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Temperature-programmed retention indices for gas chromatography–mass spectroscopy analysis of plant essential oils

Chen-Xi Zhao [a,b], Yi-Zeng Liang [a,*], Hong-Zhuang Fang [c], Xiao-Ning Li [a]

[a] *Research Center of Modernization of Chinese Herbal Medicine, College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, PR China*
[b] *Department of Applied Chemistry and Environmental Science, Changsha University, Changsha, Hunan 410003, PR China*
[c] *Department of Chinese Herbal Medicine, Jiamusi University, Jiamusi, Heilongjiang 154007, PR China*

Abstract

A total of 95 volatile compounds from the essential oil in buds of *Syringa oblata Lindl* (lilac) were identified by gas chromatography–mass spectrometry (GC–MS) combined with heuristic evolving latent projections (HELP) and moving subwindow searching (MSS). The identified compounds are mainly aliphatic, terpenes and aromatic compounds. Their temperature-programmed retention indices (PTRIs) on HP-5MS and DB-35MS at three heating rates of 2, 4 and 6 °C/min from 80 to 290 °C were obtained, which showed that aliphatic compounds give nearly constant PTRIs and PTRIs of terpenoids do not vary much at different heating rates. But PTRIs of aromatic compounds exhibit relatively large temperature dependence. PTRIs vary much more on DB-35MS than those on HP-5MS according to the compound types. In general, differences of PTRIs between the two columns increase from aliphatic compounds to terpenoids to polycyclic aromatic compounds. The PTRIs in different heating rates were used as cross-references in the identification of components in the essential oil. When they were used in analysis of essential oil from flowers of lilac, good results were obtained. These PTRIs would be a part of our PTRI database being constructed on components from plant essential oils. The results also showed that efficiency and reliability were improved greatly when chemometric method and PTRIs were used as assistants of GC–MS in identification of chemical components in plant essential oils.

Keywords: Temperature-programmed retention indices (PTRIs); Gas chromatography–mass spectrometry (GC–MS); Chemometrics; Heuristic evolving latent projections (HELP); Moving subwindow searching (MSS); Essential oil; *Syringa oblata Lindl* (lilac)

1. Introduction

With the development of botanical drugs including traditional herbal medicines, analysis of their bioactive components seems to be more and more popular. Many botanical drugs have their bioactive components in their essential oils. So it is an important and meaningful work to characterize plant essential oils, especially in the process of modernization of traditional herbal medicine. Some medicinal and chemical scientists have paid attention on it [1–3]. Gas chromatography (GC) or gas chromatography–mass spectroscopy (GC–MS) is used almost exclusively for the qualitative analysis of the volatiles.

As gas chromatography–mass spectrometry (GC–MS) becomes popular, a large number of researches have turned to mass spectra (MS) for identification of peaks. The most frequent, simple and valid identification method is the comparison of the recorded spectra with standard MS library. However, when there are peaks of isomers or of similar substances in GC–MS, the identification will be not precise enough and sometimes even wrong, from the chemical structure point of view. As we know, the natural essential oils are usually mixtures of terpenoids (mainly mono-terpenoids and sesquiterpenoids), aromatic compounds and aliphatic compounds. As mass spectra of these compounds are usually very similar, peak identification becomes very difficult and sometimes impossible. In order to address the qualitative determination of compositions of complex samples by GC–MS and to increase the reliability of the analytical results, it seems necessary to utilize retention indices identities [4].

Isothermal retention index (I), as a relative stable parameter in GC qualitative analysis, has been used widely since it was proposed in 1958 by Kovats [5]. There are many referenced isothermal retention index databases in some handbooks

* Corresponding author. Tel.: +86 731 8830831; fax: +86 731 8825637.
E-mail addresses: yizeng_liang@263.net, yzliang@public.cs.hn.cn (Y.-Z. Liang).

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[6] and/or literatures [7,8]. However, the natural essential oils are often analyzed in temperature-programmed instead of isothermal conditions because of their complicated chemical components. Thus, temperature-programmed retention indices (PTRIs) seem to be more useful in the practice of essential oil analysis, but there are much less reports about this [1,2]. It might be a quite meaningful work to build a PTRI database of natural volatile components for chromatographic scientists to refer all over the world.

It is common that two or even more substances co-elute at the same time in GC analysis, so the identification is practically impossible just by retention indices only. But if GC retention index and GC–MS combined together, the identity of isomers or co-eluted compounds could be performed with higher reliability and sufficient precision if the chromatography peaks are separated with sufficient resolution. Unfortunately, essential oil samples usually could not be separated well enough in GC because of the complicated components although the optimal operation conditions are used. This makes the identification of essential oil to be impossible even though retention index and GC–MS are used at the same time. In general, there exist two problems in analysis of natural essential oil samples. The first is the complexity of essential oil, which makes the baseline separation impossible such that there are always overlapped peaks existing in the obtained chromatogram [9]. The other is the rather low contents of some compounds, whose low signal to noise ratios make it difficult to identify what they are simply by the obtained mass spectra. Fortunately, chemometrics resolution methods [10] were used in multi-component system analysis and proved to be powerful in overlapped peaks resolution [11,12]. Heuristic evolving latent projections (HELP) [13] focus on selective information and uses full rank resolution to get the spectral and concentration profiles of the components with selective information obtained, a component stripping procedure is used to resolve the other components [14]. It has been successfully used to solve several different real-world samples [15–17] and it also was used in the present work to treat some overlapped peaks resolution.

Syringa oblata Lindl, lilac, belonging to *Oleaceae*, a plant native to China, can vegetate in different environments and it is easy to plant. Lilac can make surroundings beautiful and moreover, be used as medicament such as antimicrobial, antipyretic and antivirus and is good to liver and cholecyst [18]. The essential oil is its main bioactive substance, while the therapeutic and aromatic properties of the essential oils are directly correlated with their qualitative and quantitative composition. Analysis of the chemical components in essential oils of lilac is a basic and necessary work to the opening and using of its ample source in the near future. Reports on its essential oil study are very few [19,20], to our knowledge, the composition of the essential oil from buds of lilac has not been previously reported yet. It is the first report on its essential oil at different GC operating conditions on two-column with different polarity. PTRIs of about one hundred volatile compounds from the essential oil in buds of lilac on a slightly polar capillary column (HP-5MS) and an intermediately polar capillary column (DB-35MS) at three heating rates of 2, 4 and 6 °C/min from 80 to 290 °C were obtained using gas chromatography–mass spectrometry combined with heuristic evolving latent projections and moving subwindow searching (MSS). The PTRIs at different heating rates were used as cross-reference in the identification of components in the essential oil. Hereby the word cross-reference means that the PTRIs obtained from different experimental conditions could be used alternately as references for each other. When they were used in analysis of essential oil from flowers of lilac, good result was obtained. These retention indices would be a part of our PTRI database being constructed on components from plant essential oils. The results also showed that efficiency and reliability were improved greatly when chemometric method and temperature-programmed retention indices were used as assistants of GC–MS in identification of chemical components in plant essential oils.

2. Experiments and methodology

2.1. Plant materials and n-alkane standard solutions

The buds and flowers of *Syringa oblata Lindl* were collected from the campus of Jiamusi University, located in Jiamusi, Heilongjiang province of China. The voucher specimens were made and identified by Professor Liu Juan working at Department of Chinese Herbal Medicine, Jamusi University, PR China.

n-Alkane standard solutions C_8–C_{20} (mixture no. 04070) and C_{21}–C_{40} (mixture no.04071) were purchased from *Fluka Chemika*.

2.2. Extraction of essential oils

Essential oil samples were extracted by water distillation for 12 h from dried materials, using a set of standard apparatus, according to the procedure described in the Chinese Pharmacopoeia [21]. The essential oils were stored at 4 °C in the dark prior to analysis.

2.3. Gas chromatography–mass spectrometry

Analyses were carried out in a Hewlett-Packard 6890 gas chromatograph interfaced with a Hewlett-Packard mass selective detector 5973N (Agilent Technologies, USA) operated by HP Enhanced ChemStation software, G1701DA MSD ChemStation Rev. D.00.00.38, using a slightly polar column, HP-5MS fused silica capillary column coated with 5% phenyl methyl polysiloxane and an intermediately polar column, DB-35MS fused silica capillary column coated with 35% phenyl methyl polysiloxane. The two columns are of the same size of 30 m × 0.25 mm i.d, film thickness 0.25 μm. The oven temperature was programmed linearly from 80 to 290 °C at three heating rates of 2, 4 and 6 °C/min. Other chromatographic operating conditions were as follows: injector temperature, 280 °C; carrier gas: helium, adjusted to a volumetric column velocity of flow, 1.0 mL/min splitting ratio: 20:1. The mass spectrometric operating conditions were as follows: interface temperature, 280 °C; MS source temperature, 230 °C; MS quadrupole temperature, 150 °C; electron impact (EI) modes with an electron energy of

70 eV and full scan modes in range of *m/z* 30–500 units, mass spectra recorded with 3.12 scans/s velocity.

2.4. Retention indices

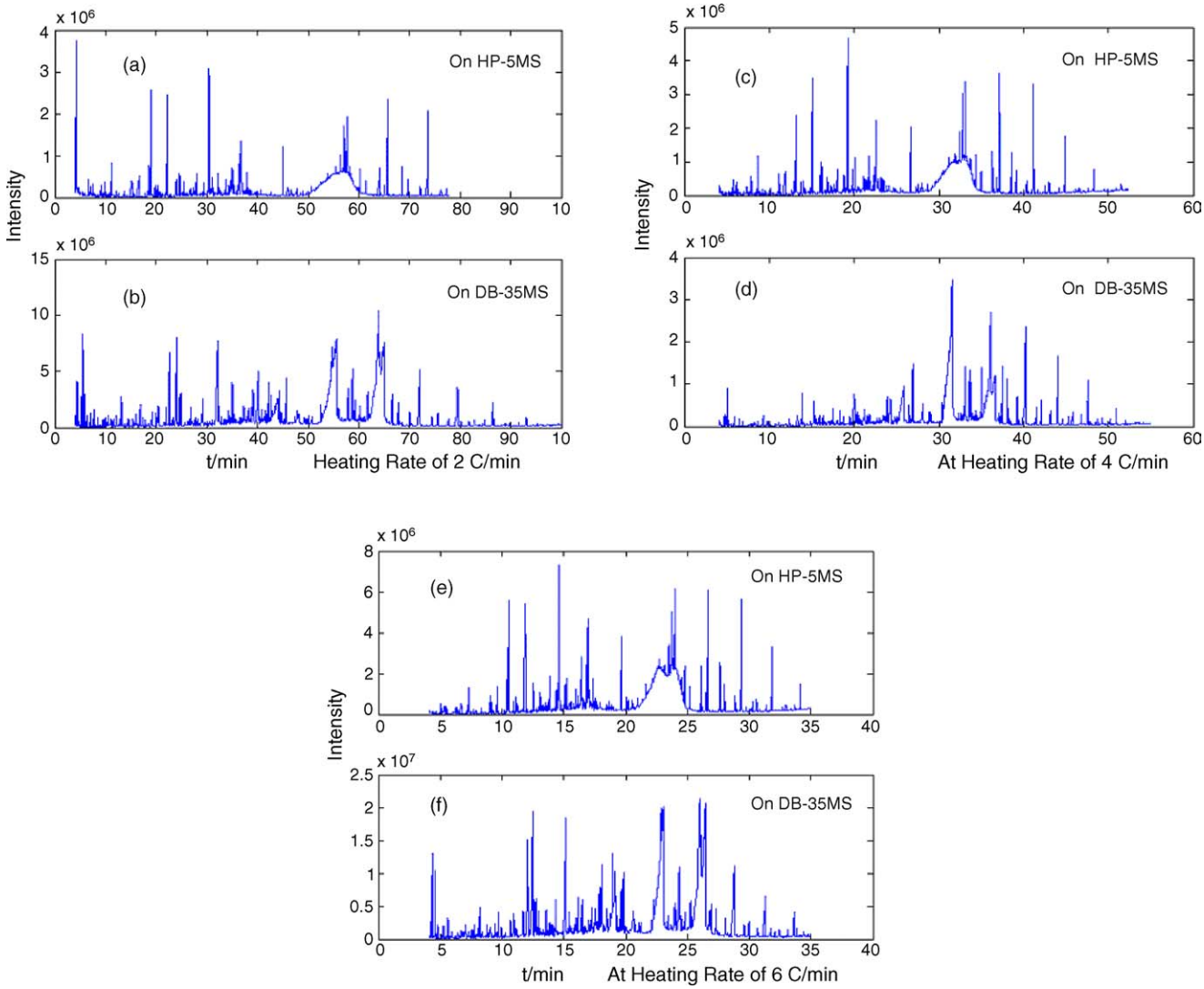
Unlike Kovats isothermal retention indices, TPRIs are varied in different chromatographic operating conditions such as carrier flow-rate and temperature program. van den Dool and Kratz [22] proposed a quasi-linear equation as follows:

$$I_u^{\mathrm{T}} = 100\left(\frac{t_u - t_n}{t_{n+1} - t_n} + n\right) \quad (1)$$

where I_u^{T} is the temperature-programmed retention index of the interest and t_n, t_{n+1} and t_u the retention time in minute of the two standard *n*-alkanes containing *n* and $n+1$ carbons and the interest, respectively. This equation was used to calculate retention indices in the present work, linear temperature-programmed GC operating conditions.

2.5. Qualitative analysis and comparing analysis between two chromatograms

Identification of oil components was performed by searching mass spectra database (NIST02) through G1701DA mass spectrum ChemStation or by a comparison of mass spectra with literature data and by a comparison of their relative retention times with those of authentic compounds, or by comparison of their temperature-programmed retention indices with those in other heating rates. Because overlapped peaks existed in the total ion current (TIC) chromatographs, HELP was used to extract pure chromatographs and mass spectra. The pure mass spectra obtained were then compared with those in the standard mass spectra library (NIST02) to be identified. When the pure spectrum of a component is obtained at certain operating condition, moving subwindow searching can be used to search this target component at other operating conditions.



Figs. 1–3. Total ion currents of essential oil in buds of lilac. (Fig. 1) Curve a: TIC at heating rate of 2 °C/min on HP-5MS. Curve b: TIC at heating rate of 2 °C/min on DB-35MS. (Fig. 2) Curve c: TIC at heating rate of 4 °C/min on HP-5MS. Curve d: TIC at heating rate of 4 °C/min on DB-35MS. (Fig. 3) Curve e: TIC at heating rate of 6 °C/min on HP-5MS. Curve f: TIC at heating rate of 6 °C/min on DB-35MS.

Moving subwindow searching, a new method, proposed on the basis of the method for identification of elution region in hyphenated chromatography [23], was used in this work in order to obtain the accurate PTRIs of the components in essential oil from buds of lilac at different temperature-programmed conditions.

There are two steps to be performed to identify a component in the MSS method. Firstly, a spectrum from a pure peak, which was obtained at one temperature-programmed condition, is selected as a target region, say $\mathbf{s}_t$. Secondly, a moving subwindow searched should be conducted along with the retention time direction for the other two-way chromatogram at different temperature-programmed conditions. Then, one could do a comparison to check if there is the same component in the searched regions with the help of subwindow factor analysis. If we denote a searching subwindow as $\mathbf{Y}_i$, the whole calculation procedure can be described as follows:

(1) Selected a target spectrum from a pure peak, say $\mathbf{s}_t$, in one two-way chromatogram;
(2) Set $i = 1, 2, \ldots$;
(3) Decompose the searched subwindow matrix $\mathbf{Y}_i$ in the other two-way chromatogram measured at different temperature-programmed conditions, that is,

$$\mathbf{Y}_i = \mathbf{T}_i \mathbf{P}_i^t$$

here $\mathbf{T}_i$ and $\mathbf{P}_i^t$ are orthogonal scores and loadings matrices, respectively, obtained by principal component analysis;

(4) Calculate a predicted spectrum, denoted by $\mathbf{s}_p$, using the information from the loadings matrix, that is,

$$\mathbf{s}_{pi} = (\mathbf{P}_i^t \mathbf{P}_i)^{-1} \mathbf{P}_i^t \mathbf{s}_t;$$

(5) Calculate the norm of the differences between $\mathbf{s}_{pi}$ and $\mathbf{s}_t$, that is, $d_i = ||\mathbf{s}_{pi} - \mathbf{s}_t||$;
(6) Set $i = i + 1$, and go back to step 3);
(7) Plot $\mathbf{d} = [d_1, d_2, \ldots, d_n]^t$ along with the retention time points.

When there is any common component in the subwindow searched, d_i would be equal to zero. With the help of the graph of $\boldsymbol{d}$ vector versus corresponding retention time points, one can easily find the elute region of the target compound, say $\boldsymbol{s}_t$. In real complex system, d_i is not always equal to zero but to noise level. MSS can be used to search the target component at other operating conditions. Thus, the retention indices of the compounds in overlapping peaks can be identified by MSS.

3. Results and discussion

3.1. Identification of components in bud essential oil of lilac and their PTRIs

The total ion currents shown in Figs. 1–3 at the three heating rates on two columns exhibit that the chemical composition of bud essential oil of lilac is very complicated. In a whole, it seems that there were more peaks with greater signal intensity on DB-35MS column than those on HP-5MS column. Different separations result at different heating rates but the separation is not always improved with the reducing of heating rate because chromatography resolution is affected by both thermodynamics and dynamics simultaneously. Therefore, even though optimal operating conditions are used, completely separation is impossible to such complicated samples. Chemometric resolution method can extract pure chromatograms and mass spectra from overlapped peaks, which then be compared with standard mass spectra library and be identified.

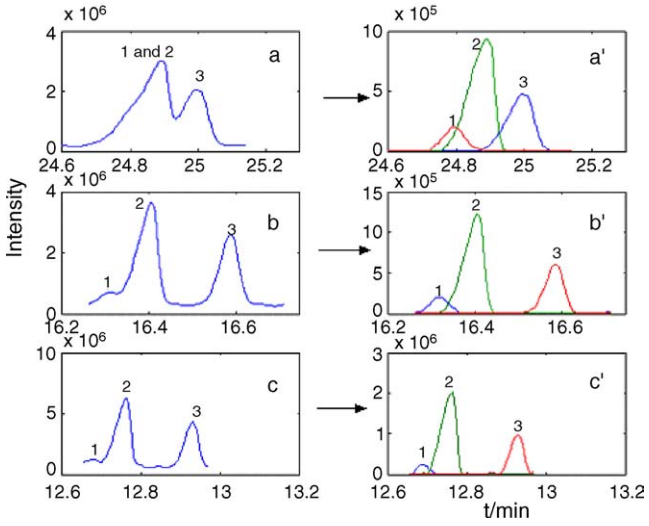


Fig. 4. Original TIC clusters and their resolution results. Curve a–c: the same TIC peak cluster at heating rates of 2, 4 and 6 °C/min on DB-35MS, respectively. Curve a′–c′: is the result of curve a–c resolved by chemometric resolution method, respectively.

For example, curves a–c shown in Fig. 4 are three original TIC peak clusters at heating rates of 2, 4 and 6 °C/min on DB-35MS, respectively. Peak 3 and 2 in curve b (4 °C/min) and curve c (6 °C/min) could be identified as α-caryophyllene and 3-pyridinecarboxylic acid, 5-ethenyl-methyl ester by searching the standard mass spectra library (NIST02) and comparing their PTRIs, with high match quality (0.91 and 0.97, respectively). But peak 1 in them could not be identified because of the low signal to noise ratios. While through chemometric resolution, extracting the pure chromatography and mass spectrum, this small peak can be identified as 1-[1,5-dimethylhexyl]-4-methylbenzene, with match qualities of 0.91 and 0.85, respectively, as seen in Fig. 4 curves b′ and c′. The PTRIs can be used as cross-reference at different heating rates if the chromatographic peaks are separated well enough. But when the heating rate was varied to be 2 °C/min, as shown in curve a of Fig. 4, peak 1 and 2 were overlapped and could not be identified, as the match quality varies from 0.49 to 0.78 at different place of the overlapped peak using library searching and its two-dimensional plot can shows us more clearly that it is indeed a double peak. With the help of the chemometric resolution technique the overlapped peak could be resolved into two pure peaks, say peak 1 and peak 2, as shown in Fig. 4a′, which could be identified by library searching. The library searching result showed that peak 3 in

Table 1
Components in essential oils of *Syringa oblata Lindl*

I.D. in		Compound name	MF
Buds	Flowers		
1		2-Pentyl-furan	$C_9H_{14}O$
2		3-Ethyl-pyridine	C_7H_9N
3	1	Limonene	$C_{10}H_{16}$
	2	β-Phellandrene	$C_{10}H_{16}$
4		Benzyl Alkohol	C_7H_8O
5	3	Benzene acetaldehyde	C_8H_8O
6	4	3-Ethenyl-pyridine	C_7H_7N
	5	Eucalyptol	$C_{10}H_{18}O$
7	6	Benzaldehyde	C_7H_6O
8	7	1-Methyl-4-[1-methylethyl]-1,4-cyclohexadiene	$C_{10}H_{16}$
9		1-Octanol	$C_8H_{18}O$
10	8	Linalool oxide [fr.1]	$C_{10}H_{18}O_2$
	9	1-Methyl-4-[1-methylethylidene]-coyclohexene	$C_{10}H_{16}$
11	10	3,7-Dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$
12	11	Nonanal	$C_9H_{18}O$
13	12	6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$
14		Tetrahydro-4-methyl-2-[2-methyl-1-propenyl]-2H-pyran	$C_{10}H_{18}O$
15	13	Phenylethyl alcohol	$C_8H_{10}O$
16		Methyl nicotinate	$C_7H_7NO_2$
17	14	Borneol	$C_{10}H_{18}O$
18	15	Camphor	$C_{10}H_{16}O$
19		1-Ethenyl-4-methoxy benzene	$C_9H_{10}O$
20		2-Nonenal,[E]	$C_9H_{16}O$
21	16	p-Menth-1-en-8-ol	$C_{10}H_{18}O$
22	17	6,6-Dimethyl-bicyclo[3,1,1]hept-2-ene-2-methanol	$C_{10}H_{18}O$
23		E,E-2,6-Dimethyl-3,5,7-octatriene-2-ol	$C_{10}H_{16}O$
24	18	[1S]-4,6,6-Trimethyl-bicyclo[3,1,1]hept-3-en-2-one	$C_{10}H_{16}O$
25		6,6-Dimethyl-bicyclo[3,1,1]hept-2-ene-2-carboxaldehyde	$C_{10}H_{14}O$
26	19	4,6,6-Trimethyl-bicyclo[3,1,1]hept-3-en-2-ol	$C_{10}H_{16}O$
27		Cis-2-Methyl-5-[1-methylethenyl]-2-cyclohexen-1-ol	$C_{10}H_{16}O$
28		Trans-2-Methyl-5-[1-methylethenyl]-2-cyclohexen-1-ol	$C_{10}H_{16}O$
29		3,7-Dimethyl-2,6-octadien-1-ol	$C_{10}H_{18}O$
30	20	Isobornyl acetate	$C_{12}H_{20}O_2$
31	21	[1R,3S,6R]-1,3,7,7-Tetramethyl-2-oxabicyclo[4,4,0]dec-9-ene	$C_{13}H_{22}O$
32		[2α,4aα,8a,α]-2H-1-Benzopyran,3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl	$C_{13}H_{22}O$
33		1-Cyclohexene-1-methanol,4-[1-methylethenyl]-	$C_{10}H_{16}O$
34	22	1-Methoxy-4-[1-propenyl]-benzene	$C_{10}H_{12}O$
35		2,6,10,10-Tetramethyl-1-oxa-spiro[4.5]-dec-6-ene	$C_{13}H_{22}O$
	23	2-Undecanone	$C_{11}H_{22}O$
36	24	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
37	25	Eugenol	$C_{10}H_{12}O_2$
38	26	α.-Cubebene	$C_{15}H_{24}$
39	27	[1S-[1α,3aα,3bβ.,6aβ,6bα]]-cyclobuta[1,2:3,4]dicyclopentene-decahydro-3a-methyl-6-methylene-1-[1-methylethyl]	C15H24
40		Tetradecane	$C_{14}H_{30}$
	28	Isocaryophyllene	$C_{15}H_{24}$
41	29	Caryophyllene	$C_{15}H_{24}$
42	30	Copaene	$C_{15}H_{24}$
	31	1,1,7-Trimethyl-4-methylene-1H-cyclopropa[e]azulene,decahydro	$C_{15}H_{24}$
43		Benzene, 1-[1,5-dimethylhexyl]-4-methyl-	$C_{15}H_{24}$
44	32	5-Ethenyl-3-pyridinecarboxylic acid methyl ester	$C_9H_9NO_2$
45	33	α-Caryophyllene	$C_{15}H_{24}$
46		4,11-Dimethyl-tetradecane	$C_{16}H_{34}$
47		[1α,4aα,8aα]-Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-[1-methylethyl]	$C_{15}H_{24}$
48		[3aS-[3aα.,3bβ,4β,7α7aS*]]-Octahydro-7-methyl-3-methylene-4-[1-methylethyl]1H-cyclopenta[1,3]cyclopropa[1,2]-benzene	$C_{15}H_{24}$
49		[E]-4-[2,6,6-trimethyl-1-cyclohexen-1-yl]-3-buten-2-one	$C_{13}H_{20}O$
50		Pentadecane	$C_{15}H_{32}$
51		[1α,4aα.,8aα]-1,2,4a,5,6,8a-hexahydro-4-7-dimethyl-1-[1-methylethyl]naphthalene	$C_{15}H_{24}$

Table 1 (*Continued*)

I.D. in		Compound name	MF
Buds	Flowers		
52	34	α-Farnesene	$C_{15}H_{24}$
	35	[S]-1-Methyl-4-[5-methyl-1-methylene-4-hexenyl]-cyclohexene	$C_{15}H_{24}$
	36	2-Tridecanone	$C_{13}H_{26}O$
	37	[1α,4aα,8aα]-1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-[1-methylethyl]naphthalene	$C_{15}H_{24}$
53		[1S,cis]-Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-[1-methylethyl]	$C_{15}H_{24}$
	39	2,4-Bis[1,1-dimethylethyl]phenol	$C_{14}H_{22}O$
54	40	Hexadecane	$C_{16}H_{34}$
55		[1R-[1α,3α,4β]]-4-Ethenyl-α.,α,4-trimethyl-3-[1-methylethenyl]cyclohexanemethanol	$C_{15}H_{26}O$
56	41	3,7,11-Trimethyl-1,6,10-Dodecatrien-3-ol	$C_{15}H_{26}O$
57	42	Caryophyllene oxide	$C_{15}H_{24}O$
58	43	Ledol	$C_{15}H_{26}O$
59		[2R-cis]-1,2,3,4,4a,5,6,7-Octahydro-α.α.,4a,8-tetramethyl-2-naphthalenemethanol	$C_{15}H_{26}O$
60		.tau..-Muurolol	$C_{15}H_{26}O$
61		Decahydro-α,α,4a-trimethyl-8-methylene-2-naphthalenemethanol	$C_{15}H_{26}O$
62		1,2,3,4,4a,5,6,7-Octahydro-α.α,4a,8-tetramethyl-2-naphthalenemethanol	$C_{15}H_{26}O$
	44	Cedrol	$C_{15}H_{26}O$
63	45	α-Cadinol	$C_{15}H_{26}O$
64		2,2′,5,5′-Tetramethyl-1,1′-biphenyl	$C_{16}H_{18}$
65		α-Bisabolol	$C_{15}H_{26}O$
	46	Tetradecanoic acid	$C_{14}H_{28}O_2$
	47	2′,3′,4′-Trimethoxyacetophenone	$C_{11}H_{14}O_4$
	48	Pentadecanoic acid	$C_{15}H_{30}O_2$
66	49	Heptadecane	$C_{17}H_{36}$
67		Nocurdione	$C_{15}H_{24}O_2$
68	50	Phenanthrene	$C_{14}H_{10}$
69	51	Octadecane	$C_{18}H_{38}$
70	52	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$
71	53	1,2-Benzenedicarboxylic acid bis[2-methylpropyl]ester	$C_{16}H_{22}O_4$
72	54	Nonadecane	$C_{19}H_{40}$
73	55	Hexadecanoic acid methyl ester	$C_{17}H_{34}O$
74	56	Dibutyl phthalate	$C_{16}H_{22}O_4$
75	57	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
76	58	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$
77		Isophytol	$C_{20}H_{40}O$
78	59	Eicosane	$C_{20}H_{42}$
79		2-Methyl eicosane	$C_{21}H_{44}$
80		E-15-Heptadecenal	$C_{17}HH_{32}O$
	60	[Z,Z]-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$
81	61	[Z,Z]-9,12-Octadecadienoic acid methyl ester	$C19H_{34}O_2$
82	62	[Z,Z,Z]-9,12,15-Octadecadienoic acid methyl ester	$C_{19}H_{32}O_2$
	63	[Z,Z,Z]-9,12,15-Octadecatrienoic acid ethyl ester	$C_{20}H_{34}O_2$
83	64	Heneicosane	$C_{21}H_{44}$
84	65	Phytol	$C_{20}H_{40}O$
85	66	Docosane	$C_{22}H_{46}$
86		n.i	
87		[E]-5-Eicosene	$C_{20}H_{40}$
88	67	Tricosane	$C_{23}H_{48}$
89	68	Tetracosane	$C_{24}H_{50}$
90	69	Pentacosane	$C_{25}H_{52}$
91	70	Hexacosane	$H_{26}H_{54}$
92	71	Heptacosane	$C_{27}H_{56}$
93		Octacosane	$C_{28}H_{58}$
94	72	Nonacosane	$C_{29}H_{60}$
95		Hentriacontane	$C_{30}H_{62}$

Fig. 4a′ responding to peak 3 in Fig. 4 a is α-caryophyllene with a match quality of 0.99, peak 2 and peak 1 in Fig. 2a′ which were resolved from peak 1 and 2 in Fig. 4a, are 3-pyridinecarboxylic acid, 5-ethenyl-methyl ester with a match quality of 0.99 and 1-[1,5-dimethylhexyl]-4-methyl-benzene, with a match quality of 0.94. Other overlapped peaks can be identified in the same way. The identified results are listed in Table 1 according to the substance eluting order on HP-5MS.

Many overlapped peaks could be identified by MSS in this work because of the different separation efficiency at the three heating rates on the two columns could provide enough pure spectrum information for us. The following three examples

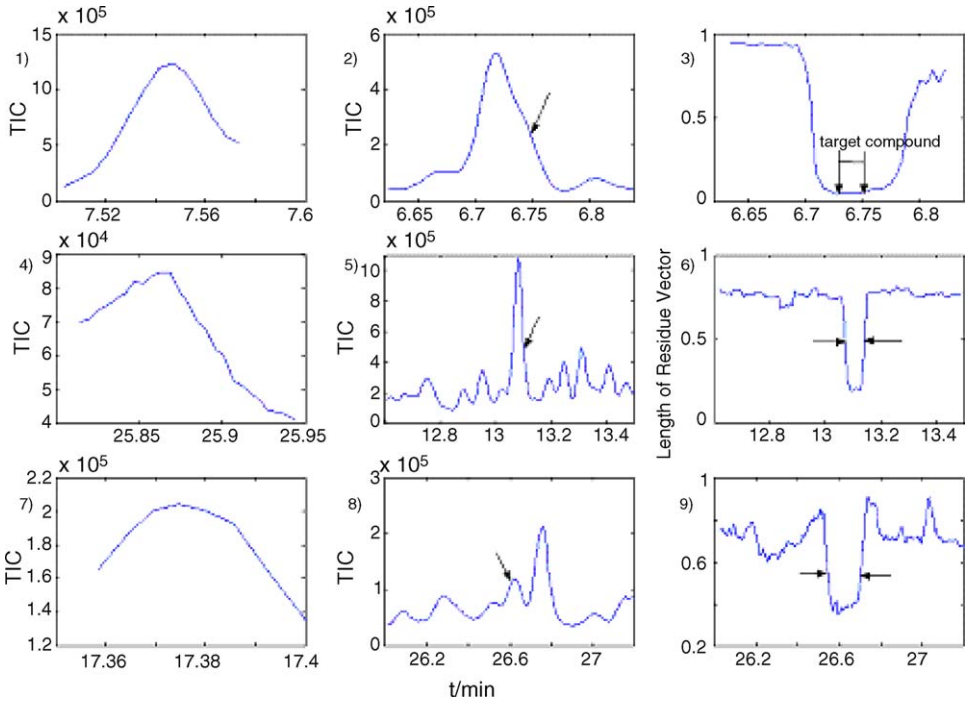


Fig. 5. (1) Pure spectrum of borneol at 6 °C/min on DB-35MS. (2) Spectra including borneol at heating rate of 6 °C/min on HP-5MS. (4) Pure spectrum of [E]-4-[2,6,6-Trimethyl-1-cyclohexen-1-yl]-3-buten-2-one at 2 °C/min on HP-5MS. (5) Spectra including [E]-4-[2,6,6-Trimethyl-1-cyclohexen-1-yl]-3-buten-2-one at heating rate of 6 °C/min on HP-5MS. (7) Pure spectrum of [1α, 4aα, 8aα]- 1,2,4a,5,6,8a-Hexahydro-4-7-dimethyl-1-[1-methylethyl] naphthalene at heating rate of 4 °C/min on HP-5MS. (8) Spectra including [1α, 4aα, 8aα]- 1,2,4a,5,6,8a-Hexahydro-4-7-dimethyl-1-[1-methylethyl] naphthalene at heating rate of 2 °C/min on HP-5MS. (3), (6) and (9) Searching results of the three compounds, respectively.

can illustrate the MSS method. The first one is using the pure spectrum of borneol located in retention time of 7.50–7.57 min (responding to 634–647 scan point range, Fig. 5(1)) of TIC at heating rate of 6 °C/min on DB-35 column as standard spectrum to search a subwindow located in retention time of 6.60–6.80 min (respond to 467–513 scan point range, Fig. 5(2)) of TIC at heating rate of 6 °C/min on HP-5MS column according to the prior PTRI information. This can mitigate greatly the searching burden otherwise the whole retention time range should be searched. The searching result is shown in Fig. 5(3). It can be seen that the retention time of target compound (borneol) can be obtained, with t_R being equal to 6.745 min or PTRI equal to 1171.8. The second example is using spectrum of [E]- 4-[2,6,6-trimethyl-1-cyclohexen-1-yl]-3-buten-2-one, located in retention time of 25.80–25.84 min (4025–4032 scan point range, Fig. 5(4)) of TIC at heating range of 2 °C/min as standard spectrum to search subwindow located in retention time of 12.6–13.5 min (1579–1746 scan point range, Fig. 5(5)) of TIC at heating rate of 6 °C/min on HP-5MS. The searching result (t_R = 13.107 min or I^T = 1493.0) is shown in Fig. 5(6). The last pure spectrum, [1α,4aα,8aα]-1,2,4a,5,6,8a-hexahydro- 4-7-dimethyl-1-[1-methylethyl]-naphthalene, located in retention time of 17.35–17.40 min (2459–2468 scan point range, Fig. 5(7)) of TIC at heating rate of 4 °C/min, was used as standard spectrum to search the retention time of 26.0–27.2 min (4062–4285 scan point range, Fig. 5(8)) of TIC at heating rate of 2 °C/min on HP-5MS column. The searched result (t_R = 26.601 min or I^T = 1497.6) was shown in Fig. 5(9). PTRI values calculated from three heating rates can be used as cross-reference to some compounds identified at one heating rate but unidentified in another one, since the order of elutes will not change at the same column for different heating rates.

In this way, other compounds and their PTRIs can be obtained. All the results were listed in Tables 1 and 2. In Table 2, most of the retention times were obtained by GC–MS and some of them were obtained through HELP and MSS marked by ‘a’ and ‘b’, respectively.

Ninty-five compounds were identified in the oil from buds of *Syringa oblata Lindl*. And six retention indices at three heating rates on two columns of 76 compounds were obtained. Near 60 (57) indices marked by ‘a’ (18) and ‘b’ (39) were obtained by HELP and MSS, respectively. This shows us that with the help of chemometric methods, much more information with improved reliability can be obtained.

Terpenes (13) and oxygenated terpenes (26), aromatic compounds (12), a series of alkanes (19) and heterocyclic compounds (8) are its main components. And there are some other compounds including ester (5), aldehyde (3), ketone (3) and alcohol (3), acid (1) and alkene (1). It is composed of about ten classes of compounds. The majority of them are oxycarbides.

It seems that separation efficiency of essential oil in buds of lilac is better on DB-35MS capillary column than that on HP-5MS from the signal intensity of TICs and the total components identified in Table 1. All the PTRIs of the 95 compounds obtained at the three heating rates on the two capillary columns were listed in Table 2.

Table 2
PTRIs of 95 volatile compounds from buds of lilac determined on HP-5MS and DB-35MS capillary columns at three heating rates

I.D.	I^T on HP-5MS			I^T on DB-35MS		
	2 °C/min	4 °C/min	6 °C/min	2 °C/min	4 °C/min	6 °C/min
1	998.3	998.5	–	–	–	–
2	–	–	–	1087.2	1087.7	1088.4
3	1031.4	1032.5	1033.2	1094.7	1095.0	1095.7
4	1034.5	1035.6	1035.7	1179.1	1183.3	1186.4
5	1045.0	1046.3	1047.1	1204.5[b]	1206.8[b]	1206.1[b]
6	–	–	–	1108.6	1109.4	1111.4
7	–	–	–	1115.0	1118.2	1120.4
8	–	–	–	1125.3	1127.6	1129.1
9	1066.9	1066.9	1066.5	1141.3	1142.3	1143.5
10	1075.3	1079.1	1080.8	1149.0	1151.9	1153.8
11	1102.3	1101.7	1101.0	1173.2	1175.0	1176.6
12	1105.9	1104.8	1104.2	1193.2	1194.4	1194.7
13	1106.9	1106.5	1106.3	1236.4	1237.9	1239.3
14	1112.3	1112.7	1112.7	1188.4	1190.9	1192.7
15	1115.1	1116.2	1116.8	1264.0	1267.3	1266.7[a]
16	1137.4	1139.2	1140.1	–	–	–
17	1166.0[b]	1170.8[b]	1171.8[b]	1272.7	1276.1[a]	1279.5
18	1145.3	1149.3	1151.8	1275.7	1279.2	1282.8
19	1151.6	1153.9	1155.1[a]	1295.2[b]	1296.3	1297.8
20	1157.6	1158.5	1158.3[a]	–	–	–
21	1192.9	1194.4	1195.6	1304.3	1305.1	1307.1
22	1199.4	1201.3	1202.1	1309.3	1311.0	1314.2
23	1209.2[b]	1208.9	1207.8	1319.3[b]	1319.3[b]	1319.4
24	1211.1	1214.0	1215.6	1240.5	1244.1	1247.1
25	–	–	–	1336.4	1340.8	1344.5
26	–	–	–	1354.6	1366.0	1371.6
27	1219.4	1220.9	1221.8	1338.5	1340.4	1354.4
28	1230.1	1232.4	1233.4	1352.7	1355.5	1358.7[a]
29	1253.1	1253.2	1252.9	1354.0	1354.0	1351.2
30	1285.6	1288.0	1289.3	1381.6	1384.9	1389.6
31	1288.1	1292.4	1294.9	1366.4	1372.5	1374.7[a]
32	1293.6	1297.8	1300.3	1378.0	1378.4[b]	1378.6
33	–	1300.6	1301.7[b]	1429.6	1436.3	1439.1[b]
34	–	–	–	1432.4	1437.6	1441.0[b]
35	1298.1	1302.5	1305.2	1401.0[b]	1406.8	1409.4
36	1313.6	1316.1	1317.5	1475.3	1481.8	1486.0
37	1358.7	1362.4	1364.6	1513.2	1523.8	1521.7
38	1373.9	1380.3	1384.5	1392.0	1396.8	1402.2
39	1382.7	1389.0	1394.5	1439.6	1448.6	1454.1
40	1400	1400	1400	1400	1400	1400[b]
41	1417.6	1426.0	1431.8	1486.8	1496.6	1502.7
42	–	–	–	1424.5	1432.2	1437.3
43	–	–	–	1525.4[a]	1530.8[a]	1534.8[a]
44	–	–	–	1527.1[a]	1533.8[a]	1538.9[a]
45	1449.7	1458.7	1463.1	1528.7	1543.1	1547.2
46	1462.0	1462.5	1464.4	1448.6	1446.9[b]	1448.7
47	1473.8	1481.0[b]	1485.2	1551.8	1507.6	1552.7
48	1477.4	1486.0	1491.7	1561.6	1573.0	1578.9
49	1483.9	1488.9	1493.0[b]	1613.1	1621.0	1625.2
50	1500	1500	1500	1500	1500	1500
51	1497.6[b]	1504.0	1504.4	1578.6	1610.8	1593.4
52	1508.1[b]	1509.5	1511.4[a]	1574.7	1578.0	1582.7[b]
53	1527.5	1527.0	1530.8	1603.8	1613.1	1618.7
54	1600[b]	1600[b]	1600[a]	1600	1600	1600[b]
55	1553.4	1552.4	1556.3	–	–	–
56	1572.5	1567.7	1568.9	1657.3	1659.1	1662.8
57	1584.3	1586.8	1593.0	1710.6	1723.6	1732.9
58	–	–	–	1719.8	1733.1	1742.1
59	1627.2	1635.3	1641.2	1753.1	1764.4	1772.6
60	1638.2	1645.7	1651.2	–	–	–
61	1645.0	1655.0	1662.4	1782.5	1798.1[b]	1809.7
62	–	–	–	1782.6	1795.4	1804.4
63	1651.1	1659.2	1665.4	1787.7	1798.3	1789.0
64	1656.5	1663.2	1668.5	1887.7	1895.4	1868.0
65	1682.0	1688.7	1695.7	1805.3	1813.6	1821.0
66	1700	1700	1700	1700	1700	1700[b]
67	1711.8	1728.9[b]	1734.4[b]	1896.1	1905.2	1914.6[b]
68	1759.6	1778.4	1790.1	2019.8	2045.9	2062.8[b]
69	1800	1800	1800	1800	1800[b]	1800
70	1846.0	1847.9	1848.9	1917.6	1913.1	1919.7
71	1867.8	1870.9	1874.3	2053.9	2060.3	2063.6
72	1900	1900	1900	1900	1900[b]	1900
73	1927.0	1927.3	1927.7	2005.3	1999.4	2005.7
74	1959.7	1965.0	1968.4	2165.5	2173.1	2177.2
75	–	–	–	2082.7	2087.6	2092.4
76	1996.0	1995.7	1995.7	2065.8	2069.2	2074.0[b]
77	–	–	–	1993.3	1987.5	1994.5
78	2000	2000	2000	2000	2000	2000[b]
79	2063.0	2062.8	2062.8	2055.6	2055.6[a]	2055.7[a]
80	2083.2	2084.5	2085.5	2169.2[b]	2170.7	2171.9
81	2092.1	2094.5	2095.6[a]	2202.3	2206.2	2207.4
82	–	–	–	2221.3	2226.9	2227.6
83	2100	2100	2100	2100	2100	2100
84	2112.9	2115.6	2117.7	2190.4	2192.6	2195.1
85	2200	2200	2200	2200	2200	2200
86	2261.9	2261.7	2262.3	2253.5	2256.2	2256.3
87	2285.0	2286.4	2287.6[a]	2662.4[b]	2663.1[b]	2663.2[b]
88	2300	2300	2300	2300	2300	2300
89	2400	2400	2400	2400	2400	2400
90	2500	2500	2500	2500	2500	2500
91	2600	2600	2600	2600	2600	2600
92	2700	2700	2700	2700	2700	2700
93	2800	2800	2800	2800	2800	2800
94	2900	2900	2900	2900	2900	2900
95	3100	3100	3100	3100	3100	3100

–: Unidentified or unknown.
[a] Identified by CRM.
[b] Identified by MSS.

3.2. Temperature dependence of retention indices

Table 3, generated from Table 2, exhibits the temperature dependence of retention indices of components in essential oil from buds of lilac on HP-5MS and DB-35MS. It shows that aliphatic compounds give nearly constant PTRIs and those of terpenoids do not vary much at different heating rates. But PTRIs of aromatic compounds exhibit relatively large temperature dependence. Secondly, generally speaking, PTRIs vary much more on DB-35MS than those on HP-5MS according the compound type. The differences of PTRIs between the two columns, in general, increase from aliphatic compounds to terpenoids to aromatic compounds. This temperature dependence tendency is consistent with that reported by Lai and Song [24].

3.3. Identification of components in flower essential oil of lilac

The unambiguous identification of the lilac flower essential oil components was achieved using the temperature-

Table 3
PTRIs for essential oil in buds of lilac on HP-5MS and DB-35MS

S. no.	I.D.	HP-5MS					DB-35MS				
		I^T range (i.u.)		T range (°C)		$\Delta I^T/\Delta T$ (i.u./°C)	I^T range (i.u.)		T range (°C)		$\Delta I^T/\Delta T$ (i.u./°C)
1.	1	998.3	998.5	89.4	96.5	0.03					
2.	2						1087.2	1088.4	90.4	105.5	0.08
3.	3	1031.4	1033.2	91.0	106.5	0.12	1094.7	1095.7	90.8	106.1	0.06
4.	4	1034.5	1035.7	91.2	106.7	0.08	1179.1	1186.4	95.9	114.7	0.39
5.	5	1045.0	1047.1	91.8	107.8	0.13	1204.5	1206.1	97.5	117.1	0.08
6.	6						1108.6	1111.4	91.5	107.4	0.18
7.	7						1115.0	1120.4	91.9	108.6	0.32
8.	8						1125.3	1129.1	92.5	109.2	0.23
9.	9	1066.9	1067.0	92.9	109.5	0.006	1141.3	1143.5	93.5	110.6	0.13
10.	10	1075.3	1080.8	93.3	110.8	0.31	1149.0	1153.8	94.0	111.6	0.27
11.	11	1102.3	1103.0	94.8	112.7	0.04	1173.2	1176.6	95.5	113.8	0.19
12.	12	1105.9	1106.2	95.1	113.0	0.02	1193.2	1194.7	96.7	115.5	0.08
13.	13	1106.9	1107.3	95.2	113.2	0.02	1236.4	1239.3	100.1	120.6	0.14
14.	14	1112.3	1112.7	95.6	114.0	0.02	1188.4	1192.7	96.4	115.3	0.23
15.	15	1115.1	1116.8	95.8	114.4	0.09	1264.0	1266.7	102.4	124.1	0.12
16.	16	1137.4	1140.1	97.5	117.0	0.14					
17.	17	1166.0	1171.8	99.6	127.4	0.21	1272.7	1279.5	103.1	125.3	0.31
18.	18	1145.3	1151.8	98.1	118.3	0.32	1275.7	1282.8	103.3	125.6	0.32
19.	19	1151.6	1155.1	98.5	118.6	0.17	1295.2	1297.8	104.9	127.4	0.12
20.	20	1157.6	1258.3	99.0	119.0	0.04					
21.	21	1192.9	1195.6	101.7	123.1	0.13	1304.3	1307.1	105.7	128.5	0.12
22.	22	1199.4	1202.1	102.2	123.8	0.12	1309.3	1314.2	106.2	129.3	0.21
23.	23	1209.2	1209.3	103.1	124.5	0.005	1319.3	1319.4	120.5	130.0	0.01
24.	24	1211.1	1215.6	103.3	125.4	0.20	1240.5	1247.1	100.4	121.5	0.31
25.	25						1336.4	1344.5	109.0	132.9	0.34
26.	26						1354.6	1371.6	111.6	136.1	0.69
27.	27	1219.4	1221.8	104.0	126.2	0.11	1338.5	1359.3	109.2	134.7	0.82
28.	28	1230.1	1233.4	105.1	127.6	0.15	1352.7	1358.7	110.7	134.8	0.25
29.	29	1253.1	1253.4	107.3	130.0	0.01	1354.0	1356.0	110.8	134.3	0.09
30.	30	1285.6	1289.3	110.4	134.3	0.12	1381.6	1389.6	113.7	138.3	0.34
31.	31	1288.1	1294.9	110.6	135.0	0.28	1366.4	1374.7	112.1	136.7	0.32
32.	32	1293.6	1300.3	111.1	135.6	0.27	1378.0	1378.6	113.3	136.8	0.03
33.	33	1300.6	1301.7	125.8	135.8	0.11	1429.6	1439.1	118.9	144.5	0.37
34.	34						1432.4	1441.0	119.2	144.7	0.34
35.	35	1298.1	1305.2	111.6	136.2	0.29	1401.0	1411.4	117.5	140.9	0.44
36.	36	1313.6	1317.5	113.2	137.7	0.16	1475.3	1486.0	124.0	150.2	0.41
37.	37	1358.7	1364.6	118.0	143.3	0.23	1513.2	1521.7	128.2	154.6	0.32
38.	38	1373.9	1384.5	119.6	145.6	0.41	1392.0	1402.2	114.8	139.8	0.41
39.	39	1382.7	1394.5	120.6	146.8	0.45	1439.6	1454.1	120.0	146.3	0.55
40.	41	1417.6	1431.8	124.4	151.3	0.53	1486.8	1502.7	125.3	152.3	0.59
41.	42						1424.5	1437.3	118.3	144.2	0.49
42.	43						1525.4	1534.8	129.6	156.1	0.35
43.	44						1527.1	1538.9	129.8	156.6	0.44
44.	45	1449.7	1463.1	127.9	155.0	0.49	1528.7	1547.2	130.0	157.6	0.67
45.	46	1462.0	1464.4	129.3	155.2	0.09	1448.6	1448.7	121.0	145.6	0.004
46.	47	1473.8	1485.2	130.6	157.7	0.42	1551.8	1552.7	132.5	158.2	0.04
47.	48	1477.4	1491.7	131.0	158.5	0.52	1561.6	1578.9	133.6	161.3	0.62
48.	49	1483.9	1493.0	131.7	158.6	0.34	1613.1	1625.2	139.3	166.7	0.44
49.	51	1497.6	1504.4	133.2	160.4	0.25	1578.6	1593.4	135.5	163.0	0.54
50.	52	1508.1	1509.5	134.5	160.8	0.01	1574.7	1582.7	135.0	161.7	0.30
51.	53	1527.5	1530.8	135.8	163.2	0.12	1603.8	1618.7	138.3	166.0	0.54
52.	55	1553.4	1556.3	138.6	166.3	0.10					
53.	56	1572.5	1572.9	140.8	167.8	0.01	1657.3	1662.8	144.2	171.1	0.20
54.	57	1584.3	1593.0	142.0	170.7	0.30	1710.6	1732.9	150.0	179.1	0.77
55.	58						1719.8	1742.1	151.0	180.4	0.76
56.	59	1627.2	1641.2	147.4	176.0	0.49	1753.1	1772.6	154.6	183.5	0.67
57.	60	1638.2	1651.2	148.6	177.0	0.46					
58.	61	1645.0	1662.4	149.3	178.2	0.26	1782.5	1809.7	157.8	187.6	0.91
59.	62						1782.6	1804.4	157.8	187.1	0.75
60.	63	1651.1	1665.4	150.0	178.6	0.50	1787.7	1789.0	158.4	185.4	0.05
61.	64	1656.5	1668.5	150.5	178.9	0.42	1887.7	1907.8	168.5	197.7	0.69
62.	65	1682.0	1695.7	153.3	181.8	0.48	1805.3	1821.0	160.2	188.8	0.55

Table 3 (*Continued*)

S. no.	I.D.	HP-5MS					DB-35MS				
		I^T range (i.u.)		T range (°C)		$\Delta I^T/\Delta T$ (i.u./°C)	I^T range (i.u.)		T range (°C)		$\Delta I^T/\Delta T$ (i.u./°C)
63.	67	1711.8	1734.4	156.4	185.5	0.78	1896.1	1914.6	169.3	198.4	0.97
64.	68	1759.6	1790.1	161.3	191.8	1.00	2019.8	2062.8	181.4	213.4	1.34
65	70	1846.0	1848.9	170.0	197.9	0.10	1917.6	1919.7	171.5	198.9	0.08
66.	71	1867.8	1874.3	172.0	200.5	0.23	2053.9	2063.6	184.8	213.5	0.34
67.	73	1927.0	1927.7	178.0	205.8	0.02	2005.3	2005.7	180.0	207.6	0.01
68.	74	1959.7	1968.4	181.1	209.7	0.30	2165.5	2177.2	195.4	224.3	0.40
69.	75						2082.7	2092.4	190.2	216.4	0.34
70.	76	1996.0	1996.3	184.5	212.4	0.01	2065.8	2074.0	186.0	214.5	0.29
71.	77						1993.3	1994.5	178.8	206.4	0.04
72.	79	2063.0	2063.4	190.7	218.8	0.01	2055.6	2055.7	185.0	212.7	0.004
73.	80	2083.2	2085.5	192.6	221.0	0.08	2169.2	2171.9	195.8	223.8	0.096
74.	81	2092.1	2095.6	193.4	222.0	0.12	2202.3	2207.4	198.7	227.1	0.18
75.	82						2221.3	2227.6	200.5	229.0	0.22
76.	84	2112.9	2117.7	195.3	223.9	0.17	2190.4	2195.1	197.6	226.0	0.16
77.	86	2261.9	2262.3	208.1	236.7	0.01	2253.5	2256.3	203.5	231.5	0.10
78.	87	2285.0	2287.6	210.1	239.0	0.09	2662.4	2663.2	204.3	232.4	0.03

programmed retention indices obtained earlier and their standard electronic impact (EI) mass spectra data in combination with chemometric methods. Seventy-two compounds were identified and listed in Table 1, too. Of all the identified 72 compounds, there are 15 terpenes and 14 oxygenated terpenes, 10 aromatic compounds and 13 *n*-alkanes. Other compounds classes are ester (6), ketone (5), acid (4), heteroatomic ring compounds (3), aldehyde (1) and alcohol (1).

4. Conclusions

Firstly, GC–MS combined with retention indices can perform much more reliable qualitative analysis of complicated essential oil samples than using GC–MS alone if the chromatographic peaks are separated each other satisfying. Secondly, chemometric resolution method is a useful and valid tool to overlapped peaks and/or peaks with low ratios of signal to noise. When pure mass spectra extracted by chemometric resolution were compared with standard mass spectra, the identity of components could be gained with much higher match quality than directly library searching and the qualitative analysis result could be much more reliable. Thirdly, moving subwindow searching is more efficiency than resolution methods when pure spectrum could be obtained. Finally, PTRI database constructed in this work could be useful and convenient in other essential oil analysis and the knowledge of PTRI on temperature dependence and polarity of capillary column can be applied in other researches.

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References

[1] C. Cavaleiro, L.R. Salgueiro, M.G. Miguel, A. Proceca da Cunha, J. Chromatogr. A 1003 (2004) 187.
[2] M. Hudaib, E. Speroni, A.M. Di Pietra, V. Cavrini, J. Pharma. Biomed. Anal. 29 (2002) 691.
[3] A.J. Maticha, H. Young, J.M. Allen, M.Y. Wang, S. Fielder, M.A. McNeilage, E.A. MacRae, Phytochemistry 63 (2003) 285.
[4] C. Wagner, M. Sefkow, J. Kopka, Phytochemistry 62 (2003) 887.
[5] E.Sz. Kovats, Helv. Chim. Acta. 41 (1958) 1915.
[6] P.Z. Cong, K.M. Su, Mass-Spectrometry Fascicule of Analytical Chemistry Handbook, The ninth fascicule, (second ed.) [M], Chemical Industry Press, Beijing, 2000.
[7] K.E. Miller, T.J. Bruno, J. Chromatogr. A 1007 (2003) 117.
[8] Y.P. Du, Y.Z. Liang, C.J. S Wu, Chinese 8th Computers and Applied Chemistry Conference, 2001, Huangshan, 2001, p. 147.
[9] J.M. Davis, J.C. Giddings, Anal. Chem. 55 (1983) 418.
[10] Y.Z. Liang, M. Olav, Kvalheim, Fresenius, J. Anal. Chem. 370 (2001) 694.
[11] F. Gong, Y.Z. Liang, H. Cui, F.T. Chau, B.T.-P. Chan, J. Chromatogr. A 909 (2001) 237.
[12] C.J. Xu, Y.Z. Liang, Y.Q. Song, J.S. Li, Fresenius, J. Anal. Chem. 371 (2001) 331.
[13] Y.Z. Liang, White, Gray and Black Multicomponent Systems and Their Chemometrics Algorithms, Hunan Publishing House of Science and Technology, Changsha, Hunan, 1996.
[14] Y.Z. Liang, Ol.M. Kvalheim, Chemo Intelli Lab Sys. 20 (1993) 115.
[15] H.L. Shen, Y.Z. Liang, R.Q. Yu, F. Li, X.X. Sun, Sci. China (Series B), 41 (1998) 21.
[16] H.L. Shen, H. Cui, Y.Z. Liang, F. Li, Acta Chim. Sin. 56 (1998) 378.
[17] A.W.M. Lee, W.F. Chan, Fion, C.H. Lo, Y.Z. Liang, Anal. Chim. Acta 339 (1997) 123.
[18] Jiangsu New Medicinal Academy. Chinese Traditional Medicinal Big Thesaurus, Shanghai Demos Press House, Shanghai, 1981.
[19] D. Lu, A.P. Lu, P.Y. Li, Chinese Special Wild Economic Animal and Plant Research 4 (2003) 41.
[20] D. Lu, P.Y. Li, J.H. Li, Chinese Traditional and Herbal Drugs 34 (8) (2003) 688.
[21] Chinese Pharmacopoeia Committee, Chinese Pharmacopoeia-Appendix 62, Publishing House of People's Health, 2000.
[22] H. van den Dool, P.D. Kratz, J. Chromatogr. 11 (1963) 463.
[23] C.J. Xu, Y.Z. Liang, F. Gong, H. Cui, Anal. Chim. Acta 428 (2001) 235.
[24] W.C. Lai, C.S. Song, Fuel 74 (1995) 1436.