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Determination of volatile components in peptic powder by gas chromatography–mass spectrometry and chemometric resolution

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Abstract

Gas chromatography–mass spectrometry (GC–MS) coupled with chemometric resolution upon two-dimensional data was proposed as a method for the analysis of volatile components in a traditional Chinese medicinal preparation peptic powder which contains *Rhizoma Atractylodis*, *Pericarpium Citri Reticulatae*, *Cortex Magnoliae Officinalis* and *Radix Glycyrrhizae*. Ninety-three components were separated and 65 of them were qualitatively and quantitatively analyzed which represented about 90.28% of the total content. With the help of chemometric resolution, the data were resolved into a pure chromatogram and a mass spectrum of each chemical component. The accuracy of qualitative and quantitative results was greatly improved by using the two-dimensional comprehensive information of chromatograms and mass spectra. The example showed that chemometric resolution could greatly enhance separation ability. This makes it possible to analyze complicated practical systems like traditional Chinese medicinal preparations with the help of coupled instruments and chemometric resolution methods.

Keywords: Chemometrics; Pharmaceutical analysis; Peptic powder; Oils; Volatile organic compounds

1. Introduction

There is a growing interest in traditional Chinese medicine (TCM) in recent years because of the advantages of low toxicity, rare complications [1] and pharmacological activity. Qualitative and quantitative studies on the constituents of TCM have generally started from the examination of components in a single herb and then proceeded with the analysis of concentrated herbal preparations [2–7]. However, traditional Chinese medicinal preparations usually contain too many components and form a very complex system. Usually, only the so-called fingerprint plots of the herbs are obtained. Therefore, new analysis methods should be developed for quality control purpose of the herbs and Chinese medicinal preparations. With the advance of coupled methods, such as GC–MS, LC–MS, HPLC–DAD (diode array detection) and CE–DAD, two-dimensional data are available to provide more information

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for the same chemical analysis. Besides the retention time, peak height and peak area information from the chromatographic technique, the spectral information from mass selective detector or diode array detector enhances identification of components from both the spectral and chromatographic directions. The evolving approaches recently developed in chemometrics, such as evolving factor analysis (EFA) [8–10], window factor analysis (WFA) [11,12], heuristic evolving latent projections (HELP) [13–15], orthogonal projection resolution (OPR) [16], and subwindow factor analysis (SFA) [17,18], have progressed rapidly. Since all the methods mentioned above are essentially window-dependent methods, the chemometric methods how to define reasonably the elution windows of the components were also developed quickly [19–22]. As a result, hyphenated instruments coupled with chemometric resolution make it possible to quantify one complicated system clearly and accurately, since the chromatographic profiles of chemical components possess an advantage of first-in-first-out, which makes it possible to resolve them in an evolving way. Such methods have been applied to many fields [23–28].

Peptic powder is an ancient concentrated preparation in China [29]. It can clear away pathogenic dampness, moisturize spleen, promote the circulation of *qi* and regulate the stomach. This formulation is composed of four single herbs. They are *Rhizoma Atractylodis* (atractylodes rhizome), *Pericarpium Citri Reticulatae* (tangerine peel), *Cortex Magnoliae Officinalis* (magnolia bark) and *Radix Glycyrrhizae* (licorice root) [30]. The volatile constituents of peptic powder are pharmacological active.

Some work has been done upon the essential oil composition of the former three single herbs with GC or GC–MS [31–33]. The identification of components is conducted only with retention indices or through the direct similarity searches in the MS library. Thus, only very few chemical components could be identified, since the overlapping chromatographic peaks always present a problem for similar search from the MS library. As a result, the qualitative and quantitative results are not reliable. Unfortunately, this case inevitably occurs for a complicated system even under good separating conditions and optimization of chromatographic separating conditions is not a trivial task at all.

In this paper, the volatile components of peptic powder were studied with GC–MS, followed by the resolution of the two-dimensional data with the HELP method. Mass spectra of each component become accessible through unique resolution. Then, qualitative identification of these constituents was performed using retention times and mass spectra. Next, the quantitative analyses were carried out with the overall volume integration method [23–25].

2. Theory

A two-dimensional data $\mathbf{A}_{m\times n}$ produced by GC–MS can be represented as follows:

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

where $\mathbf{A}_{m\times n}$ denotes an absorbance matrix expressing N components of m chromatographic scan points at n atom mass units or wavelength points. **C** is the pure chromatographic matrix, and **S** is the pure mass spectral matrix. **E** represents the noise. t is the transform of matrix **S**. The unique resolution of a two-dimensional data into chromatograms and spectra of the pure chemical constituents is carried out with local full rank analysis in the HELP method. Only concise theoretical explanation is showed here for the sake of brevity, while detail description could be found in Refs. [13–15].

1. Confirm the background and correct a drifting base line.
2. Determine the number of components, the selective region and zero-component region of each component by the use of the evolving latent projective graph and rankmap on the basis of the eigenstructure tracking analysis.
3. With the help of the selective information and zero-component region, conduct a unique resolution of two-dimensional data into pure chromatographic profiles and mass spectra by means of local full rank analysis.
4. Verify the reliability of the resolved result.

After the pure chromatogram and spectrum of the ith component have been resolved, this component can be determined qualitatively by comprehensive use of the chromatographic retention time and mass

spectrum. Next, the term $\mathbf{c}_i\mathbf{s}_i^t$ in Eq. (1) is taken as the overall volume integration value. Similar to the general chromatographic quantitative method with peak area, $\mathbf{c}_i\mathbf{s}_i^t$ is directly proportional to the mass of the ith component and so it is quantified.

3. Experimental

3.1. Materials

All single herbs that are used in peptic powder were purchased from the market, and identified by a researcher from Institute of Materia Medica, Hunan Academy of Traditional Chinese Medicine and Materia Medica, Changsha, Hunan, China.

3.2. Extraction of volatile oil

All these crude herbs were dried for about 60 min under 40°C at first. Subsequently, 200 g *Rhizoma Atractylodis*, 96 g *Pericarpium Citri Reticulatae*, 96 g *Cortex Magnoliae Officinalis* and 64 g *Radix Glycyrrhizae* were pre-mixed, then swollen with over 1000 ml of distilled water in a standard extractor for extracting volatile oil and allowed to stand for 30 min under room temperature. Next, about 100 ml of distilled water was added. Then, the essential oil was prepared according to the standard extracting method for the volatile oil in Traditional Chinese Medicines in *Chinese Pharmacopoeia* [34].

3.3. Instruments

A GC-17A gas chromatograph and a QP-5000 mass spectrometer (both from Shimadzu) was employed in this study.

3.4. Detection of volatile oil

In the gas chromatographic system, an OV-17 capillary column (30 m×0.25 mm I.D.) was used. Column temperature was maintained at 60°C for 2 min, and then programmed from 60°C to 270°C at the rate 20°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 40 to 426 u mass range with 0.2 s/scan velocity. The ionization source temperature was set at 230°C.

3.5. Data analysis

Data analysis was performed on a Pentium based IBM compatible personal computer. All programs of HELP and other methods were coded in MATLAB 5.1 for Windows. The library search and spectral matching of the resolved pure components were conducted on the National Institute of Standards and Technology (NIST) MS database containing about 62000 compounds.

4. Results and discussion

4.1. Qualitative analysis

The curve in Fig. 1 shows the real total ionic chromatogram (TIC) of the volatile oil of peptic powder. As can be seen from this profile, 45 chromatographic peaks appear and some of them overlap with one another. However, the similarity indices (SIs) obtained from direct searching with the NIST MS database are quite low for many of these chromatographic peaks. On the other hand, the same component is possibly searched at different chro-

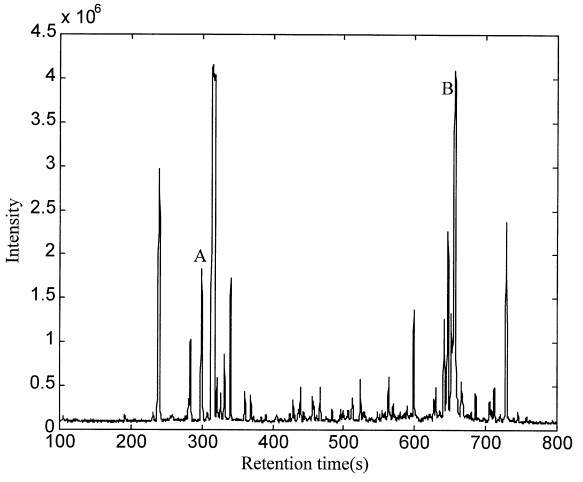

Fig. 1. TIC curve of volatile oil of peptic powder. The A and B peak clusters are investigated.

matographic scan points. All these indicate the great complexity of the system under study, or saying concretely, most of the chromatographic peaks are overlapped. If these overlapping peaks are not resolved, the simple search with the NIST MS database will definitely fail, because the mass spectrum of mixtures measured can never get a good matching index with that of a pure component in the NIST MS database. Furthermore, for the component with low content, it is also very difficult to be identified correctly with the NIST MS database, since a two-dimensional data obtained by mass spectral measurement unavoidably contains peaks associated with column background and residual gases. Without background correction, both the resolution of the overlapped peaks and the identification of the components with low content are impossible. For commercial GC–MS systems, background subtraction is usually performed as follows. First, a scan point, which only contains the background mass spectrum, is subjectively found. Next, the intensities of the same inter mass numbers appearing in the target and background spectra are subtracted, and so the practical target mass spectrum is obtained. Obviously, the practical target mass spectrum strongly depends on the selection of the background point. If this selection is wrong, different target mass spectra may be obtained. The genuine mass spectrum to be searched is surely confused with the subtracted spectrum. As for the HELP method, the local rank analysis of the zero-component regions, which contain no components eluting, before elution of the first chemical component starts and after the last chemical constituent has eluted, can together provide sufficient information for accurately correcting a drifting baseline [13–15]. Hence, a much better background subtraction could be obtained. After background subtraction, the resolution of the overlapping peaks becomes possible. Now, the peak cluster A within 4.91–5.07 min (or between 1475 and 1520 scan points) and B within 10.81–11.05 min (or between 3241 and 3351 scan points) of Fig. 1 are taken as examples to illustrate the data analysis process mentioned above.

Fig. 2 shows the TIC curve of peak cluster A which looks like a pure peak with only one constituent. Furthermore, only one component named α-phllandrene can be searched in the NIST MS

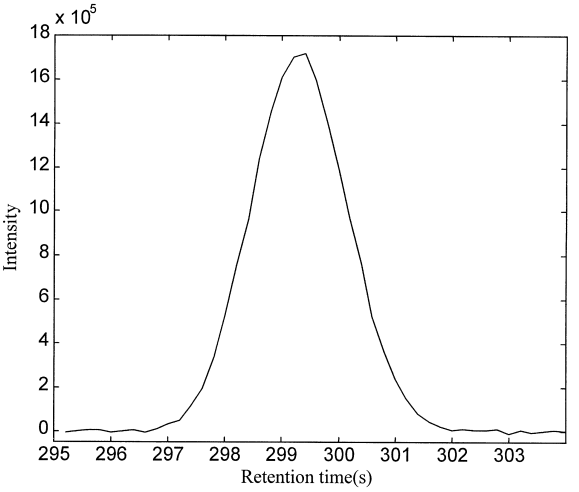

Fig. 2. TIC curve for A peak cluster (within 295–305 s).

library. Therefore, peak cluster A is generally regarded as a one-component system in a classic analytical way. However, if HELP resolution method is applied to the two-dimensional data matrix of peak cluster A, two isomeric components named α-phellandrene and *trans*-ocimene with the molecular formula $C_{10}H_{16}$ can be resolved even their mass spectra are quite similar. How this result becomes accessible is introduced in detail in the following part.

Peak purity can be identified by a fixed size moving window evolving factor analysis (FSWMEFA) [35] or so-called eigenstructure tracking analysis [15]. In the fixed size window method (FSWM) plot, the noise level is characterized by eigenvalue curves which have similar numerical values and appears together at the bottom. Eigenvalue curves higher than the noise level represent the appearance of new components. If a studied system contains only one species, there is only one eigenvalue curve higher than the noise level in its FSWM plot. From the FSWM plot of peak cluster A as shown in Fig. 3, there are four eigenvalue curves higher than the noise level within the peak region. This suggests that the peak cluster A may not be a pure one. However, the heteroscedastic noise produced by GC–MS instruments may also give rise to such a situation [36]. After a special pretreatment described in Ref. [36] is conducted on the heteroscedastic noise, the new results are shown in Fig. 4

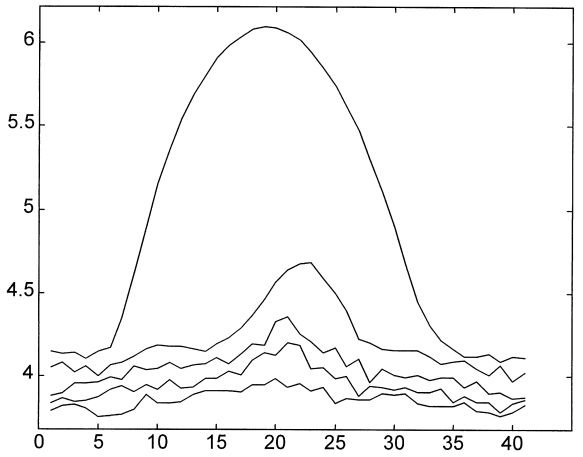

Fig. 3. FSWM plot for A peak cluster before correcting heteroscedastic noise.

from which one can distinctly conclude that the region of 1+2 is the overlapping region of the first and second components. The regions of 1 and 2 are possibly the regions of the pure first and second components, respectively.

The stepwise eluting information of chemical components in peak cluster A can be further confirmed by evolving latent projection graph (ELPG) [13–15]. This technique is based on the use of ordered nature of hyphenated data and that the selective regions appears as straight-line segments in bivariate score plots of principal component analysis. Thus, the ELPG is essentially a principal component projective curve from chromatographic or spectral spaces. There are several advantages to use the ELPG: (i) In a bivariate score plot a straight line segment pointing to the origin suggests selective information in the retention time direction, while in a bivariate loadings plot a straight line segment pointing to the origin suggests selective information in the spectral direction. The concept of ''straight line'' here is, of course, under sense of least squares; (ii) The evolving information of the appearance and disappearance of the chemical components in retention time direction can be also provided in ELPG. In the ELPG from the chromatographic space, the straight line section represents the pure selective region of one component while the curving section denotes the overlapping region of at least two constituents; (iii) Information enabling the detection of shifts of the chromatographic base line and instrumental background is also provided in ELPG. If there is a offset in chromatography the points cannot concentrate on the origin in the plot even if one includes the zero-component regions in data; (iv) ELPG is also a very good diagnostic tool to identifying the embedded peaks in chromatogram. This information is very important for resolution of concentration profiles of embedded peaks (see examples in Ref. [37]). The ELPG likes a data scope to see the insight of data structure of the two-way data. Fig. 5 shows the ELPG of peak cluster A. From this plot, one can see that this peak cluster is a two-component system. The marks, say a, b and c in the plot, indicate respectively the pure region of the first component, the overlapping region of the first and second components and the pure region of the second component in the chromatographic direction. This is consistent with the results obtained from the FSWM plot after subtracting the heteroscedastic noise.

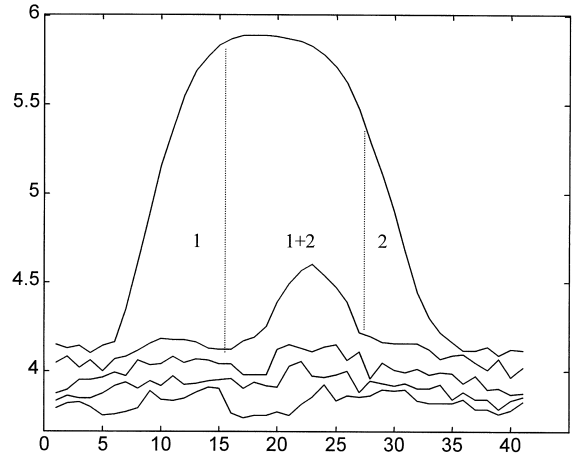

Fig. 4. FSWM plot for A peak cluster after correcting heteroscedastic noise. The regions 1, 1+2 and 2 indicate the region of the pure component 1, the overlapping region of components 1 and 2 and the region of the pure component 2, respectively.

From the discussion above, the chromatographic eluting order can be determined and so the number of components in the system, the selective regions and zero-concentration regions[13–15] of all the constituents. With all the information determined, the two-dimensional data matrix can be uniquely resolved into pure chromatographic profiles and mass

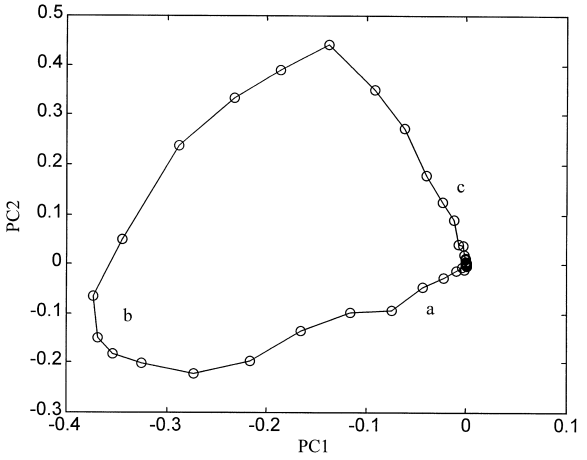

Fig. 5. Evolving latent projection graph (ELPG) for A peak cluster. The regions a, b and c indicate the region of the pure component 1, the overlapping region of components 1 and 2 and the region of the pure component 2, respectively.

spectra of all components. It should be pointed out here that for some component without available selective region, the component stripping technique may be applied to the unique resolution of this component [23].

The qualitative of the chemical composition of the sample determination can be directly performed by means of similarity searches in the NIST mass library now, as the pure chromatographic curve and mass spectrum of each component have been resolved. The result shows that these two components in peak cluster A are α-phellandrene and *trans*-

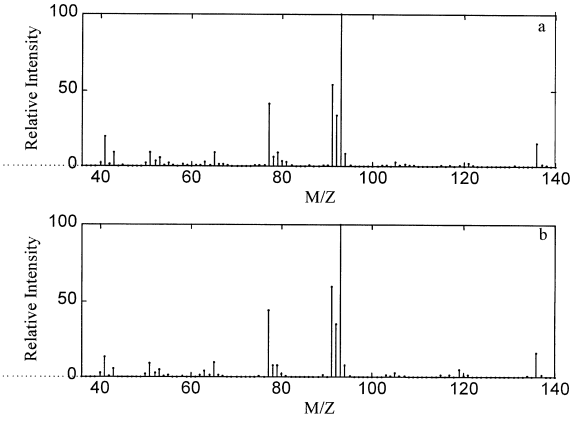

Fig. 7. Resolved mass spectrum of component 1(a) and standard mass spectrum of α-phellandrene (b, $C_{10}H_{16}$).

ocimene. Their corresponding chromatographic profiles are shown in Fig. 6, and the resolved mass spectra together with the standard spectrum of each component from the NIST MS library are also given in Figs. 7 and 8. From these two figures, one can easily see that the resolved results are quite reasonable. It is worth noting that the ability of the method to work with is strongly determined by the reproducibility of the intensities in the MS. It is found that the MS intensities have strong heteroscedastic noise [38]. Thus, the influence from the heteroscedastic noise on the intensities of MS should be taken into account even when the qualitative analysis is carried out by matching similarity between the resolved and standard spectra. In our experience the m/e values in

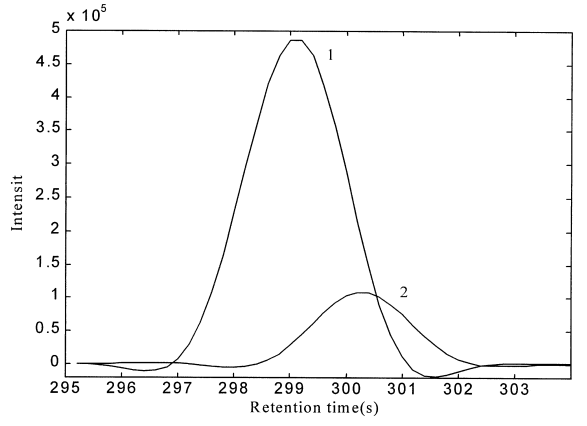

Fig. 6. Resolved chromatograms for A peak cluster containing components 1 and 2.

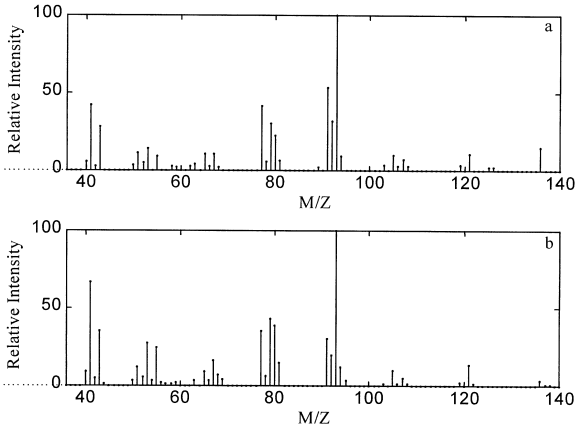

Fig. 8. Resolved mass spectrum of component 2(a) and standard mass spectrum of *trans*-ocimene (b, $C_{10}H_{16}$).

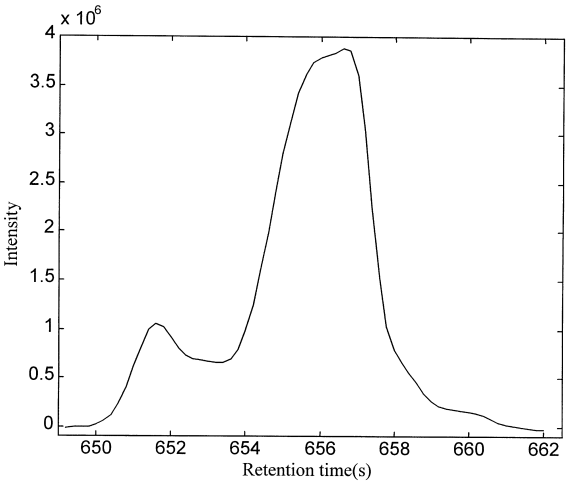

Fig. 9. TIC curve for B peak cluster (within 645–665 s).

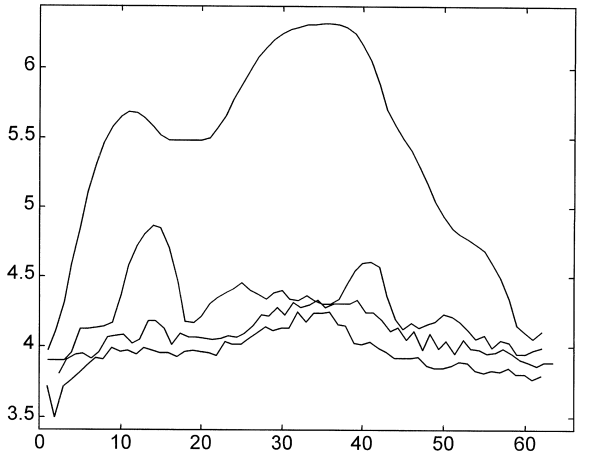

Fig. 11. FSWM plot for B peak cluster before correcting heteroscedastic noise.

MS are more important than the intensities of the MS for qualitative purpose since the heteroscedastic noises exist always in the measurements.

Likewise, Fig. 9, representing the TIC curve of peak cluster B, appears to be a mixed system of only two constituents. The small peak at the 3300th scan point is possibly a tailing peak of the adjacent big one. On the other hand, only two components named α-bisabolol and β-eudesmol can be directly matched in the NIST MS database. However, four isomeric components which are α-bisabolol, α-eudesmol, β-eudesmol and eudesmol with the molecular formula $C_{15}H_{26}O$ can be resolved by means of the HELP resolution method with the procedure described below. Moreover, the relative content of these constituents is much different from each other and the mass spectra of the later three are similar.

The instrumental background also presents in peak

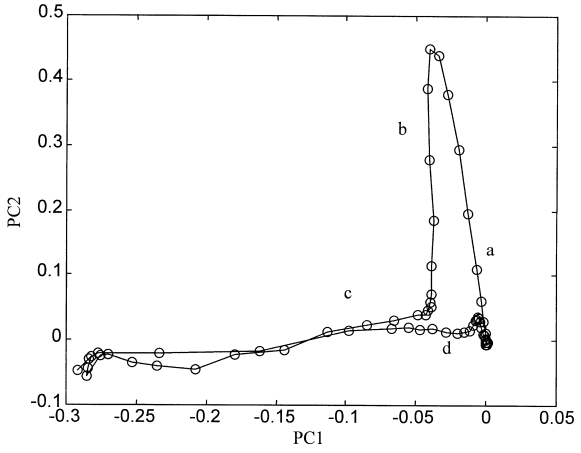

Fig. 10. Evolving latent projection graph (ELPG) for B peak cluster. The regions a and d indicate the regions of two pure components while the regions b and c possibly represent the appearances of new components.

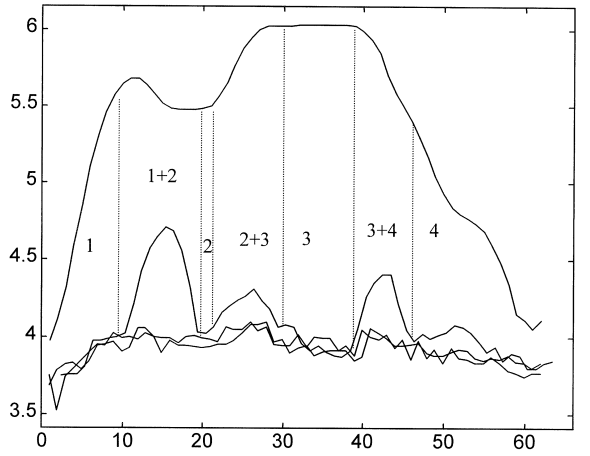

Fig. 12. FSWM plot for B peak cluster after correcting heteroscedastic noise. The regions, marked by 1, 1+2, 2, 2+3, 3, 3+4 and 4, indicate the region of the pure component 1, the overlapping region of components 1 and 2, the region of the pure component 2, the overlapping region of components 2 and 3, the region of the pure component 3, the overlapping region of components 3 and 4, and the region of the pure component 4, respectively.

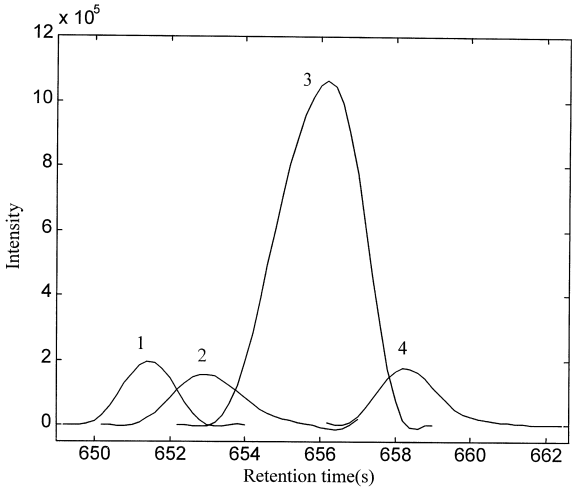

Fig. 13. Resolved chromatograms for B peak cluster containing components 1, 2, 3 and 4.

cluster B as in peak cluster A. After the background is removed, the ELPG is plotted. The ELPG in Fig. 10 suggests that the peak cluster B is much complex than the peak cluster A. It seems to be a four-component system with only two selective regions (marks a and b in the plot).

The FSWM plots before and after correction of the heteroscedastic noises for peak cluster B are shown in Figs. 11 and 12, respectively. The FSWM plot obtained after correcting the heteroscedastic noise (Fig. 12) clearly shows that there are four components in peak cluster B. After the eluting regions (Fig. 12) of all components are determined the

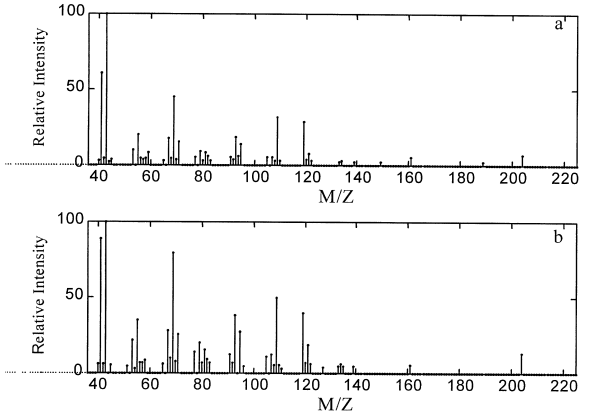

Fig. 14. Resolved mass spectrum of component 1(a) and standard mass spectrum of α-bisabolol (b, $C_{15}H_{26}O$).

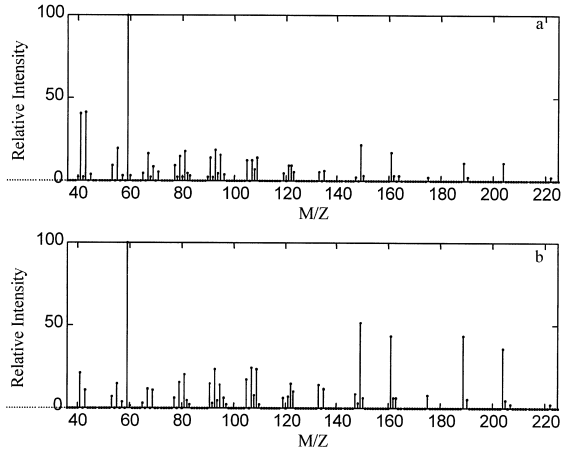

Fig. 15. Resolved mass spectrum of component 2(a) and standard mass spectrum of α-eudesmol (b, $C_{15}H_{26}O$).

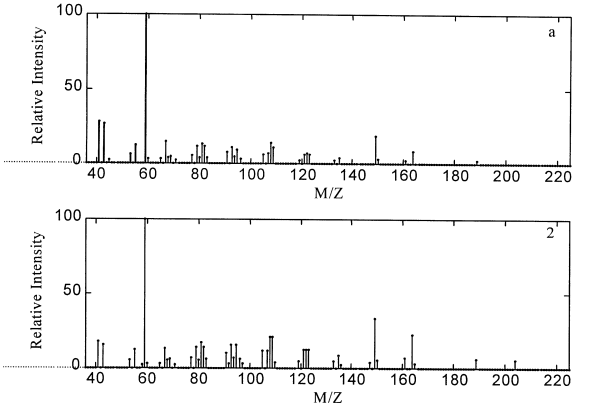

Fig. 16. Resolved mass spectrum of component 3(a) and standard mass spectrum of β-eudesmol (b, $C_{15}H_{26}O$).

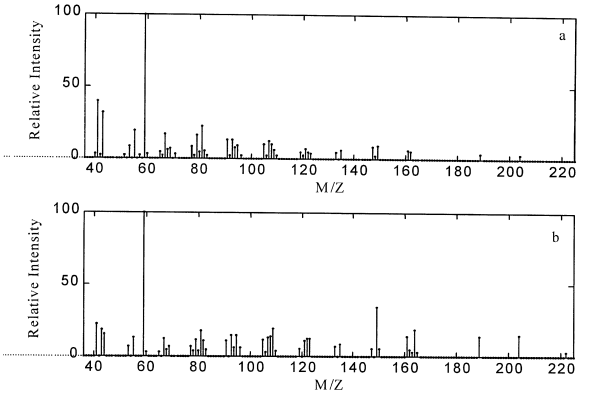

Fig. 17. Resolved mass spectrum of component 4(a) and standard mass spectrum of eudesmol (b, $C_{15}H_{26}O$).

Table 1
Qualitative and quantitative results of volatile oil from peptic powder

Retention time (s)	Name of component	Molecular formula	Relative concentration (%)
105.0	3-Methyl-3-buten-1-ol	$C_5H_{10}O$	0.08
191.0	*n*-Hexanal	$C_6H_{12}O$	0.09
231.2	Tricyclene	$C_{10}H_{16}$	0.16
239.4	*cis*-Ocimene	$C_{10}H_{16}$	9.51
255.4	*trans*-2,7-Dimethyl-3-octen-5-yne	$C_{10}H_{16}$	0.10
259.0	Camphene	$C_{10}H_{16}$	0.15
277.2	1,1-Dimethyl-2-[3-methyl-1,3-butadienyl] -cyclopropane	$C_{10}H_{16}$	0.08
281.2	β-Pinene	$C_{10}H_{16}$	0.60
283.6	β-Myrcene	$C_{10}H_{16}$	1.89
299.2	α-Phellandrene	$C_{10}H_{16}$	3.76
301.0	*trans*-Ocimene	$C_{10}H_{16}$	0.96
307.2	2-Carene	$C_{10}H_{16}$	0.21
315.6	D-Limonene	$C_{10}H_{16}$	26.68
321.0	[+]-Sabinene	$C_{10}H_{16}$	0.73
323.0	6-Methyl-5-heptene-2-one	$C_8H_{14}O$	0.06
326.2	3,7-Dimethyl-1,3,7-octatriene	$C_{10}H_{16}$	0.43
331.4	m-Cymene	$C_{10}H_{16}$	1.32
340.2	γ-Terpinene	$C_{10}H_{16}$	2.79
359.4	*trans*-Linaloloxide	$C_{10}H_{18}O_2$	0.11
360.6	Terpinolene	$C_{10}H_{16}$	0.53
368.8	3,7-Dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	0.49
390.2	*cis*-1-Meythyl-4-[1-methylethyl]-2-Cyclohexen-1-ol	$C_{10}H_{18}O$	0.11
403.0	2-Phenylethanal	C_8H_8O	0.04
405.0	*trans*-1-Methyl-4-[1-methylethyl]-2-cyclohexen-1-ol	$C_{10}H_{18}O$	0.12
424.0	*n*-Decanal	$C_{12}H_{20}O$	0.10
428.4	L-4-Terpioeol	$C_{10}H_{18}O$	0.36
430.8	L-Borneol	$C_{10}H_{18}O$	0.09
435.6	Myrcenol	$C_{10}H_{18}O$	0.15
436.6	*exo*-Isocamphanone	$C_{10}H_{16}O$	0.23
439.2	α-Terpineol	$C_{10}H_{18}O$	0.61
443.2	[*R*]-[+]-Citronellal	$C_{10}H_{18}O$	0.19
452.6	β-Citronellal	$C_{10}H_{18}O$	0.03
455.8	[−]-*cis*-Sabinol	$C_{10}H_{16}O$	0.38
457.8	3-Decyn-2-ol	$C_{10}H_{18}O$	0.26
461.8	[+]-Verbenol	$C_{10}H_{18}O$	0.09
466.8	*cis*-Geraniol	$C_{10}H_{18}O$	0.61
475.2	*trans*-Verbenol	$C_{10}H_{16}O$	0.11
483.6	Limoneon monoxide	$C_{10}H_{16}O$	0.28
496.0	Undecyne	$C_{10}H_{18}O$	0.13
500.6	α-Phenylallyl alcohol	$C_9H_{10}O$	0.03
506.4	Aromadendrene	$C_{15}H_{24}O$	0.21
511.4	2-[2-Butynyl]-cyclohexanone	$C_{10}H_{14}O$	0.13
512.2	Limonene epoxide	$C_{10}H_{16}O$	0.27
523.6	2,7-Dimethyl-2,6-octadien-1-ol	$C_{10}H_{18}O$	0.74
528.4	Isocaryophyllene	$C_{15}H_{24}$	0.28
530.6	Germacreme	$C_{15}H_{24}$	0.14

Table 1. Continued

Retention time (s)	Name of component	Molecular formula	Relative concentration (%)
547.8	α-Humulene	$C_{15}H_{24}$	0.17
563.6	β-Elemene	$C_{15}H_{24}$	0.08
569.8	*cis*-β-Farnesene	$C_{15}H_{24}$	0.27
580.0	3,3,7,7-Tertramethyl-5-[2-methyl-1-propenyl]-tricyclo 4.1.0.02,4 heptane	$C_{15}H_{24}$	1.25
590.0	4,11,11-Trimethyl-8-methylene-bicyclo-7.2.0 undec-4-ene	$C_{15}H_{24}$	0.28
599.0	Elemol	$C_{15}H_{24}$	2.12
627.2	D-Nerolidol	$C_{15}H_{26}O$	0.42
630.0	Decahydro-1,1,4,7-tetramethyl-1H-cycloprope azulen-4-ol	$C_{15}H_{26}O$	0.65
640.6	[−]-Globulol	$C_{15}H_{26}O$	0.24
641.6	Juiper camphor	$C_{15}H_{26}O$	2.08
651.4	α-Bisabolol	$C_{15}H_{26}O$	1.60
652.8	α-Eudesmol	$C_{15}H_{26}O$	2.02
656.4	β-Eudesmol	$C_{15}H_{26}O$	13.41
658.2	Eudesmol	$C_{15}H_{26}O$	1.87
668.0	Diethyl Phthalate	$C_{13}H_{24}O_2$	0.29
686.0	[−]-Spathulenol	$C_{15}H_{24}O$	0.56
705.8	Solavetivone	$C_{15}H_{22}O$	0.51
708.6	Nootkatone	$C_{15}H_{22}O$	0.32
729.0	4-Formylbiphenyl	$C_{13}H_{10}O$	5.70

unique resolution into chromatograms and mass spectra can be then conducted on the two-dimensional data. Figs. 13–17 show the resolved results. These figures definitely support the presence of four isomeric components in peak cluster B.

Other peaks in the studied sample at other chromatographic scan points (Fig. 1) were also determined qualitatively in the same way as described above (see Table 1). Ninety-three components were found but only 65 were analyzed here. It is because the ratios of signal to noise of some components are too low, or some of them in the sample are not included in the NIST MS database. This results in 28 components not being identified qualitatively. If some of these undetermined constituents are of interest, further study seems to be necessary. The retention indices and mass spectra obtained can only be used to roughly deduce possible structures of them here.

4.2. Quantitative analysis

Generally, quantitative analysis of GC–MS is carried out with peak area while overlapping peaks are approximately treated by peak split. However, the results thus obtained will be definitely inaccurate and even wrong in some of the present case. For example, if the above unresolved peak cluster B is vertically splitted at the division point and considered as a system with only two compounds namely α-bisabolol and β-eudesmol, the quantitative results are shown in Table 2. After applying the HELP method to resolve the TIC curve in Fig. 9 into pure chromatographic curves and mass spectra of four

Table 2
Quantitative results of B peak cluster

Component	α-Bisabolol (%)	α-Eudesmol (%)	β-Eudesmol (%)	Eudesmol (%)
Vertical splitting	2.75	–	16.22	–
Overall volume integration	1.60	2.02	13.41	1.87

components, much more accurate results can be reached (see Table 2). The quantitative results for all other components in the peptic powder sample can be obtained in the same way as described above, and are shown in Table 1.

5. Conclusion

With the use of HELP resolution method upon two-dimensional data together with the large mass spectral database, one can see from the results obtained that real complex systems, like the traditional Chinese medicine peptic powder, can be elegantly analyzed qualitatively and quantitatively. This demonstrated that the combination of hyphenated instruments and relevant chemometric methods opens a new way for quick and accurate analysis of real unknown complex samples. The method developed in this work paper cannot only greatly enhance the separation ability of the hyphenated chromatography but also its ability in qualitative identification. This implies shows the prosperous prospect for analysts to directly address very difficult problems in analytical chemistry.

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