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Research Article

Characterization of Chemical Composition of Pericarpium Citri Reticulatae Volatile Oil by Comprehensive Two-Dimensional Gas Chromatography with High-Resolution Time-of-Flight Mass Spectrometry

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Pericarpium Citri Reticulatae (Chenpi in Chinese) has been widely used as an herbal medicine in Korea, China, and Japan. Chenpi extracts are used to treat indigestion and inflammatory syndromes of the respiratory tract such as bronchitis and asthma. This thesis will analyze chemical compositions of Chenpi volatile oil, which was performed by comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (GC × GC-HR-TOFMS). One hundred and sixty-seven components were tentatively identified, and terpene compounds are the main components of Chenpi volatile oil, a significant larger number than in previous studies. The majority of the eluted compounds, which were identified, were well separated as a result of high-resolution capability of the GC × GC method, which significantly reduces, the coelution. β-Elemene is tentatively qualified by means of GC × GC in tandem with high-resolution TOFMS detection, which plays an important role in enhancing the effects of many anticancer drugs and in reducing the side effects of chemotherapy. This study suggests that GC × GC-HR-TOFMS is suitable for routine characterization of chemical composition of volatile oil in herbal medicines.

1. Introduction

Pericarpium Citri Reticulatae (Chenpi in Chinese) has been widely used as an herbal medicine for a long time in China, Korea, and Japan, for its pharmacologic activity, rich resources, low toxicity, and costs. Chenpi is the dried ripe fruit peel of *Citrus reticulata* Blanco and its cultivars, gathered from September to December [1]. Their main cultivars are *Citrus reticulata* "Chachi," Citrus Reticulata "Dahongpao," and *Citrus erythrosa* Tanaka. In Chinese people's traditional use, Chenpi is mostly utilized to eliminate phlegm and strengthen spleen [2]. Moreover, Chenpi is extensively added to food as a condiment.

It is well known that Chenpi contains various bioactive compounds, such as flavonoids, phenolic acids, and limonoids [2, 3]. In the present study, most reports on Chenpi focus on phenolic compounds and flavonoids [3–7], but few focus on volatile compounds which also have strong pharmacologic bioactivities. For example, high-performance liquid chromatography (HPLC), high-speed countercurrent chromatography (HSCCC), and capillary electrophoresis (CE) have been applied for the determination of phenolic compounds and flavonoids of Chenpi [8–10]. However, reviewing the literature, it seems that the chemical composition of the volatile oil of Chenpi has been little investigated [11]. Furthermore, the volatile compounds of Chenpi may contribute

to pharmacological effects of Chenpi extracts reported above [12, 13]. Therefore, a method able to rapidly identify the volatile compounds of Chenpi could be a useful tool for the purpose of a complete phytochemical analysis.

Gas chromatography-mass spectroscopy (GC-MS) has been used for the qualitative analysis of the volatile constituents in Chenpi [11]. But it is difficult to achieve the complete separation of minor volatile components and many coelution volatile constituents. To solve these problems, it is necessary to use multidimensional gas chromatography. Comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (GC × GC-HR-TOFMS) is a new developed powerful and versatile analytical tool, which combines two powerful analytical technologies with complementary attributes [14, 15]. GC × GC separates chemical species with two capillary columns interfaced by a modulator that traps and concentrates eluents from the first column, and it then introduces them into the second column, producing a full secondary chromatogram for each single data point of a traditional one-dimensional separation [16, 17]. HR-TOFMS provides mass precision that is fine enough to distinguish elemental compositions, providing a more definitive basis for molecular identification. GC × GC is important for HR-TOFMS because the better separations significantly reduce the coelution and the problems of mass spectral mixing. And, HR-TOFMS is important for GC × GC because the structural and compositional information available with HR-TOFMS aids in the interpretation of the rich, complex data from GC × GC separations [18]. GC × GC-TOFMS has been successfully applied in the volatile oil study and greatly improves the result of component separation and identification [19, 20]. In this study, the volatile oil of Chenpi was firstly separated and detected with GC × GC-HR-TOFMS (Figure 1).

2. Materials and Methods

2.1. Samples. Chenpi sample (fruit peels of *Citrus reticulate* "Dahongpao") was collected from Zigong in Sichuan province, China. The sample was authenticated by Professor Chen Jianwei from Nanjing University of Traditional Chinese Medicine, China.

2.2. Extraction of Volatile Oil. After the sample was dried for 2 h at 45°C and smashed, 50 g of sample was swollen with 600 mL of distilled water in a standard extractor for extracting volatile oil for 3 h. Then, the volatile oil was dried over anhydrous sodium sulphate until all the water was dried and then stored in the dark glass bottle at 4°C prior to GC × GC-HR-TOFMS analysis.

2.3. GC-MS System and GC × GC-HR-TOFMS Apparatus. GC × GC separations were performed by Tofwerk AG (Thun, Switzerland) on an Agilent 7890 A GC and 7693 autosampler with: 1 μL splitless injection; column one DB-XLB (Agilent), 15 m × 0.25 mm, 0.25 μm film thickness; column two BPX-50 (SGE), 1 m × 0.1 mm, 0.1 μm film thickness; oven temperature from 50 to 230°C at 2.0°C min^{-1} ramp; inlet pressure from 35 PSI to 61.5 PSI at 0.28 PSI min^{-1}; injection temperature 250°C; transfer line temperature 300°C; Zoex ZX2 thermal modulator with a 7 s modulation period, 300 ms modulation duration, 375°C hot jet temperature, 18 L min^{-1} cold jet nitrogen flow rate, and 40 PSI hot jet nitrogen pressure. The Zoex FasTOF time-of-flight- (TOF-) HRMS system used 70 eV EI ion source, 280°C ion source temperature, a mass range of m/z 50–450 with 4000 FWHM resolution, and 100 spectra per second acquisition rate.

2.4. Data Conversion and Peak Table Generation. The final data for each chromatogram is an array of 1000 × 600 data points, each data point with a HRMS vector of 40 K intensities. Thus, each chromatogram has 24 billion values requiring 96 gigabytes for representing for single-precision floating point numbers without compression. The set of 18 chromatograms has more than 1.7 terabytes of uncompressed data. The data were compressed and stored by the Zoex FasTOF system to HDF5-format files and were processed with GC Image GC × GC Software R2.1. In order to manage such large files on computers with limited random access memory (RAM), GC Image Software maintains a chromatogram with integer mass or centroid-resampled spectra in RAM and accesses the HR-MS data from disk as needed. GC Image can export raw data and computed results to nonproprietary file formats for processing with external software. The components can be quantified by Zoex software (Zoex Corp, Lincoln, NE, USA).

All peaks with signal-to-noise ratio higher than 100 were found in the raw GC × GC chromatogram. The workstation can automatically give the parameters such as similarity, reverse, and probability of peaks via comparing them with the compounds in the library. The results were combined in a peak table. The NIST/EPA/NIH Mass Spectral Library Version 2.0 was used in this work.

3. Results and Discussion

3.1. Qualitative Analysis of Chenpi Volatile Oil. The column system is nearly orthogonal and provides a structured separation. A typical two-dimensional separation/total ion chromatogram (TIC) and three-dimensional chromatogram are shown in Figure 2. In the GC × GC system, compounds are separated by volatility difference on the first dimension nonpolar column and by polarity on the second medium-polar column. The GC × GC system accomplishes the true orthogonal separation on account for both the change of the polarity of two fixed phases and the linear temperature programming.

Using GC × GC-HR-TOFMS, the quantity of the detected components was up to 834. Compared to the traditional identification method such as GC-MS, the analysis from GC × GC-HR-TOFMS becomes more reliable, relying on the combined identification information including retention times, similarity, reverse match factor, and probability. The similarity and reverse match factors indicate how well a mass spectrum matches the library spectrum, but the isomers have similar mass spectra. In this case, the probability is used to determine whether the peaks with the same name belong to one

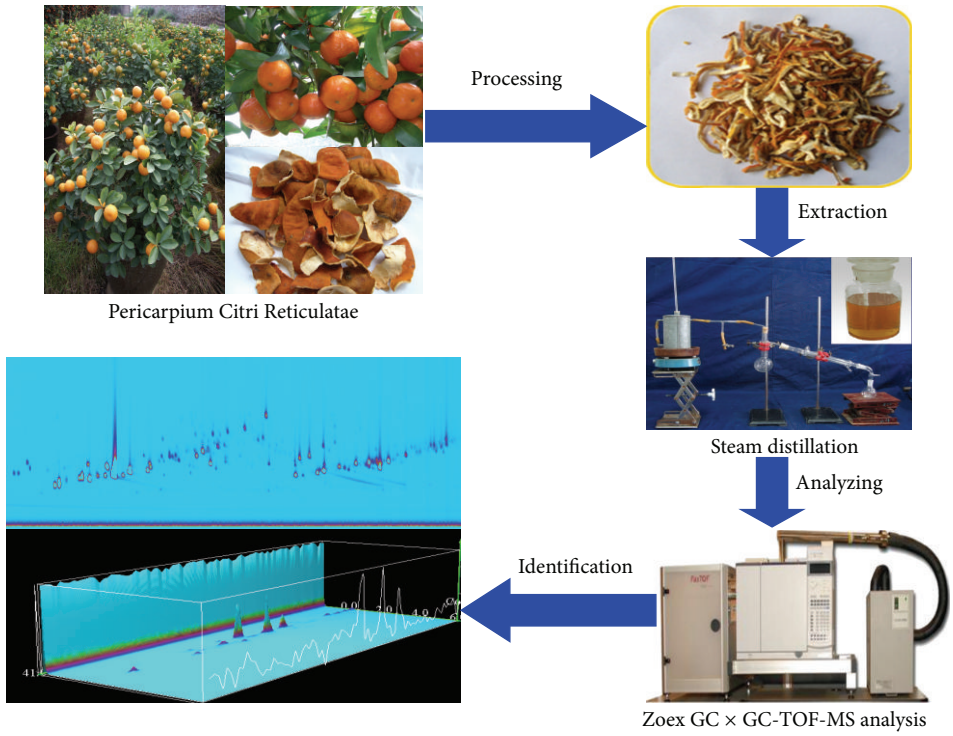
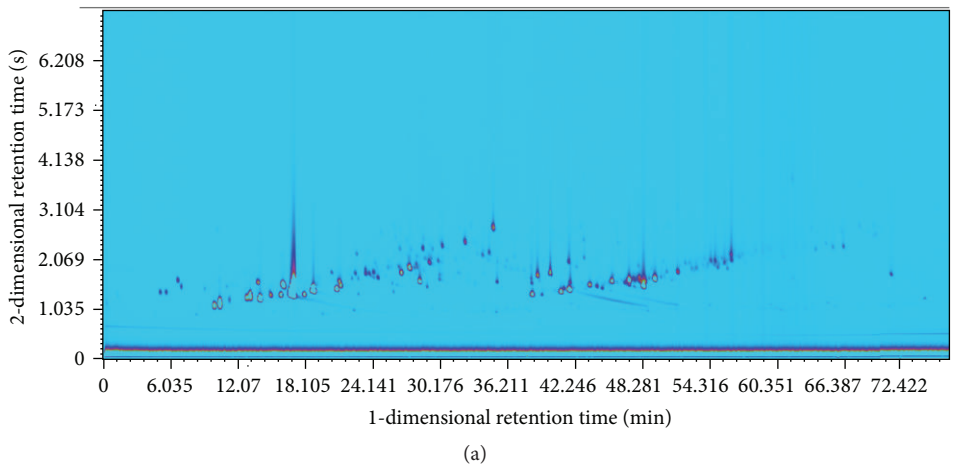
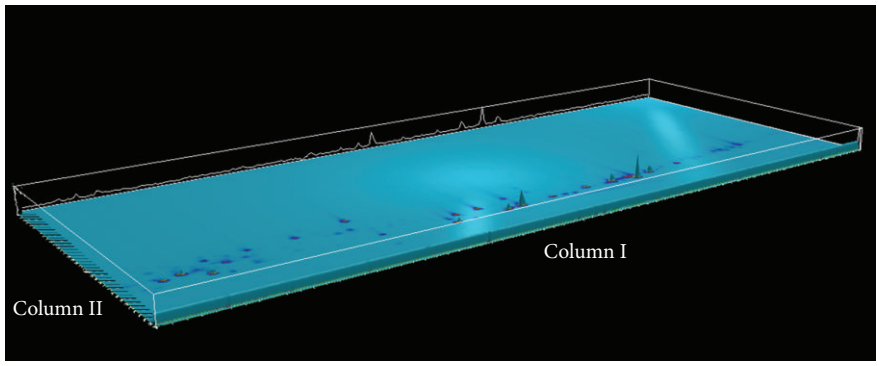


Figure 1: Flow chart of the chemical composition study of Chenpi volatile oil by GC × GC-HR-TOFMS.



(a)



(b)

Figure 2: GC × GC-HR-TOFMS chromatogram (a) and three-dimensional chromatogram (b) of Chenpi volatile oil.

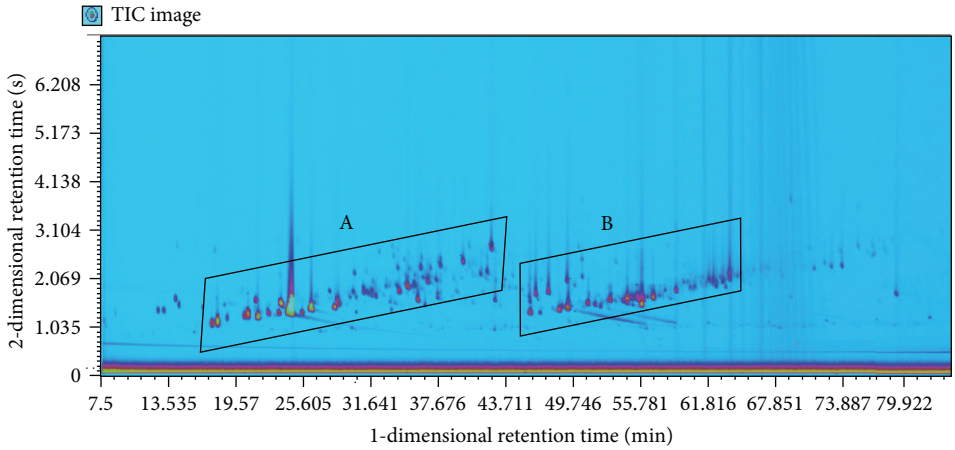


Figure 3: The GC × GC contour plot of Chenpi volatile oil group separation result. Regions marked by squares (A) and (B) were identified mainly as monoterpenes and sesquiterpenes, respectively.

compound or several compounds. The GC × GC-HR-TOF/MS software was used to find all the peaks in the raw GC × GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0, and the results were combined in a single peak table. A similarity and reverse match factor above 583 and 612, respectively, indicates that an acquired mass spectrum usually shows a good match with the library spectrum. Because of the numerous isomers present in volatile oils, especially within monoterpenes and sesquiterpenes, more attention should be paid for identification using mass spectra. In order to enhance the reliability of the identification by MS, both similarity and reverse match factor should be used. According to our experience and the literature data [18–20], 167 compounds with good match were tentatively identified including 50 monoterpenes, 36 sesquiterpenes, 31 esters and acids, 9 aldehydes and ketones, 6 alcohols, 3 ethers, 12 phenyl compounds, and 20 other components. Compounds have lower search probabilities than these counted as unknowns, and were disqualified for Kovats index comparison. Table 1 listed 167 components identified in Chenpi volatile oil. The volatile fraction is characterized by high percentages of monoterpenes, sesquiterpenes, and esters, including β-elemene, p-mentha-1(7),8(10)-dien-9-ol, and limonene. In this study, many components have also been tentatively identified, which were found in Chenpi volatile oil for the first time such as globulol and isoledene. There is high possibility that they will be literally useful for further pharmaceutical research of Chenpi volatile oil.

3.2. Group Separation of Chenpi Volatile Components. In GC × GC-HR-TOFMS analysis, the 167 identified volatile components in Chenpi volatile oil were mainly classified into two groups that can be seen in Figure 3. Based on GC × GC-HR-TOFMS, it can be found that the peaks in areas A and B are monoterpenes and sesquiterpenes, respectively. These monoterpenes and sesquiterpenes are mainly alkenes, alcohols, and ethers. It was also found that a lot of saturated and unsaturated fatty acid esters and phenyl compounds constitute the Chenpi volatile oil. This study demonstrates that GC × GC-HR-TOFMS is a powerful separation and identification tool that allows for the identification and group separation of a much larger number of complex volatile oil components.

3.3. Identification of Three Coelution Volatile Components in Chenpi Volatile Oil. The high-resolution mass spectra in the TIC can be used for accurate identification of volatile compounds in Chenpi volatile oil, and these identified compounds will be significant to the further pharmaceutical research. For example, Figure 4 compares the high resolution mass spectrum of the blob (peak) marked with 138 (49.14 min, 2.02 s), 104 (49.26 min, 1.58 s), and 77 (49.14 min, 1.45 s), head-to-tail with the mass spectrum of p-mentha-1(7),8(10)-dien-9-ol, dodecanal, and β-elemene TMS from the NIST/EPA/NIH library mass spectra. For p-mentha-1(7),8(10)-dien-9-ol, the forward match factor is 806; reverse match factor is 855; and probability is 20.12%. For dodecanal, the forward match factor is 763; reverse match factor is 773; and probability is 7.16%. For β-elemene, the forward match factor is 911; reverse match factor is 914; and probability is 17.11%. The above three volatile components cannot be clearly separated or identified by traditional one-dimensional gas chromatography or GC-MS method, because they are coelution volatile components, which have very similar chemical properties including volatility and polarity. In this study, the three coelution volatile components in Chenpi volatile oil were well separated and identified by GC × GC-HR-TOFMS, which have not been reported in other studies (Figure 4).

This study showed that GC × GC-HR-TOFMS represents a powerful separation and analysis tool for the analysis of complex volatile oils of herbal medicines. GC × GC-HR-TOFMS can give the information about the formula and structures, can provide the opportunity for differentiating different volatile oils, can give the subtle differences of the oils from different areas, and can find new compounds that have the possible pharmaceutical effect on some diseases.

Table 1: 167 main volatile components identified in the Chenpi volatile oil.

No.	Compound name	Peak I/min	Peak II/s	Volume	Library formula	Library probability	Library CAS no.
(1)	Nonanal	28.62	1.6	2367.153	$C_9H_{18}O$	50.1	124-19-6
(2)	Pyrrolizidine-3-one-5-ol, ethyl ether	53.49	2.74	53.1709	$C_9H_{15}NO_2$	17.7	0-00-0
(3)	2-Methoxy-4-vinylphenol	42.38	2.73	2959.926	$C_9H_{10}O_2$	59.39	7786-61-0
(4)	Styrene	14.14	1.65	731.272	C_8H_8	36.4	100-42-5
(5)	Octanal	21.38	1.58	2235.185	$C_8H_{16}O$	62.19	124-13-0
(6)	Ethylbenzene	12.57	1.39	484.4996	C_8H_{10}	64.86	100-41-4
(7)	Pterin-6-carboxylic acid	11.24	0.82	50.3913	$C_7H_5N_5O_3$	40.94	948-60-7
(8)	Hexadecane, 1,1-bis(dodecyloxy)-	77.27	1.2	102.3988	$C_{40}H_{82}O_2$	9.24	56554-64-4
(9)	1-Heptatriacotanol	75.09	2.76	169.2274	$C_{37}H_{76}O$	54.56	105794-58-9
(10)	Cholestan-3-ol, 2-methylene-, (3β,5α)-	64.35	2.11	268.4351	$C_{28}H_{48}O$	13.09	22599-96-8
(11)	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	66.4	2.23	151.311	$C_{28}H_{34}N_2O$	44.04	0-00-0
(12)	1,1′-(4-Methyl-1,3-phenylene)bis[3-(5-benzyl-1,3,4-thiadiazol-2-yl)urea]	23.55	2.15	106.0574	$C_{27}H_{24}N_8O_2S_2$	24.95	0-00-0
(13)	Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitro-benzofurazan-4-yl]amino-	46.49	0.79	105.5334	$C_{26}H_{27}N_5O_6$	9.48	0-00-0
(14)	Benzene, 1,1′-[3-(3-cyclopentylpropyl)-1,5-pentanediyl]bis-	7.74	1.18	149.2865	$C_{25}H_{34}$	15.18	55191-62-3
(15)	2-(2-Azepan-1-yl-2-oxoethyl)-1-hydroxy-1-phenyl-octahydro-pyrido[1,2-a]azepin-4-one	76.42	2.77	142.1083	$C_{24}H_{34}N_2O_3$	52.15	0-00-0
(16)	6,9,12,15-Docosatetraenoic acid, methyl ester	59.4	1.77	55.943	$C_{23}H_{38}O_2$	19.28	17364-34-0
(17)	2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	53.49	2.15	64.4497	$C_{23}H_{32}O$	25.57	0-00-0
(18)	Naphthalen-2-yl-acetic acid, 6-hydroxy-6-methyl-cyclodecyl ester	41.54	2.63	65.5915	$C_{23}H_{30}O_3$	27.3	0-00-0
(19)	Z-5-Methyl-6-heneicosen-11-one	80.89	1.11	115.1736	$C_{22}H_{42}O$	8.27	0-00-0
(20)	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	61.45	1.91	133.6559	$C_{22}H_{40}O_2$	14.4	56599-50-9
(21)	Doconexent	17.88	1.63	127.9331	$C_{22}H_{32}O_2$	40.98	6217-54-5
(22)	9,12,15-Octadecatrienoic acid, 2,3-dihydroxy-propyl ester, (Z,Z,Z)-	31.16	2.22	105.1463	$C_{21}H_{36}O_4$	19.78	18465-99-1
(23)	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	42.75	2.02	216.8234	$C_{21}H_{36}O_2$	8.42	21061-10-9
(24)	5,8,11-Eicosatrienoic acid, methyl ester	57.59	2.03	96.1455	$C_{21}H_{30}O_2$	43.95	0-00-0
(25)	cis-5,8,11,14,17-Eicosapentaenoic acid	24.52	4.25	53.7632	$C_{20}H_{30}O_2$	13.99	10417-94-4
(26)	5,8,11,14-Eicosatetraynoic acid	58.8	3.17	144.9613	$C_{20}H_{24}O_2$	29.78	1191-85-1
(27)	1,16-Cyclocorynan-17-oic acid, 19,20-di-dehydro-, methyl ester, (16S,19E)-	12.21	0.64	88.5548	$C_{20}H_{22}N_2O_2$	53.45	6393-66-4
(28)	Octadecane, 6-methyl-	82.09	1.25	184.9235	$C_{19}H_{40}$	18.41	10544-96-4
(29)	2-Methyl-E,E-3,13-octadecadien-1-ol	64.83	2.01	124.0941	$C_{19}H_{36}O$	9.74	0-00-0
(30)	Z,Z,Z-4,6,9-Nonadecatriene	26.81	1.7	109.996	$C_{19}H_{34}$	21.05	0-00-0
(31)	6,9,12-Octadecatrienoic acid, methyl ester	55.78	2.92	63.7153	$C_{19}H_{32}O_2$	28.52	2676-41-7
(32)	Z,Z,Z-1,4,6,9-Nonadecatetraene	21.5	1.8	65.4446	$C_{19}H_{32}$	14.64	0-00-0
(33)	12,15-Octadecadienoic acid, methyl ester	49.14	2.35	158.5734	$C_{19}H_{30}O_2$	19.33	57156-95-3
(34)	10,13-Octadecadienoic acid, methyl ester	50.47	2.47	161.0184	$C_{19}H_{30}O_2$	15.45	18202-24-9
(35)	2,5-Octadecadienoic acid, methyl ester	59.52	1.99	254.7258	$C_{19}H_{30}O_2$	10.15	57156-91-9
(36)	2,2,4,4-Tetramethyl-6-(1-oxo-3-phenylprop-2-enyl)-cyclohexane-1,3,5-trione	70.27	0.49	83.2401	$C_{19}H_{20}O_4$	21.98	0-00-0
(37)	Gentamicina	63.63	3.21	62.6103	$C_{18}H_{36}N_4O_{10}$	27.05	13291-74-2

Table 1: Continued.

No.	Compound name	Peak I/min	Peak II/s	Volume	Library formula	Library probability	Library CAS no.
(38)	Z-10-Methyl-11-tetradecen-1-ol propionate	40.33	2	70.3719	$C_{18}H_{34}O_2$	10.53	0-00-0
(39)	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	34.18	2.06	63.2456	$C_{18}H_{30}O_2$	14.6	16714-85-5
(40)	α-L-Fucopyranose 1,2:3,4-bis(benzeneboronate)	67.13	2.28	180.5156	$C_{18}H_{18}B_2O_5$	25.89	102281-26-5
(41)	3-(O-Anisidinomethyl)-5-(3-fluorobenzylidene)-2,4-thiazolidinedione	34.66	2.46	82.9072	$C_{18}H_{15}FN_2O_3S$	9.43	302954-96-7
(42)	1-Hexadecanol, 2-methyl-	44.19	1.03	65.1528	$C_{17}H_{36}O$	11.82	2490-48-4
(43)	10-Methyl-E-11-tridecen-1-ol propionate	33.33	1.56	164.2353	$C_{17}H_{32}O_2$	6.69	0-00-0
(44)	13-Heptadecyn-1-ol	40.09	2.25	78.5129	$C_{17}H_{32}O$	12.04	56554-77-9
(45)	4,7,10-Hexadecatrienoic acid, methyl ester	32.97	2.05	83.5894	$C_{17}H_{28}O_2$	7.98	17364-31-7
(46)	Methyl 5,7-hexadecadienoate	53.61	1.7	58.765	$C_{17}H_{26}O_2$	14.34	0-00-0
(47)	3-(5-Benzyloxy-3-methylpent-3-enyl)-2,2-dimethyloxirane	35.02	2.61	96.359	$C_{17}H_{24}O_2$	8.95	0-00-0
(48)	Falcarinol	37.07	1.57	55.0178	$C_{17}H_{24}O$	35.51	21852-80-2
(49)	tert-Hexadecanethiol	70.27	1.03	151.5898	$C_{16}H_{34}S$	13.87	25360-09-2
(50)	n-Hexadecanoic acid	79.08	1.74	705.7247	$C_{16}H_{32}O_2$	43.05	10-3-1957
(51)	Cyclopentaneundecanoic acid	46.49	1.62	94.519	$C_{16}H_{30}O_2$	13.97	6053-49-2
(52)	9-Hexadecenoic acid	65.56	1.91	89.144	$C_{16}H_{30}O_2$	13.29	2091-29-4
(53)	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	51.92	1.71	99.1647	$C_{16}H_{26}O_2$	23.72	0-00-0
(54)	1,3-Dioxolane, 2-heptyl-4-phenyl-	7.74	0.67	137.1547	$C_{16}H_{24}O_2$	20.64	55668-40-1
(55)	Peyonine	24.64	0.58	54.9972	$C_{16}H_{19}NO_5$	27.28	19717-25-0
(56)	12,14,14-Trimethyl-3,6,9-trioxapentadecan-1-ol	66.52	1.18	58.5099	$C_{15}H_{32}O_4$	16.94	55489-54-8
(57)	Isocalamendiol	73.65	2.76	52.3348	$C_{15}H_{26}O_2$	24.27	0-00-0
(58)	Geranyl isovalerate	53.37	1.03	77.0183	$C_{15}H_{26}O_2$	11.72	109-20-6
(59)	Cubeduel	63.26	1.88	203.2965	$C_{15}H_{26}O$	24.8	0-00-0
(60)	α-Cadinol	63.63	2.07	649.3081	$C_{15}H_{26}O$	24.36	481-34-5
(61)	Cubenol	62.42	1.9	408.4446	$C_{15}H_{26}O$	16.17	21284-22-0
(62)	.tau.-Cadinol	63.02	2	707.983	$C_{15}H_{26}O$	11.7	11-1-5937
(63)	α-Acorenol	63.63	2.17	1949.884	$C_{15}H_{26}O$	9.72	0-00-0
(64)	Globulol	60.13	1.88	278.3377	$C_{15}H_{26}O$	6.4	51371-47-2
(65)	7-Epi-cis-sesquisabinene hydrate	54.57	1.3	81.2625	$C_{15}H_{26}O$	6.4	0-00-0
(66)	Limonen-6-ol, pivalate	44.56	1.85	78.8648	$C_{15}H_{24}O_2$	24.04	0-00-0
(67)	Aromadendrene oxide-(2)	62.78	2.09	96.7312	$C_{15}H_{24}O$	15.84	0-00-0
(68)	Caryophyllene oxide	59.88	2	161.5517	$C_{15}H_{24}O$	13.15	1139-30-6
(69)	Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-	38.16	2.27	73.2272	$C_{15}H_{24}O$	7.02	39510-36-6
(70)	Humulene	53	1.61	5204.711	$C_{15}H_{24}$	44.49	6753-98-6
(71)	(Z,E)-α-farnesene	55.78	1.52	31067.6	$C_{15}H_{24}$	42.94	26560-14-5
(72)	α-Copaene	48.42	1.4	7508.707	$C_{15}H_{24}$	42.37	0-00-0
(73)	δ-Elemene	45.88	1.33	5692.139	$C_{15}H_{24}$	38.01	20307-84-0
(74)	Naphthalene, 1,2,3,5,6,8a-hexahydro- 4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	56.75	1.68	12956.64	$C_{15}H_{24}$	31	483-76-1
(75)	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	54.94	1.51	196.9983	$C_{15}H_{24}$	29.01	26560-14-5
(76)	Caryophyllene	50.95	1.54	3006.936	$C_{15}H_{24}$	17.9	87-44-5
(77)	β-Elemene	49.14	1.45	20347.87	$C_{15}H_{24}$	17.11	515-13-9
(78)	α-Guaiene	52.16	1.47	556.5085	$C_{15}H_{24}$	16.63	12-1-3691
(79)	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-	52.76	1.23	160.6527	$C_{15}H_{24}$	16.42	110823-68-2

Table 1: Continued.

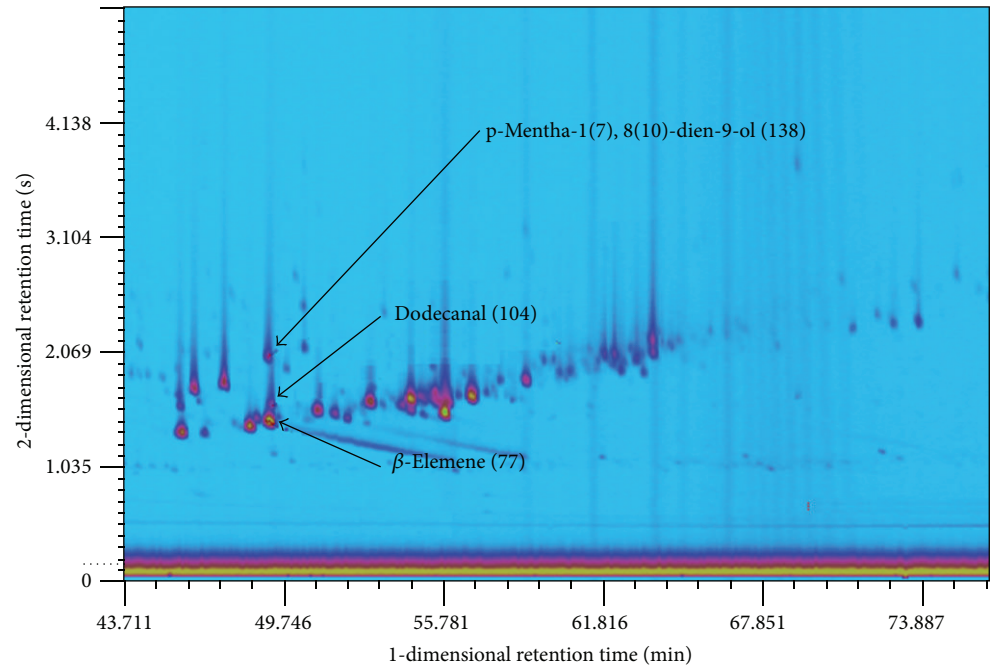
No.	Compound name	Peak I/min	Peak II/s	Volume	Library formula	Library probability	Library CAS no.
(80)	1,6-Cyclodecadiene, 1-Methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	54.45	1.65	14112	$C_{15}H_{24}$	15.25	23986-74-5
(81)	γ-Elemene	51.68	1.51	742.7373	$C_{15}H_{24}$	15.14	29873-99-2
(82)	Naphthalene, 1,2,3,4,4a,7- hexahydro-1,6-dimethyl-4-(1-methylethyl)-	57.35	1.71	200.7951	$C_{15}H_{24}$	14.55	16728-99-7
(83)	Cyclohexane, 1-ethenyl-1-methyl- 2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	48.66	1.47	549.6758	$C_{15}H_{24}$	10.66	515-13-9
(84)	β-Copaene	54.45	1.55	561.4817	$C_{15}H_{24}$	10.53	0-00-0
(85)	γ-Elemene	58.8	1.82	1698.139	$C_{15}H_{24}$	8.31	29873-99-2
(86)	β-Guaiene	57.59	1.75	60.1417	$C_{15}H_{24}$	7.16	88-84-6
(87)	Guaia-1(10),11-diene	54.82	1.67	1093.799	$C_{15}H_{24}$	6.6	0-00-0
(88)	Isoledene	54.21	1.58	405.0377	$C_{15}H_{24}$	6.27	0-00-0
(89)	4,5-Di-epi-aristolochene	47.81	1.43	90.0937	$C_{15}H_{24}$	4.2	0-00-0
(90)	trans-calamenene	56.51	1.85	84.299	$C_{15}H_{22}$	38.35	0-00-0
(91)	β-Vatirenene	73.65	2.34	437.4147	$C_{15}H_{22}$	25.71	0-00-0
(92)	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	72.68	2.32	284.3733	$C_{15}H_{22}$	11.28	79718-83-5
(93)	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	75.21	3.61	61.6952	$C_{15}H_{20}O_4$	11.61	0-00-0
(94)	Propanoic acid, 3-(2,3,6-trimethyl- 1,4-dioxaspiro[4.4]non-7-yl)-, methyl ester	33.93	2.7	84.8793	$C_{14}H_{24}O_4$	19.92	0-00-0
(95)	2,5-Furandione, 3-(2-decenyl)dihydro-	40.33	2.76	154.1135	$C_{14}H_{22}O_3$	11.38	62568-81-4
(96)	trans-(2-Decenyl)succinic anhydride	72.08	1.6	67.8793	$C_{14}H_{22}O_3$	10.39	81949-64-6
(97)	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	77.51	0.5	70.9573	$C_{14}H_{22}O_2$	7.13	2444-28-2
(98)	Tetraacetyl-d-xylonic nitrile	58.32	1.62	100.275	$C_{14}H_{17}NO_9$	18.48	0-00-0
(99)	α-Ionol	30.19	2.18	277.9312	$C_{13}H_{22}O$	15.16	25312-34-9
(100)	1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	73.28	0.5	55.2116	$C_{13}H_{21}N_3O_3$	8.78	0-00-0
(101)	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	33.21	1.89	138.6104	$C_{13}H_{20}O_2$	6.45	0-00-0
(102)	Pyrimidin-2-one, 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl	68.94	0.38	54.3663	$C_{13}H_{19}N_5O_5$	23.12	0-00-0
(103)	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	55.3	1.93	203.2335	$C_{13}H_{18}O_2$	28.93	0-00-0
(104)	Dodecanal	49.26	1.58	1282.104	$C_{12}H_{24}O$	6.6	112-54-9
(105)	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	46.37	1.74	3059.872	$C_{12}H_{20}O_2$	37.64	141-12-8
(106)	Geranyl acetate	47.45	1.79	3772.587	$C_{12}H_{20}O_2$	28.7	105-87-3
(107)	(R)-Lavandulyl acetate	46.37	2.25	81.7472	$C_{12}H_{20}O_2$	14.19	0-00-0
(108)	Geranyl vinyl ether	36.83	1.77	76.0262	$C_{12}H_{20}O$	14.31	0-00-0
(109)	3′-Hydroxyquinalbarbitone	39.61	1.5	51.4505	$C_{12}H_{18}N_2O_4$	10.72	839-21-4
(110)	Non-1-yn-5-en-9-aldehyde, 4-carbethoxy-	44.31	2.11	115.1319	$C_{12}H_{16}O_3$	17.87	0-00-0
(111)	Undecanal	42.75	1.59	704.5031	$C_{11}H_{22}O$	33.86	112-44-7
(112)	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-	30.31	1.34	135.2002	$C_{11}H_{18}$	23.83	95452-08-7
(113)	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	42.02	2.22	591.2578	$C_{11}H_{18}$	14.36	5293-90-3
(114)	Acetic acid, octyl ester	36.47	1.49	323.6557	$C_{10}H_{20}O_2$	17.84	112-14-1
(115)	Decanal	35.74	1.61	7031.098	$C_{10}H_{20}O$	61.62	112-31-2
(116)	Cephrol	37.55	1.66	328.2375	$C_{10}H_{20}O$	12.34	40607-48-5
(117)	Linalol	28.74	1.52	3645.373	$C_{10}H_{18}O$	81.59	78-70-6

Table 1: Continued.

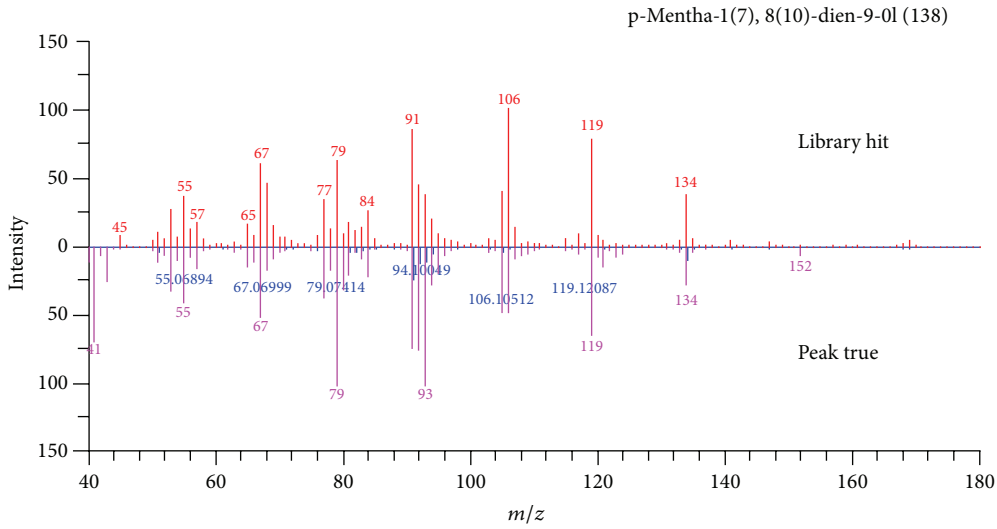
No.	Compound name	Peak I/min	Peak II/s	Volume	Library formula	Library probability	Library CAS no.
(118)	α-Terpineol	34.9	1.89	7440.985	$C_{10}H_{18}O$	67.75	98-55-5
(119)	Terpinen-4-ol	34.18	1.79	3293.372	$C_{10}H_{18}O$	50.03	562-74-3
(120)	Citronellal	32.12	1.69	615.3934	$C_{10}H_{18}O$	42.59	106-23-0
(121)	2-Cyclohexen-1-ol, 1-methyl-4-(1-methyl-ethyl)-, cis-	30.31	1.67	159.2231	$C_{10}H_{18}O$	39.11	29803-82-5
(122)	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	31.64	1.8	726.8632	$C_{10}H_{18}O$	10.59	7299-41-4
(123)	2-Cyclohexen-1-ol, 2-methyl-5-(1-methyl-ethyl)-, (1S-cis)-	36.11	1.85	101.2405	$C_{10}H_{18}O$	10.53	536-30-1
(124)	exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	32.97	1.89	169.9902	$C_{10}H_{18}O$	8.15	0-00-0
(125)	5-Hepten-1-ol, 2-ethenyl-6-methyl-	45.76	1.68	397.1537	$C_{10}H_{18}O$	7.75	18479-48-6
(126)	Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-, [1S-(1α, 3β,6α)]-	45.76	1.58	368.8305	$C_{10}H_{18}$	9.72	2778-68-9
(127)	Desulphosinigrin	66.64	1.06	73.3518	$C_{10}H_{17}NO_6S$	8.92	5115-81-1
(128)	R-Limonene	39.37	2.73	97.0311	$C_{10}H_{16}O_3$	40.37	0-00-0
(129)	Limonene oxide, trans-	31.28	1.78	570.8168	$C_{10}H_{16}O$	54.24	4959-35-7
(130)	Limonene oxide, cis-	30.92	1.78	1068.476	$C_{10}H_{16}O$	40.43	13837-75-7
(131)	trans-p-Mentha-2,8-dienol	30.07	1.78	448.7691	$C_{10}H_{16}O$	34.35	0-00-0
(132)	3-Cyclohexene-1-acetaldehyde, α,4-dimethyl-	36.35	2.09	181.7561	$C_{10}H_{16}O$	31.38	29548-14-9
(133)	cis-p-Mentha-1(7),8-dien-2-ol	37.43	2.05	398.5489	$C_{10}H_{16}O$	26.42	0-00-0
(134)	(Z)-Carveol	35.5	1.97	452.2402	$C_{10}H_{16}O$	25.48	1197-06-4
(135)	trans-Carveol	36.71	2	1591.657	$C_{10}H_{16}O$	22.66	1197-07-5
(136)	cis-p-Mentha-1(7),8-dien-2-ol	33.93	1.91	336.4337	$C_{10}H_{16}O$	21.5	0-00-0
(137)	1-Cyclohexene-1-methanol, 4-(1-methyl-ethenyl)-	35.62	1.89	195.9113	$C_{10}H_{16}O$	20.76	536-59-4
(138)	p-Mentha-1(7),8(10)-dien-9-ol	49.14	2.02	1162.984	$C_{10}H_{16}O$	20.12	29548-13-8
(139)	cis-p-Mentha-2,8-dien-1-ol	43.47	1.91	99.0278	$C_{10}H_{16}O$	7.83	3886-78-0
(140)	2,6-Dimethyl-3,5,7-octatriene-2-ol, E,E-	31.88	1.98	187.0291	$C_{10}H_{16}O$	7.6	0-00-0
(141)	(S)-(-)-(4-Isopropenyl-1-cyclohexenyl)-methanol	35.38	1.89	172.7702	$C_{10}H_{16}O$	5.86	18457-55-1
(142)	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methyl-ethyl)-	17.4	1.11	7918.412	$C_{10}H_{16}$	65.04	28634-89-1
(143)	β-Pinene	20.66	1.31	19964.34	$C_{10}H_{16}$	40.8	127-91-3
(144)	β-Ocimene	25.48	1.34	4519.345	$C_{10}H_{16}$	37.56	13877-91-3
(145)	α-Phellandrene	22.47	1.32	2085.008	$C_{10}H_{16}$	33.42	99-83-2
(146)	β-Myrcene	21.5	1.27	69260.99	$C_{10}H_{16}$	31.3	123-35-3
(147)	α-Pinene	17.88	1.16	48376.96	$C_{10}H_{16}$	23.82	80-56-8
(148)	Camphene	18.85	1.22	406.4708	$C_{10}H_{16}$	19.93	79-92-5
(149)	β-Phellandrene	20.29	1.27	4173.575	$C_{10}H_{16}$	19.68	555-10-2
(150)	D-Limonene	24.52	1.69	1784450	$C_{10}H_{16}$	19.59	5989-27-5
(151)	α-Terpinene	23.43	1.33	4905.547	$C_{10}H_{16}$	17.58	99-86-5
(152)	Isoterpinene	28.38	1.46	14607.02	$C_{10}H_{16}$	16.01	586-62-9
(153)	γ-Terpinene	26.33	1.5	189808.1	$C_{10}H_{16}$	15.28	99-85-4
(154)	1,5,5-Trimethyl-6-methylene-cyclohexene	26.33	1.86	305.9981	$C_{10}H_{16}$	9.81	514-95-4
(155)	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	24.52	3.31	51.7426	$C_{10}H_{16}$	9.58	5989-54-8
(156)	γ-Pyronene	23.07	1.27	60.3834	$C_{10}H_{16}$	5.33	514-95-4
(157)	5-Isopropenyl-2-methylcyclopent-1-enecarbo-xaldehyde	36.11	2.3	711.2013	$C_{10}H_{14}O$	34.31	0-00-0
(158)	(-)-Carvone	37.8	2.35	909.8454	$C_{10}H_{14}O$	32.76	6485-40-1

Table 1: Continued.

No.	Compound name	Peak I/min	Peak II/s	Volume	Library formula	Library probability	Library CAS no.
(159)	1-Cyclohexene-1-carboxaldehyde, 4-(1-methyl ethenyl)-	39.85	2.43	1404.104	$C_{10}H_{14}O$	31.76	2111-75-3
(160)	3,5-Heptadienal, 2-ethylidene-6-methyl-	36.95	2.28	238.0085	$C_{10}H_{14}O$	28.28	99172-18-6
(161)	Benzenemethanol, α,α,4-trimethyl-	34.05	2.28	288.3869	$C_{10}H_{14}O$	16.59	1197-01-9
(162)	Cyclohexanone, 2-(2-butynyl)-	42.38	3.18	59.1028	$C_{10}H_{14}O$	15.02	54166-48-2
(163)	D-Verbenone	41.42	2.16	475.2141	$C_{10}H_{14}O$	11.63	18309-32-5
(164)	3,5-Heptadienal, 2-ethylidene-6-methyl-	36.11	2.5	184.88	$C_{10}H_{14}O$	8.97	99172-18-6
(165)	o-Cymene	23.55	1.55	35991.09	$C_{10}H_{14}$	47.51	527-84-4
(166)	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	31.64	1.71	164.8089	$C_{10}H_{14}$	20.19	460-01-5
(167)	Benzene, 1-methyl-4-(1-methylethenyl)-	27.9	1.8	271.6784	$C_{10}H_{12}$	20.42	1195-32-0



(a)



(b)

Figure 4: Continued.

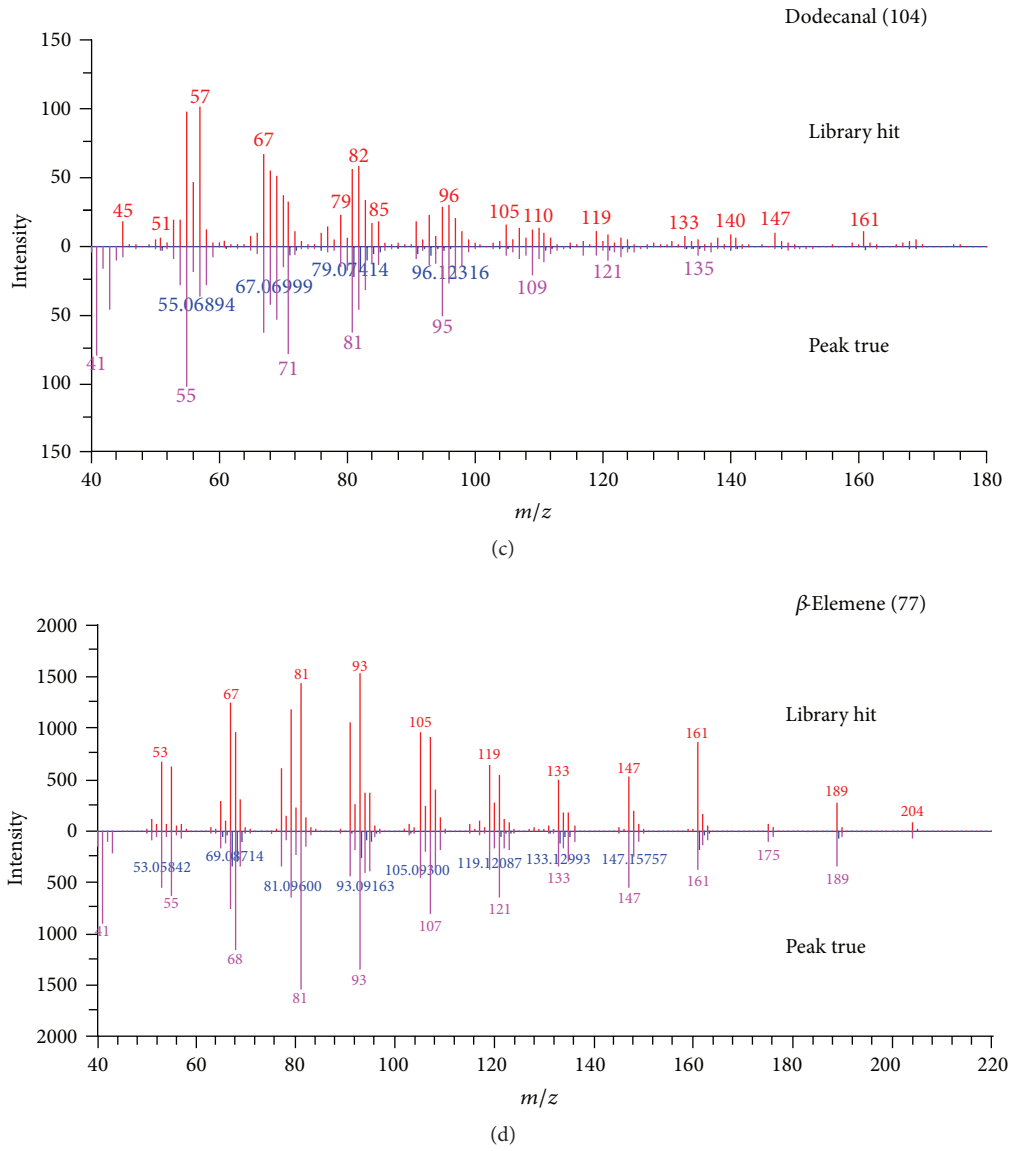


Figure 4: Details of three coelution volatile components (peak 77, 104, 138) in GC × GC chromatogram. The spectra of β-elemene (peak 77), dodecanal (peak 104), and p-mentha-1(7),8(10)-dien-9-ol (peak 138) in sample and in NIST library, respectively.

4. Conclusions

In this study, GC × GC-HR-TOFMS not only tentatively identified 167 volatile components in Chenpi volatile oil, but also provided several kinds of identification information that make the result more reliable. Among 167 components, there are 50 monoterpenes, 36 sesquiterpenes, 31 esters and acids, 9 aldehydes and ketones, 6 alcohols, 3 ethers, 12 phenyl compounds, and 20 other components. Monoterpenes and sesquiterpenes are the main components of Chenpi volatile oil. This study demonstrates a dependable method for the qualitative analysis of volatiles, which can achieve an accurate and comprehensive chromatographic profile with a low contamination risk and cost, as well as shortened sample preparation time. GC × GC-HR-TOFMS will play an important role in the analysis of volatile oils of herbal medicines in the future.

Conflict of Interests

The authors explicitly declare that this paper has no conflict of interests.

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