H.T. Chang by a researcher from institute of materia medica, hunan academy of traditional Chinese medicine and materia medica.

3.2. Extraction of the essential oil

The essential oil was prepared according to the Chinese pharmacopoeia (Chinese pharmacopoeia committee publishing house of people's Health, 2000, Appendix 64) [21], 5×10^{-2} kg NI powder was put into extract apparatus and subjected to hydro-distillation for 16 h, 1.9×10^{-6} m^3 canary clear oil-like essential oil gained, the yield (v/w) of the sample was 3.8%, the obtained essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analysis.

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 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 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, ionization source temperature were 280 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 (NIST), which contains 107886 compounds.

4. Results and discussion

4.1. Evaluation of separation quality

The total ion chromatogram (TIC) of essential oil of NI is shown in Fig. 1, and it is indeed a complicated mixture. Some of the chromatographic peaks reach baseline separation, but the other peaks overlap with each other. Furthermore, some peaks, which seem to be single component, are actually several compounds co-eluting. There are 46 roughly detached peaks apparently, and then the measured matrix $\boldsymbol{X}$ is divided into 46 submatrices by zero component regions along elution sequence. Among these 46 peaks clusters, according to eigenvalue analysis, there are 19 single component peaks, which can be easily identified and quantified by chromatography researchers. Beside, there are still 27 overlap peaks. How to resolve these overlapped peaks with high efficiency and accepted accuracy? Firstly, to avoid effect of background and baseline shift in measured data, it is necessary to remove background and baseline shift. Secondly, one can calculate OVI values of each submatrix, to obtain the overlap degree of each peak cluster. The OVI value is related to the overlapping degree of chromatography profiles, in the meantime, it can also reflect the degree of co-linearity of spectra in the overlapped peaks. Table 1 shows the overlap index of each sub-system. Finally, the sub-systems are resolved by two kinds of method, iterative one OPA and/or non-iterative one EWOP, respectively.

To our knowledge and experience, when the clusters are slightly overlapped, both the iterative and non-iterative methods can provide a satisfactory result. However, when the clusters are heavily overlapped, the manual non-iterative method is preferred. At this time, careful elution window identification is usually needed.

4.2. Resolution and comparison

In OPA, only one parameter, that is component number of peak clusters, should be defined beforehand. The dissimilarity plot of the OPA method could give an indication of the number of the peak cluster.

In EWOP, the selective region and zero concentration region of certain component is first identified, and then chromatographic profile and spectra can be

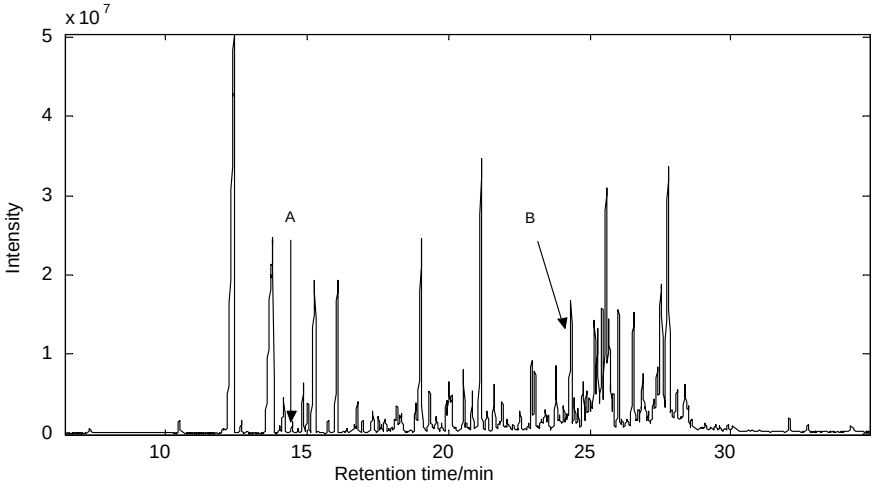

Fig. 1. The total ion chromatogram of the essential oil from NI.

obtained by local orthogonal projection. Here, the zero concentration graph (ZCG) is proposed to identify the elution window with little trial.

Table 1 shows that the similarity of spectra obtained from two methods. In most cases, the spectra obtained from two methods are very similar, especially in slightly overlapped condition. At this time, the OPA algorithm is preferred in practice for easy use and automatic realization.

When the overlap index is bigger, i.e. the eluting component is often strongly overlapped with each other. At this time, big difference will appear between spectra obtained by the two methods.

Three problems might appear when using OPA method for resolution. Firstly, the method sometimes does not converge. For instance, there are four cases, in which the OPA did not converge in this study (as indicated in Table 1). Secondly, the resolved chromatogram and spectra obtained by OPA will not meet the common chemically sensible criteria, for instance, un-unimodel of chromatogram. Thirdly, for some cases of strong overlapping between the spectra and/or chromatograms of the pure components, the resolution results became unaccepted. When the above-mentioned problems appeared, we might resort to other non-iterative methods.

The chromatographic segment (13.9–14.4 min) is taken as an example to demonstrate the whole procedure of our approach. Fig. 2 is a section of chromatogram from 13.9 to 14.4 min (named peak cluster A). It can be seen that the frontal small peak is not baseline separated from the latter extend forward bigger peak. If relevant resolution technique is not used, one had to resort to search directly from mass library. By this the front small peak might be 2-*n*-pentylfuran or 2,4-*trans*,*trans*-nonadienal with almost the same similarity degree of 0.90. Obviously, it is very difficult to determine which one is the correct component. The later bigger peak is not a single component because it is provided with different spectra at different position of the peak. It seems like an overlapping peak of two components. The former part looks like beta-myrcene

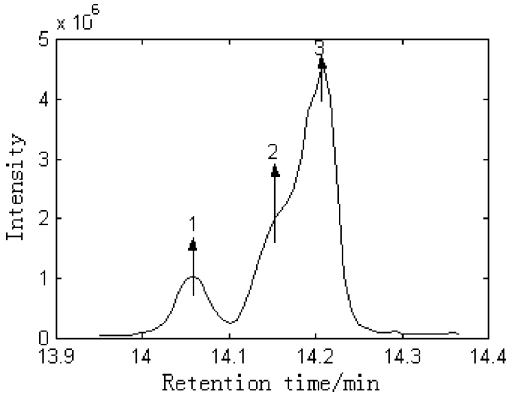

Fig. 2. A section of chromatogram from 13.9 to 14.4 min.

Table 1
Separation quality and similarity of OPA and EWOP

Scan point	Number of components	Separation quality	Similarity
5.775–5.945	1		
10.352–10.689	1		
12.021–12.514	1		
12.605–12.774	1		
13.437–13.932	1		
13.938–14.270	3	0.6427	0.9991
14.273–14.602	1		
14.606–14.772	1		
14.768–14.935	1		
14.935–15.114	1		
15.114–15.354	2	0.4592	0.9978
15.691–15.854	1		
15.857–16.186	1		
16.520–16.894	5	1.2002	0.9519
16.894–17.063	1		
17.107–17.441	2	0.9242	0.9980
17.441–17.684	2	0.3010	0.9978
17.684–17.854	3	0.9122	0.9412
18.021–18.476	4	2.6762	No convergence[a]
18.688–19.186	3	0.5214	No convergence
19.186–19.439	1		
19.439–19.688	2	0.3010	0.9725
19.857–20.270	3	1.022	0.9923
20.437–20.689	1		
20.771–20.934	1		
21.023–21.268	1		
21.268–21.475	2	0.6106	0.9923
21.520–21.731	3	0.5733	0.9838
21.731–22.229	4	0.7107	0.9941
22.356–22.601	2	0.3408	0.9846
22.853–23.184	4	1.5020	0.9897
23.607–24.018	2	0.3065	0.9894
24.018–24.395	4	1.082	No convergence
24.395–24.646	2	0.3010	0.9997
24.646–24.853	2	0.5288	0.9992
24.853–25.023	3	0.9486	0.9970
25.023–25.354	3	0.6325	0.9569
25.354–25.522	1		
25.482–25.812	3	0.5667	0.9999
26.350–26.688	2	0.8156	0.9997
26.770–27.025	4	2.7258	0.9430
27.271–2935	4	1.030	No convergence
27.608–27.940	3	1.6259	0.9702
27.940–28.688	3	0.7697	0.9875
31.939–32.188	1		
34.104–34.355	1		

[a] No convergence by OPA.

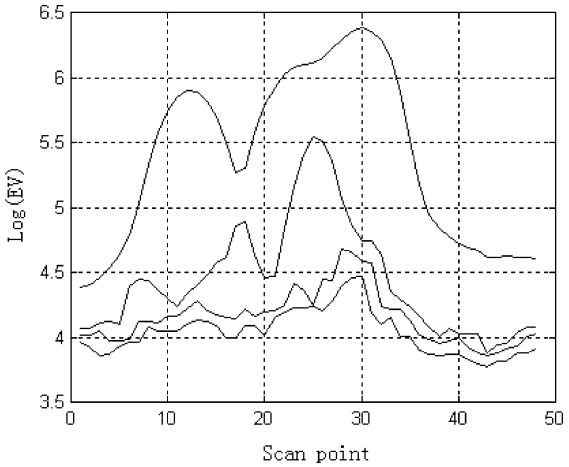

Fig. 3. The evolving eigenvalues obtained using FSMWEFA with a window size of 4 of peak cluster A.

or *trans*,*cis*-2,6-monadienal with almost the same similarity of 0.85, and the hinder part looks like octanal with similarity of 0.82. From the results obtained so far, the conclusion of what components are involved in this peak cluster is really difficult to reach. Furthermore, the qualitative results are irresponsible and inaccurate. Thus, the quantitative analysis of this peak cluster seems also impossible, because the area of each component is not known.

In this paper, OPA and EWOP are used to resolve the overlapping peak. At first, it is necessary to determine the number of the components in the peak cluster, the elution sequence of each component is estimated by fixed size moving window evolving factor analysis (FSMWEFA) [22], the rank map of window size of 4 derived from FSMWEFA is shown in Fig. 3. It is an information distribution graph of the component in the time direction the map tells the local rank in the elution sequence. If the local rank is one, there is one component, and if the local rank is two, there are two components co-eluting in this region. The stepwise eluting information of chemical components in peak cluster A can be further confirmed by evolving latent projection graph (ELPG) [9,10,23]. Fig. 4 shows the ELPG of peak cluster A, the straight lines pointing to the origin represent pure regions of the components. It can be also seen that it is a three-component system. The components will be marked by numbers 1, 2 and 3, according to their elution sequences from the plot.

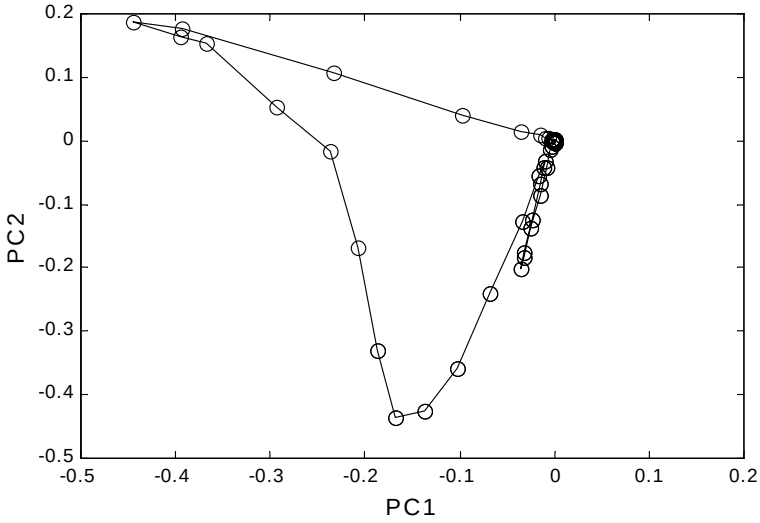

Fig. 4. Evolving latent projection graph (ELPG) for peak cluster A.

According to Table 1, OVI of peak cluster A is 0.64, the pure spectra and concentration profiles of the peak cluster can be derived by OPA easily. Spectra at the retention tine at of 14.06, 14.15 and 14.21 min were selected as key spectra 1, 2 and 3, which was shown in Fig. 2.

It can also resolved by EWOP, firstly, their pure spectra are extracted, and then each component in the cluster can be identified by similarity searches in the NIST mass library. The quantitative results can be calculated in case the concentration profiles are obtained.

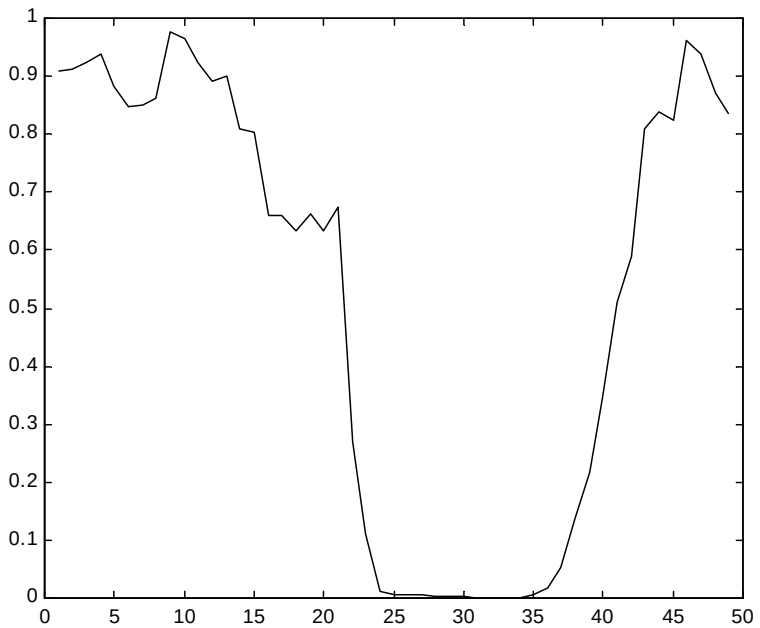

Fig. 5. Zero-concentration graph with a window size of 4 for component 3.

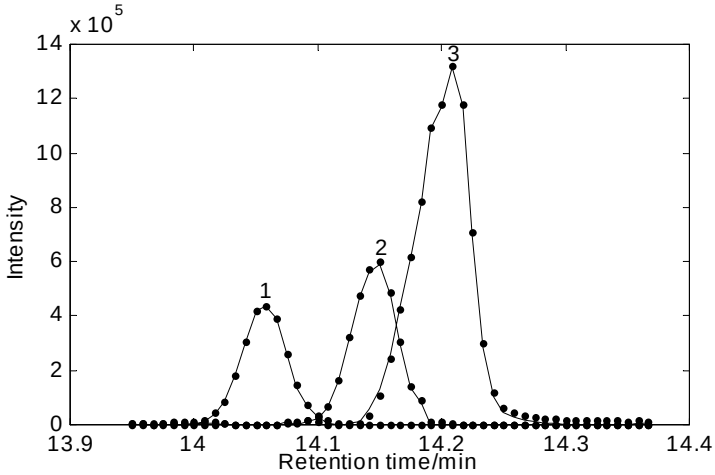

Fig. 6. The resolved chromatogram of cluster A by EWOP and OPA: dotted line denotes OPA and solid line, EWOP.

Zero concentration graph for a particular component, is obtained by projecting the pure mass spectra to orthocomplemental space created by moving alone the retention time. Fig. 5 shows the ZCG with a window size of 4 for component 3, in which the range where ordinate is zero is its elution range, i.e. 21–42 (scan point) in the plot. Moreover, the plot can also provide the information of zero concentration region of component 3. As seen in the plot, the ranges are 5–21 and 42–50, respectively. Accurate ascertain of zero concentration is in favor of stripping and resolution of the component afterwards. The chromatographic profiles can be obtained by local orthogonal projection. The chromatogram resolved of cluster A is shown in Fig. 6 (expressed by solid line). The residual is about two magnitudes less than the content of each component, and the residual representing noise level is comparatively higher at the position with higher content. It indicates that heteroscedastic noise is effectively avoided by using EWOP. The resolved chromatograph is shown in Fig. 6 (expressed by dotted line). It is shown that the result derived by EWOP is primarily the same as that of OPA. After obtaining the pure mass spectra, the similarity searches in the NIST mass library for the three resolved components are conducted. They may be 2-*n*-pentylfuran, beta-myrcene, and octanal with the correlation coefficient of 0.956,

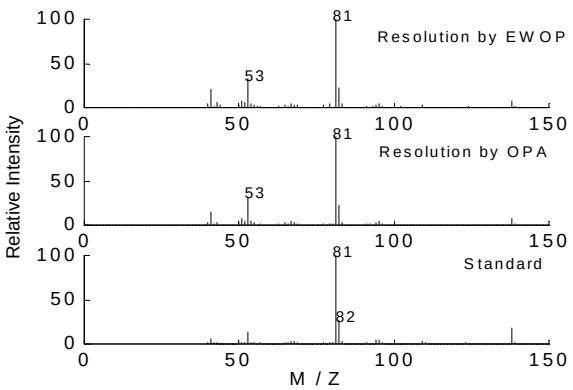

Fig. 7. Standard mass spectrum of 2-*n*-pentylfuran ($C_9H_{14}O$(138)) and resolved mass spectrum of component 1 by EWOP and OPA.

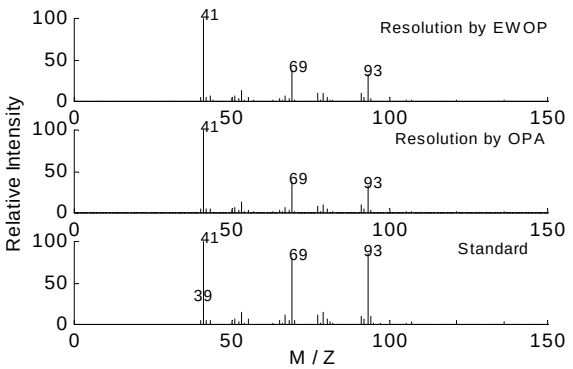

Fig. 8. Standard mass spectrum of beta-myrcene $C_{10}H_{16}$(136) and resolved mass spectrum of component 2 by EWOP and OPA.

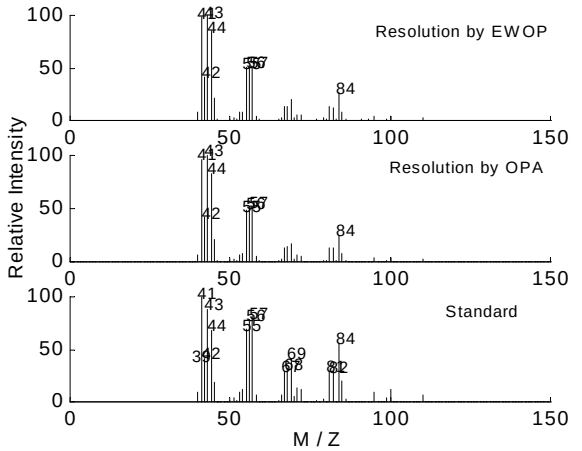

Fig. 9. Standard mass spectrum of octanal $C_8H_{16}O$(128) and resolved mass spectrum of component 3 by EWOP and OPA.

0.934 and 0.964, respectively. Compared with the results of direct search mentioned above, the correlation coefficients such obtained have been improved greatly. The resolved spectra by OPA and EWOP and standard mass spectra of the three components are displayed in Figs. 7–9, respectively. The similarity of the two methods achieves 0.9991, which might also indicate that the results obtained are more credible.

Although most of the overlapped peaks can be resolved by OPA easily, the data of some peaks with poorer separation quality or by the reason of co-linearity of spectra cannot converge to true value, hence lead to inaccurate results. The cluster of 24–24.45 min is such an example, which is marked as B in Fig. 1. It can be resolved by EWOP, and the resolved chromatogram is shown in Fig. 10. The comparative results are shown in Table 1 and the qualitative results are shown in Table 2. Ninty-eight constituents are resolved, and 65 components are identified. Unfortunately, 33 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. We will keep the data in case better methods and bigger library are available. Most of them are

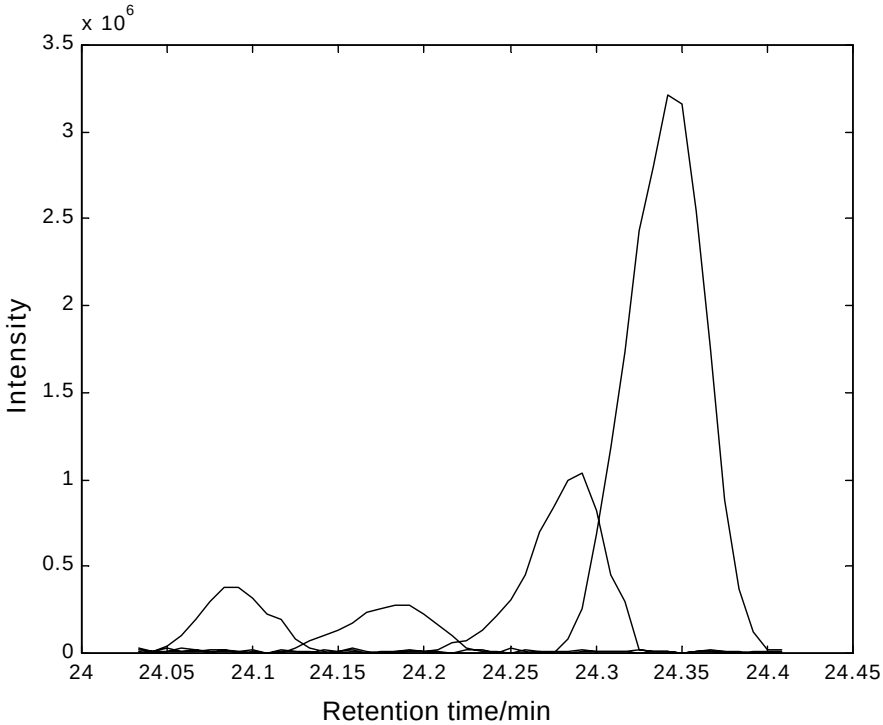

Fig. 10. The resolved chromatogram of peak cluster B.

Table 2
Composition of the essential oil from NI

Number	Retention time (min)	Name of component	Molecular formula	Relative content (%)
1	5.875	Hexanal	$C_6H_{12}O$	0.04
2	10.538	*n*-Heptanal	$C_7H_{14}O$	0.30
3	12.383	α-Pinene	$C_{10}H_{16}$	15.06
4	12.692	Santolina triene	$C_{10}H_{16}$	0.67
5	13.710	β-Pinene	$C_{10}H_{16}$	9.78
6	14.050	2-*n*-Pentylfuran	$C_9H_{14}O$	0.11
7	14.128	β-Myrcene	$C_{10}H_{16}$	0.59
8	14.200	*n*-Octaldehyde	$C_8H_{16}O$	0.16
9	14.485	α-Thujene	$C_{10}H_{16}$	0.17
10	14.693	Ocimene	$C_{10}H_{16}$	0.06
11	14.872	(+)-2-Caren	$C_{10}H_{16}$	0.77
12	15.044	*o*-Cymene	$C_{10}H_{14}$	0.72
13	15.198	Sabinene	$C_{10}H_{16}$	2.83
14	15.310	α-Limonene	$C_{10}H_{16}$	2.00
15	15.750	Ocimene	$C_{10}H_{16}$	0.16
16	16.031	γ-Terpinene	$C_{10}H_{16}$	3.23
17	16.672	Decanal	$C_{10}H_{20}O$	0.09
18	16.688	2-Nonanone	$C_9H_{18}O$	0.14
19	16.786	4-Methyl-3-[1-methylethylidene]cyclohexene	$C_{10}H_{16}$	0.45
20	16.975	2-Decen-1-ol	$C_{10}H_{20}O$	0.18
21	17.534	6-Camphenol	$C_{10}H_{16}O$	0.14
22	17.641	2-Decyne-1-ol	$C_{10}H_{18}O$	0.23
23	18.281	[*E*]-3[10]-Caren-4-ol	$C_{10}H_{16}O$	0.23
24	18.345	*trans*-2-Nonen-1-al	$C_9H_{16}O$	0.48
25	18.842	Artemiseole	$C_{10}H_{16}O$	0.62
26	19.030	4-Terpineol	$C_{10}H_{18}O$	4.85
27	19.32	*p*-Menth-1-en-8-ol	$C_{10}H_{18}O$	0.94
28	19.575	Myrtenol	$C_{10}H_{16}O$	0.26
29	20.542	[*Z*]-2-Decenal	$C_{10}H_{18}O$	0.83
30	20.852	4-[1-Methylethyl]-1-cyclohexene-1-carboxaldehyde	$C_{10}H_{16}O$	0.64
31	21.151	Bornyl acetate	$C_{12}H_{20}O_2$	5.54
32	21.619	[*E*,*E*]-2,4-Decadienal	$C_{10}H_{16}O$	0.70
33	21.891	*p*-Vinylguaiacol	$C_9H_{10}O_2$	0.73
34	22.495	[*E*]-2-Tetradecene	$C_{14}H_{28}$	0.28
35	22.554	α-Cubebene	$C_{15}H_{24}$	0.13
36	22.908	2,4-Di-ter-butyl-thiophene	$C_{12}H_{20}S$	0.98
37	22.941	Acetic acid, undec-2-enylester	$C_{13}H_{24}O_2$	0.71
38	23.003	Dihydrocarveol acetate	$C_{12}H_{20}O_2$	0.36
39	23.790	β-Sesquiphellandrene	$C_{15}H_{24}$	0.98
40	24.080	α-Chamigrene	$C_{15}H_{24}$	0.27
41	24.285	β-Farnesene	$C_{15}H_{24}$	0.54
42	24.341	[−]-Isoaromadendrene[V]	$C_{15}H_{24}$	2.12
43	24.451	Caryophyllene	$C_{15}H_{24}$	0.41
44	24.580	β-Cubebene	$C_{15}H_{24}$	0.32
45	24.765 min	Isoledene	$C_{15}H_{24}$	0.54
46	24.867	β-Chamigrene	$C_{15}H_{24}$	0.47
47	24.964	Acetic acid, undec-2-enylester	$C_{13}H_{24}O_2$	0.34
48	25.167	α-Muurolene	$C_{15}H_{24}$	1.36
49	25.275	β-Farnesene	$C_{15}H_{24}$	1.86
50	25.432	Cedrene	$C_{15}H_{24}$	1.69
51	25.583	Germacrene D	$C_{15}H_{24}$	5.10
52	25.650	*trans*-2-α-Bisabolene epoxide	$C_{15}H_{24}O$	1.93

Table 2 (*Continued*)

Number	Retention time (min)	Name of component	Molecular formula	Relative content (%)
53	25.704	Di-epi-α-cedrene	$C_{15}H_{24}$	0.34
54	26.491	Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	$C_{15}H_{26}O$	0.91
55	26.558	[−]-Spathulenol	$C_{15}H_{24}O$	1.74
56	26.800	*trans-Z*-α-Bisabolene epoxide	$C_{15}H_{24}O$	0.76
57	27.366	Cubenol	$C_{15}H_{26}O$	0.84
58	27.507	Tau-Muurolol	$C_{15}H_{26}O$	3.83
59	27.743	β-Eudesmol	$C_{15}H_{26}O$	5.32
60	27.790	α-Cadinol	$C_{15}H_{26}O$	1.51
61	27.816	β-Eudesmol	$C_{15}H_{26}O$	1.28
62	28.077	α-Bisabolol	$C_{15}H_{26}O$	0.77
63	28.352	Isogeraniol	$C_{10}H_{18}O$	0.48
64	32.032	Palmitic acid	$C_{16}H_{32}O_2$	0.22
65	34.221	9,12-Octadecadienal	$C_{18}H_{32}O$	0.04

monoterpene, monoterpene alcohol and sesquterpene alcohol species compound.

4.3. Quantitative analysis

With the pure chromatographic profile and mass spectrum obtained for each component, the total two-way response of each component can be obtained from the outer product of the concentration vector and the spectrum vector for each component. The total relative 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. Quantitatively analyzed representing about 92.13% of the total content. The final relative quantitative results are listed in Table 2.

5. Conclusion

In general, when separation performances is good, an iterative method OPA is employed to resolving the mixture system, and when separation performances is not good, the manual method, such as EWOP is used to solve the measure data. Such approaches will greatly alleviate the burden of chromatographic separation in conventional chromatographic analysis. Moreover, the proposed approaches can yield good solutions with acceptable accuracy and high efficiency. It will also be a fundamental work in building large TCM constitute database, and then the chemical knowledge of TCM can be acquired more easily than hitherto.

Acknowledgements

Financial support from National Natural Science Foundation of PR China (Grant nos. 20175036 and 20235020) is gratefully acknowledged.

References

[1] K.C. Wen, C.Y. Huang, F.L. Lu, J. Chromatogr. 631 (1993) 241.
[2] C.J. Xu, Y.Z. Liang, Y.Q. Song, J.S. Li, Fresenius J. Anal. Chem. 371 (2001) 331.
[3] Y.Z. Liang, O.M. Kvalheim, Fresenius J. Anal. Chem. 370 (2001) 694.
[4] P.J. Gemperline, J. Chem. Inf. Comput. Sci. 24 (1984) 206.
[5] F.C. Sanchez, S.C. Rutan, M.D.G. Garcia, D.L. Massart, Chemom. Intell. Lab. Syst. 36 (1997) 153.
[6] R. Manne, B.V. Grande, Chemom. Intell. Lab. Syst. 50 (2000) 35.
[7] M. Maeder, A. Zilian, Chemom. Intell. Lab. Syst. 3 (1988) 205.
[8] E.R. Malinowski, J. Chemom. 6 (1992) 29.
[9] O.M. Kvalheim, Y.Z. Liang, Anal. Chem. 64 (1992) 936.
[10] Y.Z. Liang, O.M. Kvalheim, H.R. Keller, D.L. Massart, P. Kiechle, F. Erni, Anal. Chem. 64 (1992) 946.
[11] R. Manne, H.L. Shen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 171.
[12] C.J. Xu, J.H. Jiang, Y.Z. Liang, Analyst 124 (1999) 1471.
[13] F. Gong, Y.Z. Liang, H. Cui, F.T. Chau, B.T.P. Chan, J. Chromatogr. A 905 (2001) 193.
[14] Z. Zhao, E.R. Malinowski, Anal. Chem. 71 (1999) 602.

[15] H.L. Shen, Y.Z. Liang, R.Q. Yu, X.C. Li, X.X. Sun, Sci. China (Part B) 41 (1998) 21.
[16] F. Gong, Y.Z. Liang, Q.S. Xu, F.T. Chau, K.M. Ng, Anal. Chim. Acta 450 (2001) 99.
[17] M. Kozawa, M. Fukumoto, Y. Matsuyama, K. Baba, Chem. Pharm. Bull. 31 (1983) 2712.
[18] Z.M. Gu, D.X. Zhang, X.W. Yang, M. Hattori, T. Namba, Chem. Pharm. Bull. 38 (1990) 2498.
[19] E.R. Malinowski, Factor Analysis in Chemistry, second ed., Wiley, New York, 1991.
[20] C.J. Xu, Y.Z. Liang, Y. Li, Y.P. Du, Analyst 128 (2003) 75.
[21] Chinese Pharmacopoeia Committee, Chinese Pharmacopoeia, Publishing House of People's Health, 2000, Appendix 64.
[22] H.R. Keller, D.L. Massart, Anal.Chim. Acta 246 (1991) 379.
[23] Y.Z. Liang, O.M. Kvalheim, A. Rahmani, R.G. Brereton, J. Chemom. 7 (1993) 15.