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Study of traditional Chinese medicine volatile oils from different geographical origins by comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS) in combination with multivariate analysis

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Abstract

Chemical constitutes of volatile oil in the rhizomes and radixes of *Notopterygium incisum Ting ex H.T. chang* (Qianghuo in Chinese) from different regions were investigated using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS) and GC × GC–flame ionization detector (FID). A total of 769 compounds were tentatively identified and quantified in a typical sample from Sichuan province, a producing area of the Genuine Medicinal Materials. An obvious group-type separation was observed in the GC × GC–TOFMS chromatogram. Identification and quantitative results showed that Qianghuo from Sichuan province has some significant differences in the chemical composition from other geographical origin of herbs, especially in monoterpenes and oxygenated sesquiterpenes. The data of all individual peaks collected by GC × GC–FID were processed using a principal component analysis (PCA) method to classify the samples from different regions, find and identify the marker compounds that lead to the differentiation. The abundances of monoterpenes and oxygenated sesquiterpenes were responsible for the differentiation, which is in good agreement with the group quantitative results of GC × GC analysis.

Keywords: GC × GC–TOFMS; Traditional Chinese medicine; Qianghuo; *Notopterygium incisum Ting ex H.T. chang*; Principal component analysis

1. Introduction

Qianghuo (the rhizomes and radixes of *Notopterygium incisum Ting ex H.T. chang*) is a traditional Chinese medicine. It distributes in Sichuan, Qinghai, Yunnan and Gansu provinces. Qianghuo from Sichuan province is generally accepted as the Genuine Medicinal Material (Daodi Yaocai in Chinese), which means the herb has steady quality and good curative effect of clinic. Qianghuo shows many pharmacological effects such as anodyne, antifebrile and anti-inflammatory activity. The 5-lipoxygenase and cyclooxygenase inhibitory active constituents from *n*-hexane extract of Qianghuo were also reported [1]. Its main pharmacologically active constitutes are coumarin and the volatile oil. The chemical constitutes of the volatile oil are very complex, which contain many active components. It was reported that the activity of anodyne, antifebrile and anti inflammation was related to the components in Qianghuo volatile oil [2].

Comprehensive two-dimensional gas chromatography (GC × GC) is considered the most powerful and versatile separation tool in GC [3]. It was widely applied in the separation of complex samples such as petroleum, environmental samples, food, fat, fragrances, and essential oils in the past few years. GC × GC has been used in the analysis of herbal drugs successfully. GC × GC with flame ionization detection (FID) was used to analyze the ginger volatiles, volatiles in peppermint and spearmint oil, enantioseparation of monoterpenes, ephedrine-type alkaloids in herbal materials, profiling

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of volatile oils in herbal mixtures, Asian and American ginseng [4–9]. Besides, GC × GC–TOFMS was used in the analysis of sub-critical water extraction of essential oils from *Thymbra spicata*, volatiles in *Pistacia vera* L., Hop volatiles, odorants in coriander and wild coriander leaves, zedoary volatile oil, volatiles in *Ziziphora taurica subsp. Taurica* [10–15].

Data processing of large volume of multidimensional data obtained by GC × GC is a time consuming and complicated work. In order to discover the significant differences of complex samples, a specific data processing method is essential. Principal component analysis (PCA) translates the raw data from multidimensional variables space into principle component (PC) space where there is one score describing each chromatogram in the data set in the much lower dimensional PC space [16]. Weckwerth et al. used PCA to distinguish GC–MS separations of metabolite extracts from different Arabidopsis plants [17]. Pierce et al. used PCA data mining method of GC × GC–TOFMS chromatograms to quickly discover the differences of organic extracts of basil, peppermint, and sweet herb stevia [18].

Determination of genuineness of traditional Chinese medicines (TCMs) is one of the most crucial issues in TCM quality control and safety. The introduction of new analytical techniques gives a tremendous promotion to TCM quality assurance. In addition novel data analysis methods are necessary. Fingerprinting quality control of Qianghuo by high-performance liquid chromatography (HPLC) has been reported. Li et al. used liquid chromatography–electrospray ionisation–multiple stage mass spectrometry (HPLC–ESI–MS^n) to identify the fingerprint components [19]; Jiang et al. developed a fingerprint method using HPLC–photodiode array detection for the quality control of Qianghuo. Raw herbs of Qianghuo from different sources and species were distinguished by chromatographic fingerprint combining similarity evaluation [20]. The aim of the current study is to classify and characterize Qianghuo from the different geographical origins on the basis of chemical compositions of volatile oil. Fifteen herb samples from five regions were analyzed using GC × GC–TOFMS and GC × GC–FID, a principal component analysis (PCA) method was also used to classify samples and find the chemical variables that contribute to the differentiation.

2. Experimental

2.1. Samples and sample preparation

Qianghuo samples were donated by Prof. Famei Li in Shenyang Pharmaceutical University of China or purchased from Meiluo Pharmacy Dalian. Fifteen samples were from five major producing regions in China: samples 01, 02 and 03 were from Sichuan, samples 04, 05 and 06 were from Ningxia, samples 07, 08 and 09 were from Inner Mongolia, samples 10, 11 and 12 were from Hunan and samples 13, 14 and 15 were from Gansu. The volatile oil was extracted with the steam distillation method described in the Chinese Pharmacopoeia [21].

2.2. Instrumentation

A GC × GC system consisted of a HP6890 GC equipped with a flame ionization detector (Agilent Technologies, Wilmington, DE, USA) and a cold-jet modulator KT-2001 Retrofit prototype (Zoex Corporation, Lincoln, NE, USA).

A LECO Pegasus 4D GC × GC–TOFMS instrument (LECO Corporation, St. Joseph, MI, USA) equipped with an Agilent 6890N GC was used to acquire mass spectral data from GC × GC using 70 eV electron impact ionization.

2.3. Column system and conditions

A column set with a non-polar one as primary column and a polar one as secondary column was used. The first dimension chromatographic column was 50 m × 0.25 mm × 0.25 μm CEC-1 (100% Dimethylpolysiloxane, Chrom Expert Company, USA). The second dimension chromatographic column was 1.8 m × 0.10 mm × 0.1 μm DB-WAX (polyethylene glycol, J&W Scientific, Folsom, CA). Ultra high purity (99.9995%) helium was used as the carrier gas in constant pressure mode. The inlet pressure was 72.4 psi. An Agilent 7683B autosampler (Agilent, Palo Alto, CA, USA) injected 0.2 μl of sample at a split ratio of 1:30 in a 250 °C inlet onto column 1. Column 1 oven was held at 50 °C for 0.5 min, then ramped at 2 to 200 °C/min. Column 2 oven was at a constant 25 °C higher than column 1 oven. The mass spectrometer was operated at an acquisition rate of 50 spectra/s. No mass spectra were collected during the solvent delay for the first 5 min of each run. The modulation period was 5 s. The transfer line and the ion source temperature was 230 and 210 °C, respectively. The detector voltage was 1650 V and the electron energy was −70 V. Mass spectra were collected from *m/z* 35–400. The pressure inside the flight tube was about 1E-7 Torr.

2.4. Data processing

The GC × GC–FID and the GC × GC–TOFMS were used to carry out the quantitative analysis and identification of peaks, respectively. The retention time differences between GC × GC–FID and GC × GC–TOFMS are not obvious if the same chromatography conditions are used. Thus, the translation of GC × GC retention time based on the peak shape and the relative position of peaks from GC × GC–FID to GC × GC–TOFMS is feasible. The raw data of either GC × GC–FID (100 Hz) or GC × GC–TOFMS (50Hz) were exported as comma separated value (*.csv) files. The *.csv files of GC × GC–FID were converted into a two-dimensional matrix and exported as *.bin files by an in-house conversion program based on the modulation frequency and sampling rate. The *.bin files were read into Transform (part of Noesys Software Package, Research Systems International, Crowthorne, UK) to generate a contour plot. In a contour plot whose *x*-axis is the number of modulation periods that had been modulated, and *y*-axis is the number of blobs that had been collected in the second dimension. The *x* and *y*-axes could be easily translated back to first and second dimensional retention time based on

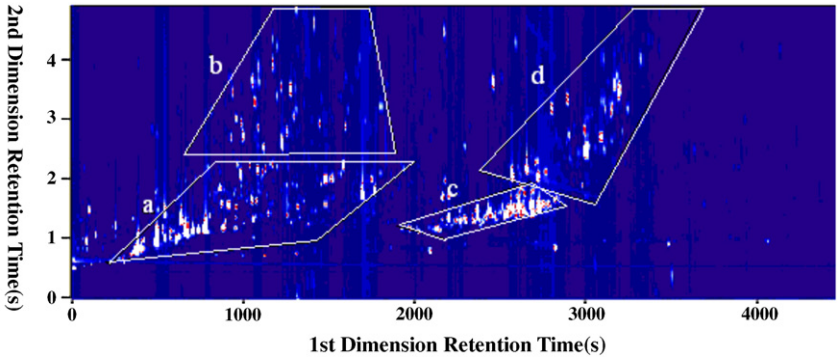

Fig. 1. The GC × GC–TOFMS contour plots of Qianghuo volatile oil. Zones (a–d) are mainly monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes, respectively.

the modulation frequency and sampling rate. In the meantime, the peaks in contour plot can be integrated after the baseline region next to each peak was subtracted and quantified by Zoex software (Zoex Corp., Lincoln, NE, USA). Since it is difficult to get the pure compounds as standard samples, the peak volume normalization was applied to approximately compare the relative content of the components.

To carry out the PCA analysis, it is necessary to make peak alignment among different chromatograms. Software was home developed with graphical user-interfaces (GUIs). Firstly, three peaks having good separation and good interval in the GC × GC–FID chromatograms were chosen as the reference peaks. Then, the relative retention times of all peaks to their neighboring reference peaks were calculated and exported into an excel table. Finally, the alignment of peaks in different chromatograms was carried out based on the relative retention times. The chromatograms of fifteen samples were handled and 1544 peaks were generated, a 15 × 1544 data matrix including their relative peak volume (vol.%) from GC × GC–FID was used for the multivariate analysis by using the SIMCA-P 10.0 Version (Umetrics AB, Umeâ, Sweden) to discriminate the fifteen samples from five producing regions and find the marker compounds with the significant difference.

In the identification analysis, the GC × GC–TOFMS software from LECO Pegasus was used to find all the peaks in the raw chromatograms. The parameters such as similarity, reverse, and probability of peaks through a library search using the NIST/EPA/NIH Version 2.0 by the workstation were combined in a single peak table.

3. Results and discussion

3.1. Separation and identification of individual compounds by GC × GC

The effect of temperature program, modulation period, and the two-dimensional column system on the resolution has been well investigated [22]. In this study, the proper temperature program rate (2 °C/min) and modulation period (5 s) for Qianghuo volatile oils were found. In our previous work, we have known that the column system with a non-polar column on the first dimension and a polar column on the second dimension can provide a group-type separation of some kinds of components in the volatile oils of TCM, a polarity reversed column system can provide the more detailed separation [23]. In this study, we are more interested in the group-type separation than a very detailed individual separation, so a column set with the non-polar one (CEC-1) as primary column and the polar one (DB-WAX) as secondary column was used. The GC × GC–TOFMS contour plot of Qianghuo volatile oil is depicted in Fig. 1.

A total of 769 compounds were tentatively identified by TOFMS and quantified by FID in sample 03 from Sichuan province, including 249 hydrocarbons, 105 ketones, 46 aldehydes, 101 esters, 131 alcohols, 4 ethers, 6 acids, 23 nitrogen-containing compounds, and 104 other components. The compounds whose similarity, reverse number and content (%) are above 800, 900 and 0.05, respectively are listed in Table 1. It is far more than the results that had been reported [24].

3.2. Classification and differentiation based on groups or fingerprints

3.2.1. Group compositional comparison of volatiled oils from different geographical origins based on GC × GC contour plot

From Fig. 1, an apparent group-type separation of some major components in the sample is displayed. The components located in the regions marked by squares (a to d) are identified mainly as monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes, respectively. It can be observed that the four chemical classes are partially overlapped in the first dimension. However, they had great differences in the second dimensional retention time, by which the separation of these chemical classes was achieved. The relative quantitative results of these four chemical types in the fifteen samples obtained by using GC × GC–FID were integrated and the contents of samples from the same region were averaged to represent the different geographical origin. The results of chemical compositions in volatile oils from five regions are listed in Table 2. It can be obviously seen from Table 2 that Qianghuo from Sichuan province, which is the Genuine Medicinal Material, has some apparent differences in chemical compositions from other regions' herbs. The content of monoterpenes in volatile oil of the samples from

Table 1
Compounds identified by GC × GC–TOFMS with good match and quantified by GC × GC–FID with high content (%) in sample 03 (from Sichuan province)

No.	Name	CAS	^1t_R, ^2t_R[a] (s)	Formula	Weight	Similarity	Reverse	*P*-value	Content[b] (%)
1	Heptane	142-82-5	190, 0.520	C7H16	100	897	931	6917	0.059
2	Furan, 2-ethyl-	3208-16-0	190, 0.660	C6H8O	96	944	944	7456	0.096
3	2-Butenal, 3-methyl-	107-86-8	240, 1.360	C5H8O	84	949	949	7216	0.190
4	Heptanal	111-71-7	410, 1.220	C7H14O	114	946	946	9492	0.557
5	Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-	464-17-5	450, 0.720	C10H16	136	934	934	7578	0.152
6	Tricyclo[2.2.1.02,6]heptane, 1,7,7-trimethyl-	508-32-7	485, 0.760	C10H16	136	971	971	5290	0.233
7	2-Heptenal, (Z)-	57266-86-1	525, 1.840	C7H12O	112	962	962	6703	0.062
8	Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl)-	36262-09-6	535-895, 0.980	C10H14	134	950	950	3861	0.237
9	Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-	471-84-1	540, 0.840	C10H16	136	932	932	2838	0.918
10	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	18172-67-3	610-625, 0.940	C10H16	136	903	903	5158	4.734
11	2-Propenoic acid, 2-methyl-, oxiranylmethyl ester	106-91-2	625, 1.080	C7H10O3	142	815	936	7000	1.641
12	Furan, 2-pentyl-	3777-69-3	650, 1.200	C9H14O	138	902	902	7564	0.568
13	1,3,8-*p*-Menthatriene	21195-59-5	655-1145, 1.100	C10H14	134	901	901	3459	0.531
14	Octanal	124-13-0	660, 1.480	C8H16O	128	941	956	9053	1.297
15	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	470-67-7	725, 1.020	C10H18O	154	965	965	9484	0.500
16	Benzene, 1-methyl-3-(1-methylethyl)-	535-77-3	745-770, 1.300	C10H14	134	912	912	4086	4.484
17	Limonene	138-86-3	780, 1.100	C10H16	136	911	911	4614	1.256
18	Benzene, 1-ethyl-2,3-dimethyl-	933-98-2	915, 1.520	C10H14	134	970	970	2817	0.135
19	Benzene, 1-methyl-4-(1-methylethenyl)-	1195-32-0	925-950, 1.900	C10H12	132	933	951	4661	0.169
20	2-Nonanone	821-55-6	960, 1.640	C9H18O	142	967	967	4869	0.176
21	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9	970, 1.220	C10H16	136	949	949	6678	0.805
22	2-*n*-Heptylfuran	3777-71-7	980, 1.340	C11H18O	166	857	914	3964	0.079
23	Nonanal	124-19-6	1000, 1.620	C9H18O	142	972	972	8174	0.235
24	Benzene, 1-ethyl-4-methoxy-	1515-95-3	1020, 2.500	C9H12O	136	901	901	2789	0.584
25	Fenchol, exo-	22627-95-8	1035, 3.080	C10H18O	154	960	975	5074	0.064
26	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	38651-65-9	1060, 2.980	C9H14O	138	934	934	7004	0.073
27	4-Acetyl-1-methylcyclohexene	6090-9-1	1070, 2.660	C9H14O	138	924	924	9645	0.116
28	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, *trans*-	29803-81-4	1070, 2.780	C10H18O	154	940	940	6239	0.130
29	2,6-Dimethyl-1,3,5,7-octatetraene, E	460-01-5	1085-1115, 1.700	C10H14	134	955	976	7615	0.057
30	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-	464-48-2	1105, 2.300	C10H16O	152	971	971	5730	0.056
31	Cyclohexanol, 4-(1-methylethyl)-	4621-4-9	1120-1660, 3.600	C9H18O	142	952	952	8960	0.133
32	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	547-61-5	1120, 3.740	C10H16O	152	940	940	9287	0.137
33	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	7216-56-0	1125, 1.400	C10H16	136	926	926	2288	0.241
34	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, *cis*-	29803-82-5	1135, 3.340	C10H18O	154	938	938	6891	0.063
35	2,4-Dimethylstyrene	2234-20-0	1150, 2.080	C10H12	132	874	918	1926	0.052
36	2-Nonenal, (E)-	18829-56-6	1190, 2.240	C9H16O	140	904	925	6703	0.073
37	Borneol	10385-78-1	1190-1225, 3.620	C10H18O	154	870	906	3931	0.146
38	Bornyl chloride	464-41-5	1205, 1.500	C10H17Cl	172	947	947	8673	0.054
39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	562-74-3	1275, 2.720	C10H18O	154	918	918	7562	1.952
40	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	564-94-3	1295, 2.840	C10H14O	150	936	936	8997	0.067
41	(+)-α-Terpineol (*p*-menth-1-en-8-ol)	0-00-0	1320, 3.560	C10H18O	154	943	943	5900	0.243
42	Bicyclo[3.1.1]hept-2-ene-2-methanol,6,6-dimethyl-	515-00-4	1345-1640, 4.980	C10H16O	152	949	949	8898	0.071

Table 1(*Continued*)

No.	Name	CAS	^1t_R, ^2t_R[a] (s)	Formula	Weight	Similarity	Reverse	*P*-value	Content[b] (%)
43	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	1076-56-8	1480-1575, 2.000	C11H16O	164	950	950	8014	0.726
44	2-Decenal (Z)-	2497-25-8	1590, 2.320	C10H18O	154	943	943	4304	0.280
45	1-Cyclohexene-1-carboxaldehyde,4-(1-methylethyl)-	21391-98-0	1605, 2.920	C10H16O	152	955	965	8765	0.112
46	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	92618-89-8	1695, 1.840	C12H20O2	196	925	925	6921	1.842
47	2,4-Decadienal (E,E)-	25152-84-5	1710-1790, 3.000	C10H16O	152	939	968	6422	0.074
48	4-Carene (1S,3R,6R)-(−)-	5208-49-1	1725-1755, 1.800	C10H16	136	895	921	5026	0.687
49	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-*trans*)-	20307-84-0	1940-2620, 1.200	C15H24	204	958	968	6760	0.464
50	α-Cubebene	17699-14-8	1990, 1.140	C15H24	204	926	926	5745	0.089
51	Germacrene D	23986-74-5	2065-2575, 1.180	C15H24	204	912	912	3744	1.288
52	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2α,4α)]-	515-13-9	2110-2380, 1.380	C15H24	204	919	919	6125	1.149
53	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1α,3aα,4α,7α)]-	22567-17-5	2175-3425, 1.260	C15H24	204	905	912	3833	2.821
54	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1aR-(1aα,7α,7aα,7bα)]-	17334-55-3	2275, 1.360	C15H24	204	918	932	4288	0.234
55	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1aα,4aα,7α,7aα,7bα)]-	25246-27-9	2300, 1.380	C15H24	204	926	926	2211	0.064
56	α-Caryophyllene	6753-98-6	2350-2830, 1.560	C15H24	204	920	957	7220	1.075
57	Spiro[5.5]undec-2-ene,3,7,7-trimethyl-11-methylene-, (−)-	18431-82-8	2425, 1.600	C15H24	204	924	924	5296	0.140
58	Eudesma-4(14),11-diene	0-00-0	2465, 1.640	C15H24	204	921	921	2675	1.696
59	δ-Selinene	28624-23-9	2490, 1.460	C15H24	204	940	940	6848	0.072
60	2-Dodecanone	6175-49-1	2510, 1.820	C12H24O	184	910	910	5668	0.240
61	Benzene,1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	16982-00-6	2515, 1.940	C15H22	202	917	917	9100	0.305
62	3-Phenyl-1-propanol, acetate	122-72-5	2520-2850, 3.060	C11H14O2	178	905	905	6331	0.057
63	Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	495-61-4	2575, 1.480	C15H24	204	905	905	5281	0.485
64	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	16728-99-7	2635, 1.640	C15H24	204	914	914	7228	0.178
65	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4α)]-	639-99-6	2680, 3.240	C15H26O	222	939	947	8117	0.186
66	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-α,α,3,8-tetramethyl-	13822-35-0	2850-2940, 2.860	C15H26O	222	922	922	6004	0.169
67	.tau.-Cadinol	5937-11-1	2985, 3.140	C15H26O	222	907	913	5518	0.114
68	2-Naphthalenemethanol, decahydro-α, α, 4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aα)]-	473-15-4	3005, 3.640	C15H26O	222	931	938	8527	0.211
69	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8-tetramethyl-, [2R-(2α,4aα,8aα)]-	473-16-5	3025, 3.520	C15H26O	222	941	946	7659	0.482
70	Eicosane	112-95-8	3125-3910, 0.960	C20H42	282	957	957	909	0.081

[a] ^1t_R and ^2t_R are retention times on the first and second dimensions, respectively.

[b] Content is the peak volume percentage of compounds in Qianghuo sample.

Table 2
Content of four chemical types in samples from different regions

Chemical type	Content (%) ± S.D.				
	Sichuan	Ningxia	Inner Mongolia	Hunan	Gansu
Monoterpenes	40.17 ± 2.52	28.14 ± 2.45	15.02 ± 2.72	20.22 ± 0.13	20.29 ± 3.75
Oxygenated monoterpenes	6.45 ± 1.55	4.74 ± 1.87	5.97 ± 0.33	6.27 ± 0.22	5.42 ± 2.07
Sesquiterpenes	19.95 ± 0.89	14.69 ± 1.95	12.21 ± 0.99	16.34 ± 0.75	14.11 ± 2.58
Oxygenated sesquiterpenes	3.95 ± 1.03	27.49 ± 2.96	39.83 ± 0.69	32.49 ± 2.35	28.70 ± 2.69

Sichuan province is much higher than that from other production area. Besides, the content of oxygenated sesquiterpenes is much lower than that of others'. The contents of monoterpenes and oxygenated sesquiterpenes were the main chemical variables, which contribute to the differentiation. Since it has been reported that volatile oil is the major pharmacological activity ingredient in Qianghuo, and the quality of drug is directly affected by the chemical composition in the volatile oil. In addition, the pharmacological activity is concerned with certain sort of components with specific molecular skeleton [25]. Consequently, the better quality of Genuine Medicinal Material (sample from Sichuan province) is perhaps related to the content of monoterpenes and oxygenated sesquiterpenes in its volatile oil.

3.2.2. *Principle component analysis comparison of volatiled oils from different geographical origins based on GC × GC fingerprint*

Hundreds of component peaks were detected in each sample of Qianghuo volatile oils. Principal component analysis (PCA) method was employed to discriminate among different geographical origins and identify the marker compounds with significant difference based on the GC × GC–FID and GC × GC–TOFMS fingerprints.

The scores plot obtained from PCA is shown in Fig. 2. The first three PCs (PC1, PC2 and PC3) had the greatest eigenvalues and captured 24.0, 16.8 and 9.9% of the total variance, respectively, so PC1, PC2 and PC3 were used for data analysis. Fig. 2 shows that the samples are separated into three clusters, i.e. from Sichuan, Gansu and other regions. Especially, the group of samples from Sichuan province was dispersive from others. It indicates that the components in these samples from Sichuan province were apparently different from those of other regions. It is in good agreement with the result in the previous section. Besides, the spots of samples from Ningxia, Inner Mongolia and Hunan are located in a small area but a sample from Hunan, which indicated that the chemical components of the samples from these regions have fewer differences.

To find the marker compounds for discrimination of the regions, a parameter variable importance in the projection (VIP) [26] was introduced to reflect the variable importance. Based

Table 3
Highly loaded component peaks and the chemical classes that they belong to

Variable ID (x and y)[a]	^1t_R, ^2t_R (s)	VIP[b]	Compound name	Formula	Chemical type
330, 66	1650, 1.32	1.75792	4-Carene, (1S,3R,6R)-(−)-	C10H16	Monoterpenes
100, 54	500, 1.08	1.71576	3-Carene	C10H16	Monoterpenes
497, 147	2485, 2.94	1.70965	Globulol	C15H26O	Oxygenated sesquiterpenes
119, 58	595, 1.16	1.70332	α-Phellandrene	C10H16	Monoterpenes
61, 71	305, 1.42	1.69005	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)-	C10H16	Monoterpenes
458, 98	2290, 1.96	1.68654	Diepicedrene-1-oxide	C15H24O	Oxygenated sesquiterpenes
365, 93	1825, 1.86	1.6685	4-Carene, (1S,3S,6R)-(−)-	C10H16	Monoterpenes
57, 68	285, 1.36	1.66661	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-, (1S)-	C10H16	Monoterpenes
518, 164	2590, 3.28	1.65815	Cyclohexanemethanol, 4-ethenyl-α, α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4α)]-	C15H26O	Oxygenated sesquiterpenes
88, 50	440, 1	1.6579	Camphene	C10H16	Monoterpenes
474, 109	2370, 2.18	1.65668	Isoaromadendrene epoxide	C15H24O	Oxygenated sesquiterpenes
371, 94	1855, 1.88	1.65573	1S-α-Pinene	C10H16	Monoterpenes
90, 87	450, 1.74	1.65549	α-Pinene	C10H16	Monoterpenes
565, 184	2825, 3.68	1.65393	Epiglobulol	C15H26O	Oxygenated sesquiterpenes
463, 126	2315, 2.52	1.65025	Diepi-α-cedrene epoxide	C15H24O	Oxygenated sesquiterpenes
118, 66	590, 1.32	1.62786	1,3,8-*p*-Menthatriene	C10H14	Monoterpenes
516, 139	2580, 2.78	1.6211	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	C15H26O	Oxygenated sesquiterpenes
215, 104	1075, 2.08	1.61289	Diepi-α-cedrene epoxide	C15H24O	Oxygenated monoterpenes
461, 125	2305, 2.5	1.60118	Caryophyllene oxide	C15H24O	Oxygenated sesquiterpenes
514, 203	2570, 4.06	1.59886	Carotol	C15H26O	Oxygenated sesquiterpenes

[a] In (x and y), x is the number of modulation periods that had been modulated, and y is the number of blobs that had been collected in the second dimension. The x and y could be easily translated back to first and second dimensional retention time based on the modulation frequency and sampling rate.

[b] VIP, Variable importance in the projection, is the sum over all model dimensions of the contributions of variable influence[26].

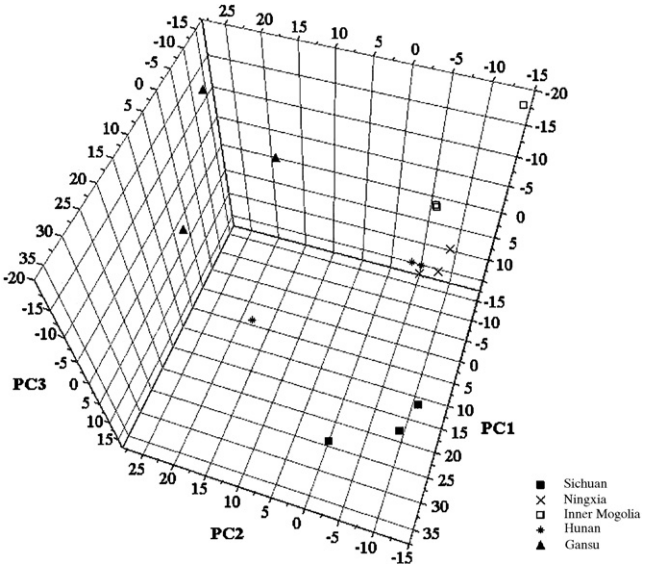

Fig. 2. Score plot of the PC1, PC2 and PC3 component scores of Qianghuo showing differentiation according to region.

on the VIP value, the most highly loaded 20 variables (marker compounds) were selected from 1544 variables. Then these 20 variables were employed to test the difference between the samples from five regions using t-test. Significant differences ($P < 0.05$) were found. The variable ID (x and y) can be translated to two-dimensional retention time based on the modulation frequency and sampling rate. Therefore, the chromatographic peaks, which were referred to the highly loaded variables, can be found out from the GC × GC–FID chromatogram easily. The GC × GC retention times may be translated from the FID to the TOFMS system. The most highly loaded variables identified by TOFMS were listed in Table 3. All of them were in the zone (a) and zone (d) in Fig. 1A, and were identified by TOFMS as monoterpenes and oxygenated sesquiterpenes. In other words, the sample types were distinguished according to their geographical origins mainly by these two types of chemical compounds. These can be supported by the result of groups' quantitative analysis in the previous section.

4. Conclusions

Classification and differentiation of TCMs according to geographical origin are important for quality control and safety. GC × GC–TOFMS methods were employed to characterize the chemical composition of Qianghuo volatile oils from different regions. A total of 769 compounds were tentatively identified and quantified in a typical sample from Sichuan province. It is far more than the results that had been reported. Based on the obvious group-type separation obtained in the GC × GC–TOFMS contour plots, major four types of compounds in Qianghuo volatile oil were identified and the relative content of these compound types was integrated and compared. Qianghuo from Sichuan province, which is the Genuine Medicinal Material, has some significant differences with those herbs from the other regions, especially in monoterpenes and oxygenated sesquiterpenes. GC × GC–TOFMS and GC × GC–FID in combination with PCA method provided the differentiation according to geographical origins, and a list of marker compounds with significant concentration difference in the different regions from the individual peak data. The results show that monoterpenes and oxygenated sesquiterpenes were related to sample differentiation. It is in good agreement with the group quantitative results of GC × GC analysis above.

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