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Analysis of volatile terpenoid compounds in *Rhododendron* species by multidimensional gas chromatography with quadrupole time-of-flight mass spectrometry

Chenyu Qian[a,1], Wenxuan Quan[a,1], Chaochan Li[a,b,*], Zhangmin Xiang[c,*]

[a] School of Life Science/Guizhou Provincial Key Laboratory of Mountainous Environmental Protection, Guizhou Normal University, Guiyang, Guizhou, People's Republic of China
[b] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou, People's Republic of China
[c] Guangdong Institute of Analysis, Guangzhou, Guangdong, People's Republic of China

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ABSTRACT

In this work, an efficient analytical method based on headspace solid phase microextraction (HS-SPME) and multidimensional gas chromatography coupled with simultaneous quadrupole time-of-flight mass spectrometry and flame ionization detection (MDGC-QTOFMS/FID) was established to analyze volatile terpenoids of *Rhododendron*. The HS-SPME method were optimized for the best extraction efficiency by using divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers at 70 °C equilibration temperature for 20 min, and extracted for 15 min. A total of 34 volatile terpenoid compounds were identified by NIST mass spectral match, and confirmed by accurate mass and retention index (RI). Quantitative analysis was performed with an internal standard (IS) 1,4-cineole. The relative standard deviations (RSD) of most identified compounds were < 19.7% for intraday and 18.8% for interday measurements, respectively. Samples from six different *Rhododendron* species were analyzed, and the results indicated that monoterpenes and their oxygenated derivatives were the major components in all species of *Rhododendron*, including D-limonene (average 2781.69 μg/kg), followed by p-cymene (average 254.52 μg/kg), linalool (average 224.40 μg/kg), 6-methyl-5-hepten-2-one (average 150.39 μg/kg) and α-terpineol (average 140.17 μg/kg). Additionally, principal component analysis (PCA) was applied to study the detailed differences in terpenoid concentrations in different parts of *Rhododendron* species.

1. Introduction

Allelopathy is an important competitive strategy of plants, and a mechanism of plant interference mediated by the production of bioactive secondary metabolites from chemical interactions [1]. And volatile terpenoids play a critical role by providing the survival advantage through affecting the growth and development of neighboring plants [2–4]. *Rhododendron delavayi*, *R. decorum*, *R. stamineum*, *R. agastum*, *R. annae* and *R. irroratum* are the species from the *Rhododendron* genus of the Ericaceae family, and the pioneer and constructive species in Baili Rhododendron National Forest Reserve in Guizhou province of China. Evidence to date has indicated that the volatilization pathway of chemicals in pure forests of the *Rhododendron* communities had an important impact on its natural regeneration [5]. Although great progress has been made, research on the analysis of volatile terpenoids in *Rhododendron* is still at an initial stage, and the volatile terpenoids of *Rhododendron* are not well understood. On the other hand, terpenoids, including the hydrocarbons and their oxygenated derivatives, are one of the most abundant and diverse compounds in nature [6]. Thus, identifying the volatile terpenoid compounds existing in plant tissues is always of great interest to the botany and chemistry community.

In this context, the headspace solid phase microextraction (HS-SPME) was used for the sampling of volatile terpenoids from *Rhododendron*. HS-SPME is characterized by several advantages well suitable for volatile sampling, such as ease of automation, solvent-free procedure, high preconcentration capacity, little manipulation of the sample and high cost-efficiency [7–9]. This technique has been successfully applied for the extraction of volatile compounds in many plant materials, such as teas [10], *Eugenia uniflora* L. [11] and *Plectranthus grandis* [12]. The optimization of SPME method is a critical step of the analysis. A series of important SPME experimental parameters, which would frequently influence the extraction efficiency, namely sample

* Corresponding authors.
E-mail addresses: wenxuanq@gznu.edu.cn (W. Quan), chaochanl@gznu.edu.cn (C. Li), xiangzm@live.com (Z. Xiang).
[1] These authors are contributed equally to this work.

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quantity, fiber coating extraction phase, equilibration temperature and time, and extraction time [13,14], were all evaluated and screened for establishing an optimal condition to extract volatile terpenoids.

One-dimensional gas chromatography mass spectrometry (1-DGC-MS) has been used for the analysis of volatile and semi-volatile organic compounds [15]. However, for a better separation of complex constituents that might exist in plant materials, comprehensive two-dimensional gas chromatography (GC × GC) stands for a more proper technique with the advantage of high resolution due to the additional separation in the secondary dimension, and enhanced sensitivity due to the re-concentration of the first-dimension effluent through modulation process, allowing the separation of co-eluted compounds and detection of compounds in trace levels [16]. High resolution time-of-flight mass spectrometry (TOFMS) has been widely used for non-target analysis with its strengths in accurate mass measurements, and good sensitivity in full scan acquisition mode [17]. In addition, the scan rate of TOFMS is sufficiently fast for profiling a typical GC × GC peak, making GC × GC -TOFMS an increasingly popular analytical technique for characterization of the chemical compositions of biological samples [18–20].

In this study, a multi-dimensional separation technique was developed shown in Fig. 1. This MDGC system combining 1-DGC and GC × GC on one instrument, with simultaneous FID and QTOFMS detection, has been employed to analyze volatile terpenoid components in different *Rhododendron*. This technique allows the identification and quantification of unknown analytes to be accomplished in one GC injection, thus provides an efficient and convenient approach for studying the volatile terpenoids of *Rhododendron* species. In this study, we labeled MDGC-QTOFMS/FID to analyze volatile terpenoids in *Rhododendron* and established a new approach for the volatile compounds analysis of *Rhododendron*.

2. Materials and methods

2.1. Samples

Methanol was purchased from Sigma-Aldrich Fluka (Buchs, Switzerland), and 1,4-cineole (internal standard; purity > 93.5%) was obtained from CATO (Oregon, U.S), both were stored at 4 °C until use. The plants materials from pure forest of the six *Rhododendron* species (*R. delavayi*, *R. decorum*, *R. stamineum*, *R. agastum*, *R. annae* and *R. irroratum*) were collected in the summer of 2018 (between July and August) in Baili Rhododendron National Forest Reserve E 105°45′–106°04′ 45; "N 27°08′ 30″–27°20′ 00″, located in northwestern of Guizhou, China. Leaves (both of fresh and litters), stems and roots of six *Rhododendron* species were collected and placed in sealed plastic bags and immediately transported in a cooler with ice to the laboratory. Afterwards, the obtained samples were smashed after frozen in a vacuum freeze dryer for a week at −70 °C (FD-1C-80; Boyikang, Beijing, China), then transferred into 50 mL vials. All samples were stored in a freezer at a temperature below −20 °C until analysis. Each type of sample (fresh leaves, litters, stems and roots of six *Rhododendron* species) were mixed in equal quantities and the mixed samples were used for the purpose of quality control (QC), and the QC sample was used for further optimization of HS-SPME parameters, analytical method establishment and methodology examination.

2.2. SPME methodology

The SPME holder for manual sampling and fibers of 85 μm carboxen/polydimethylsiloxane (CAR/PDMS), 65 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 50/30 μm divinylbenzene/polydimethylsiloxane (DVB/PDMS) and 85 μm Polyacrylate (PA) were purchased from Supelco (Aldrich, Bellefonte, PA, USA). Several experimental HS-SPME parameters were tried for the optimized condition. Firstly, four types of microextraction fibers were examined under equivalent extraction conditions. 100 mg of QC sample was accurately weighed into a 20 mL vial, and then the SPME fiber was exposed to the headspace of the bottle for 15 min at 60 °C. The SPME fiber was then introduced into the GC injector and kept there for 3.0 min to allow thermal desorption of the analytes. Subsequently, sample quantity, equilibration temperature and time, and extraction time were screened in sequence. All measurements were conducted in triplicate for each aliquot of QC sample to check the repeatability and reliability of the method development.

2.3. Multidimensional gas chromatography system

The MDGC system is consisted of a gas chromatography (7890B Agilent Technology) coupled with high resolution quadrupole time-of-flight mass spectrometry (QTOFMS) (mass resolution 20,000 and a mass accuracy specification of 3 ppm) (7250, Agilent Technology), and a flame ionization detector (FID). The samples were introduced by a split/splitless injector (SSL) system with an autosampler (PAL RSI 120, CTC Technologies). In this study, the combination of a first dimension column HP-5 MS (5% phenyl-95% dimethylpolysiloxane, 30 m × 250 μm, 0.25 μm film) and a second dimension column DB-17 MS (50% phenyl-50% dimethylpolysiloxane, 1.2 m × 180 μm, 0.18 μm film) was utilized as the two-dimensional capillary column system (both from Agilent Technologies, USA). A scheme of the MDGC system is shown in Fig. 1: the 1st column end was connected to the Deans switch (DS, Agilent Technologies) device. A solid state modulator (SSM, J&X Technologies SSM1800) was installed between DS and 2nd column. In one-dimensional gas chromatography (1-DGC) system (down), a 0.77 m × 0.18 μm film uncoated column connected to the 1st column in the other port of the Deans switch. Then the effluents of 1-DGC (down) and GC × GC (up) were first combined and then split to two detectors (QTOFMS and FID) by a 4-port splitter (4-PS). The two transfer lines to the detectors were deactivated capillary columns for the QTOFMS and FID, respectively. A split ratio of 1:2.5 between the QTOFMS and the FID was achieved.

1-DGC and GC × GC conditions were as followed: the GC injector was kept at 250 °C; helium (99.999%) was used as the carrier gas at a constant flow of 1.2 mL/min; oven temperature was programmed from initial temperature at 50 °C (held for 3 min) to 200 °C at 4 °C/min (held

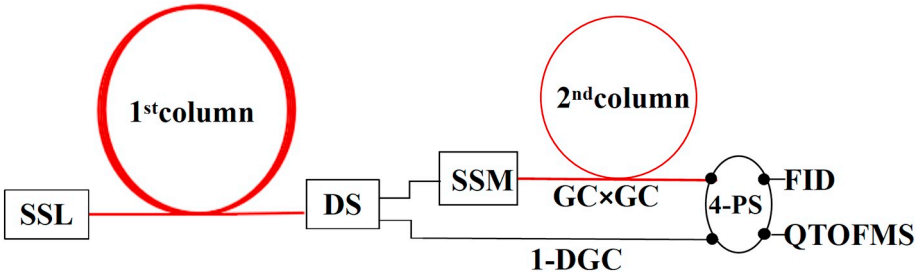

Fig. 1. A scheme of the MDGC-QTOFMS system (SSL: split/splitless injector; DS: deans switch; SSM: solid state modulator; 4-PS: 4-port splitter; 1-DGC: one-dimensional gas chromatography; GC × GC: comprehensive two-dimensional gas chromatography).

for 0.5 min), then increased to 250 °C by 10 °C/min, for a total run time of 46 min. For GC × GC system, the oven temperature was the same as the 1-DGC method; the cold zone temperature of the SSM was set at −50 °C. The temperatures of the entry hot zone and exit hot zone are +30 and +120 °C offset relative to oven temperatures, respectively, with a cap temperature of 320 °C for both hot zones. The modulation period was 4 s.

MS condition: The transfer line and ion source temperature were set to 280 °C and 250 °C, respectively. Electron impact ionization was employed, with electron energy applied at 70 eV, and a mass range was set at 50–500 *m/z* in full-scan acquisition mode. The MS scan rate was 50 Hz.

2.4. Data method

The 1-DGC data was processed using Agilent Mass Hunter Qualitative Analysis navigator B.08.00; The GC × GC data were analyzed by a dedicated GC × GC data processing software Canvas (J&X Technologies, version v1.4.0). Compounds were tentatively identified through mass spectral match based on NIST 17 Mass Spectral Library (NIST/EPA/NIH 2017), and then verified by the retention index (RI) and accurate mass [21]. RI was calculated using a series of n-alkanes (C8–C25) analyzed on a HP-5 MS column under the same chromatographic method.

1,4-cineole was used as internal standard (IS), and 1 μL of IS (31.4 μg/mL) was added to the samples. Quantitative method was performed based on previous studies [22]. The volatile terpenoids corresponding contents in various *Rhododendron* samples (up to 24 samples) were examined under optimized HS-SPME parameters. A signal-to-noise ratios (S/N) of 3.0 was set as the threshold for peak identification and integration.

Principal component analysis (PCA) was performed using SIMCA-P 11.5 software (Umetrics, Umea, Sweden), and Excel 2016 (Microsoft, USA) was used for data calculation and visualization.

3. Results and discussion

3.1. Method optimization of HS-SPME

The performance of a given SPME application is critically dependent on the properties of the selected extraction phase, which determines the selectivity and the reliability of the method. Five experimental HS-SPME parameters were screened to achieve the best extraction efficiency of volatile terpenoids in *Rhododendron*, by comparing the total GC × GC peak areas of target volatile terpenoids. First of all, four commercial fiber materials CAR/PDMS (85 μm), DVB/CAR/PDMS (65 μm), DVB/PDMS (50/30 μm) and PA (85 μm) were screened. As can be seen in Fig. 2, the results of the extracted terpenoid peak areas indicated that DVB/CAR/PDMS has the best extraction efficiency, followed by PDMS/DVB, PDMS/CAR and PA fibers. Eventually, DVB/CAR/PDMS fiber was selected for the rest of all samples.

Secondly, considering that the sensitivity of the SPME is partly influenced by the amount of plant samples, the quantity of QC samples varying from 25 mg to 300 mg is tested for the purpose of detecting the most terpenoids at a proper sample quantity. As shown in Fig. 3a, the amount of terpenoids significantly enhanced when the sample quantity increased from 25 mg to 100 mg, and experiments with low sample quantity (< 100 mg) failed to detect some trace components. However, when the tested sample quantity continually increased (> 100 mg), the total peak area slightly decreased. In conclusion, 100 mg was employed as the desired sample quantity.

Subsequently, a series of equilibration temperatures were also investigated. As Fig. 3b illustrated, the amount of extraction of terpenoids increased with increasing temperature from 40 °C to 70 °C, but remained almost constant when the equilibration temperature exceeded 70 °C. Therefore, 70 °C was chosen as the best equilibration temperature. In addition, the equilibration time was evaluated. As shown in Fig. 3c, an equilibration time of 20 min exhibited slight advantage against 5 min and 10 min, but the peak area decreased when the equilibration time increased to 30 min and 40 min. As a result, further analysis employed 20 min as the equilibration time.

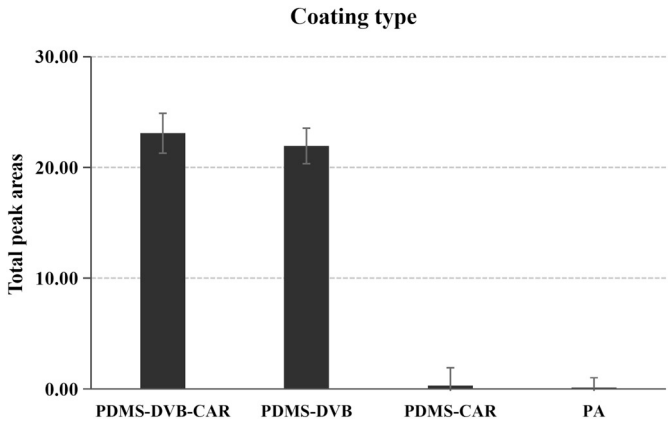

Fig. 2. Comparison of the extraction efficiency of commercial SPME coatings.

Finally, the extraction time was investigated under the optimal conditions above. Fig. 3d demonstrated that 15 min of extraction time provides the best extraction efficiency. In conclusion, the optimized SPME method with the best extraction efficiency was to use DVB/CAR/PDMS (65 μm) as the SPME fiber phase, and to employ a sample quantity of 100 mg, with an equilibration temperature of 70 °C for 20 min, and with a 15 min extraction time.

3.2. Analytical precision

It is important that analytical errors are minimized so as to ascertain that the observed differences between sample groups are indeed due to biological variations among the samples, rather than analytical variance [22]. Thus, the optimized SPME method condition was further evaluated for the stability and repeatability with QC samples. Each sample was conducted in triplicate to reduce deviation, the intra-day precision was evaluated by analyzing three equivalent QC samples on the same day, and the same procedure was performed once a day for 3 consecutive days to determine inter-day precision. The intra-day and inter-day precision were expressed as relative standard deviation (RSD %).

As can be seen in Fig. 4a, ten selected major components demonstrated that the RSD of nearly all of the tested compounds were lower than 20%. And the relatively low intra-day (3.0%) and inter-day precision (8.3%) of IS proved that stable and repeatable method conditions were satisfactorily achieved. Fig. 4b arrayed all the identified compounds to four groups with their RSD below 19.7% for intra-day and 18.8% for inter-day measurements, respectively. In conclusion, the optimized SPME method could attain acceptable precision for most of the analytes.

3.3. Comparison of the QTOFMS and FID

In conventional GC × GC systems, components in samples were subjected to MS detector and FID for quantitative and qualitative characteristics through two different separated steps. For achieving a more convenient and economical approach, the present study has developed a MDGC configuration with simultaneous detection by QTOFMS and FID. The advantage of such a configuration was that the quantification and identification of analytes were conducted in one step, by splitting the effluent to QTOFMS and FID at the same time. A split ratio of circa 1:2.5 was achieved to QTOFMS and FID. A comparison of the QTOFMS and FID GC × GC contour plots of total ion

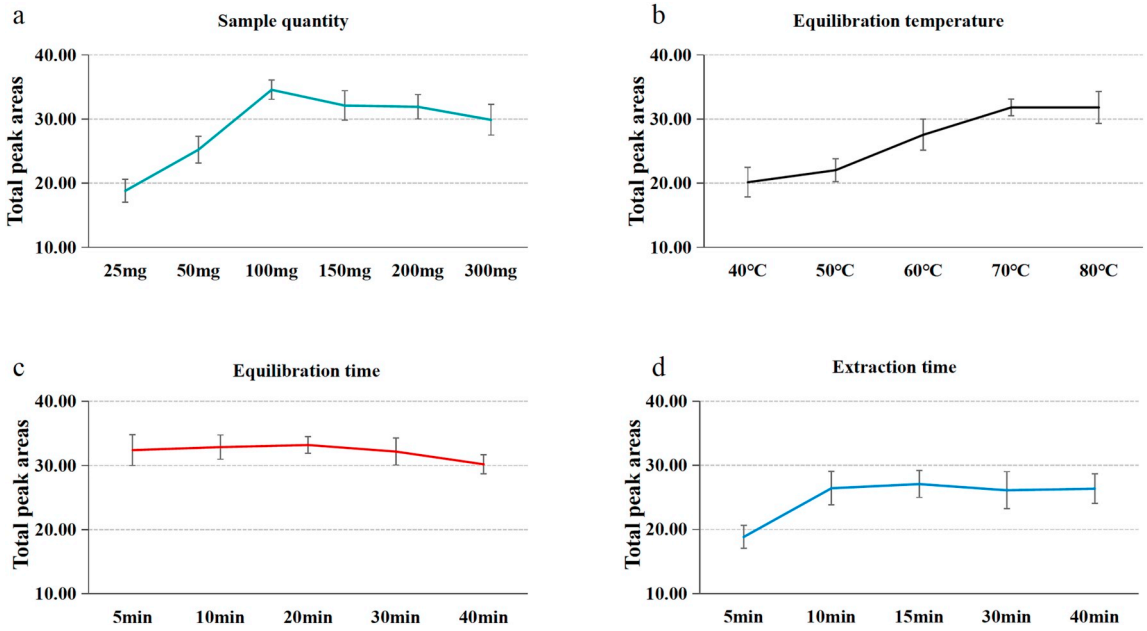

Fig. 3. The analysis parameter optimizing of HS-SPME.

chromatogram (TIC) was presented in Fig. 5. Although these two chromatographic displays look quite similar, the retention positions for most of separated components are not exactly the same. FID is a universal detection providing a relatively uniform carbon response [23], while QTOFMS is efficient to detect all kinds of compounds maximally, leading to the result that more responded peaks appear in QTOFMS chromatograms compared to FID. And small changes in column flow caused negligible retention time differences (~0.026 s) between the QTOFMS and FID traces.

3.4. Identification of analytes

Component identification was achieved by matching the QTOFMS spectral with a commercial mass spectral library (NIST 17), with a minimum match factor of 800. The QTOFMS generates the high-resolution mass spectral information, which may include the molecular ions of the compounds. Comparison between the molecular ion, if exists, and the accurate mass of the identified compound was carried out after library search for verification. Additionally, retention index information was used for further confirmation, with the acceptable RI difference between the experimental measured value (RI_{exp}) and the literature value (RI_{lit}) of 0–40 ($|RI_{exp} - RI_{lit}|$), which was considered reasonable (< 5%) in previous studies [24]. All the terpenoid compounds were tentatively identified with a relatively high values of similarity, with the average forward match factor of 862 and average reverse match factor of 891. Consequently, qualitative identification accepted by comparisons of RI and accurate mass from the NIST 17 library led to the identification of 34 terpenoids in total 93 compounds from *Rhododendron*.

GC × GC analysis was performed using a combination of two columns consisting of a non-polar stationary phase (HP-5 MS) as first-dimension column and a polar stationary phase (DB-17 MS) as second-dimension column. As exhibited in Table 1, a total of 18 volatile terpenoid compounds were detected by GC measurement, while 34 volatile terpenoid compounds were further separated by GC × GC analysis, approximately two times of that detected by 1-DGC technique, due to additional separation based on polarity in the secondary column. Some peaks in 1-DGC analysis comprised of co-eluted components were further resolved by GC × GC.

The general molecular formula for terpenoid is $(C_5H_8)_n$, and QTOFMS yields primary molecular ions providing an accurate molecular quantity. As represented in Fig. 5, a two-dimension plot of all 34

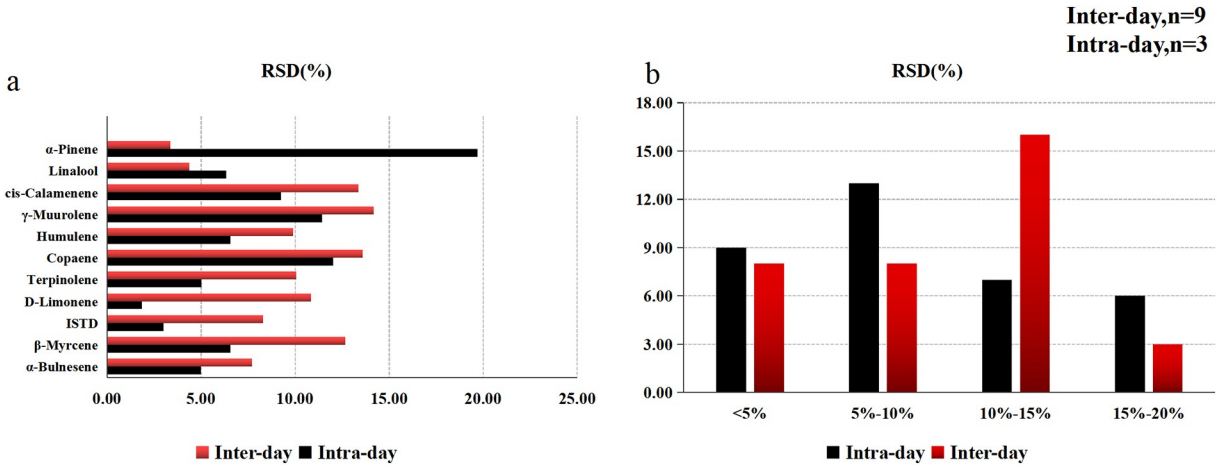

Fig. 4. Intra-day and inter-day precision (expressed as relative standard deviation RSD %) for confirmed method.

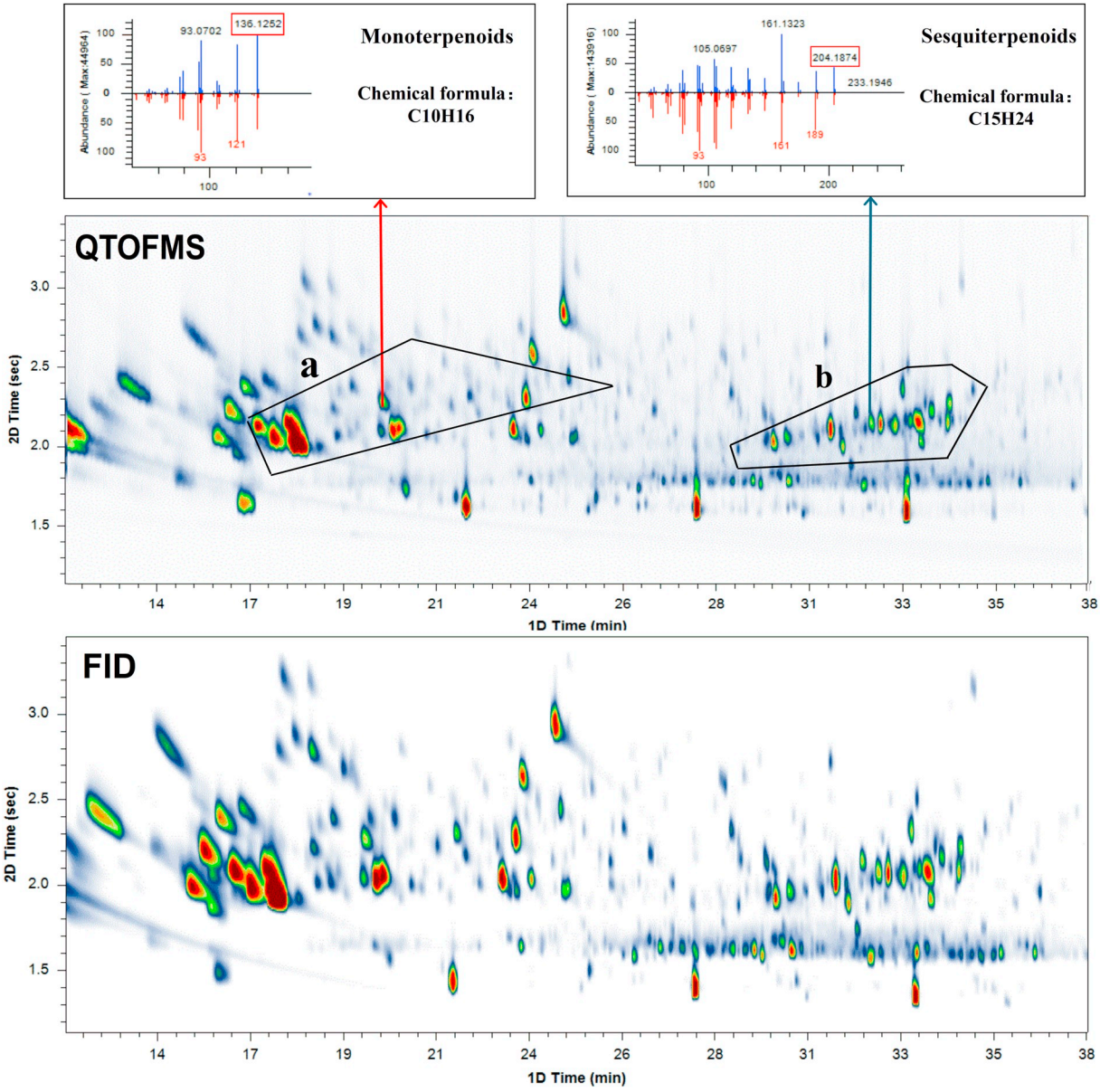

Fig. 5. Two terpenoid regions presented in GC × GC-QTOFMS/FID chromatogram.

terpenoids peaks from the QC sample using GC × GC method was illustrated. The monoterpenoids ($C_{10}H_{16}$) were displayed in Fig. 5a, while sesquiterpenoids ($C_{15}H_{24}$) were shown in Fig. 5b. Compared to monoterpenoids, the sesquiterpenoids have higher volatility, thus elute later than monoterpenoids. The monoterpenoids and sesquiterpenoids are distributed in two distinct regions with ordered structures in the GC × GC chromatogram. Additionally, a detailed mass spectra of monoterpenoids and sesquiterpenoids were demonstrated with their NIST library mass spectra displayed head-to-tail. Their accurate molecular ion masses were both marked with red boxes for comparison with their actual values.

3.5. Real sample analysis

All those samples were identified by MDGC/QTOFMS and quantified by MDGC/FID in one single run. The FID response provides reliable quantitative results of the resolved peaks due to its stable characteristics, while the QTOFMS provides the qualitative information. Quantitative analysis of plant materials has been performed to calculate the respective concentrations of volatile components according to the proportion of the peak areas of the compounds to the internal standard [25].

In this work, all samples were investigated in one replicate in order to give representative results. As demonstrated in **Supplementary Table S1**, a total of 34 kinds of terpenoids were detected in all types of *Rhododendron* samples with various concentration levels. The major compounds in each *Rhododendron* were approximately the same, with the major constituents of D-limonene (average 2781.69 μg/kg), followed by p-cymene (average 254.52 μg/kg), linalool (average 224.40 μg/kg), 6-methyl-5-hepten-2-one (average 150.39 μg/kg) and α-terpineol (average 140.17 μg/kg). Apparently, D-limonene was the dominant component in every part of all species. The D-limonene was confirmed by many researchers that it had strong allelopathy, such as respiratory inhibition activity against growth of *Microcystis* [26] or their cellular growth [27]. The concentrations of p-cymene in the roots (average 547.81 μg/kg) were significantly higher than that in the litters (average 251.24 μg/kg), stems (average 163.51 μg/kg) and fresh leaves (average 97.44 μg/kg). P-cymene has been reported to have allelopathy activity and inhibitory effect in seed germination [28]. This study indicated that the p-cymene mainly produced by root decomposition

Table 1
Compounds identified in *Rhododendron* by GC and GC × GC.

Constituents	GC		GC × GC									
	RT (min)	concentration (%)	$^{1}tR^{a}$ (min)	$^{2}tR^{b}$ (s)	Molecular ion mass(*m/z*)		Match factor		RI^{c}_{exp}	RI^{d}_{lit}	RSD(%)	
					Theoretical	Experimental	Forward	Reverse			Intraday	Interday
6-Methyl-5-Hepten-2-one			15.37	2.20	126.1039	126.1032	802	908	992	964	4.87	6.11
β-Myrcene	15.49	0.41	15.57	1.86	136.1247	136.1252	891	915	997	983	6.57	12.65
p-Cymene	16.85	0.04	17.03	2.08	134.109	134.1088	809	858	1034	1025	16.93	18.78
D-Limonene	16.94	2.27	17.10	1.94	136.1247	136.1244	876	876	1036	–	1.86	10.83
α-Pinene			17.77	1.89	136.1247	136.1252	840	859	1053	1037	19.70	3.36
γ-Terpinene	18.18	0.48	18.23	2.00	136.1247	136.1252	888	910	1065	1050	8.88	10.07
Isophorone			18.30	2.35	138.1039	138.1028	800	846	1066	1089	9.27	10.06
Terpinolene			19.43	2.03	136.1247	136.1252	894	925	1095	1079	5.02	10.06
Linalool			19.77	2.03	154.1352	154.1359	880	922	1104	1086	6.33	4.38
Allocimene			20.90	1.98	136.1247	136.1252	844	886	1134	1131	15.28	14.25
(+)-(*E*)-Limonene oxide			21.30	2.24	152.1196	152.1197	809	830	1145	1130	14.53	15.70
α-Terpineol			23.30	2.29	154.1352	154.136	816	913	1199	1175	5.16	0.86
β-Cyclocitral	24.38	0.06	24.43	2.46	152.1196	152.1197	814	816	1230	1197	4.61	11.84
Isobornyl acetate	26.67	0.04	26.70	2.20	196.1458	196.1449	886	887	1293	1273	6.72	4.78
α-Cubebene	28.86	0.21	28.97	1.89	204.1873	204.1874	851	873	1360	1351	12.74	11.19
Cyclosativene			29.70	1.96	204.1873	204.1874	882	907	1382	1373	19.88	8.21
Copaene			29.90	1.95	204.1873	204.1874	919	927	1388	1376	12.02	13.58
(−)-β-Bourbonene	30.21	0.07	30.23	2.00	204.1873	204.1874	880	901	1398	1382	10.52	6.39
β-Maaliene			31.10	1.98	204.1873	204.1874	860	872	1425	1413	5.19	3.04
Caryophyllene	31.37	0.29	31.43	2.05	204.1873	204.1874	906	933	1436	1419	17.61	10.12
Isogermacrene			31.70	2.04	204.1873	204.1874	865	881	1444	1437	10.85	5.80
trans-α-Bergamotene	31.70	0.07	31.77	1.91	204.1873	204.1874	900	920	1446	1432	8.95	7.92
Dihydropseudoionone			32.10	2.18	194.1665	194.1663	842	890	1457	1432	3.65	14.77
Humulene	32.47	0.10	32.50	2.11	204.1873	204.1874	842	897	1469	1451	6.56	9.88
9-epi-β-Caryophyllene	32.71	0.16	32.77	2.09	204.1873	204.1874	905	934	1477	1464	11.18	15.32
γ-Muurolene	33.09	0.11	33.17	2.08	204.1873	204.1874	866	923	1490	1472	11.44	14.16
β-Ionone	33.29	0.03	33.37	2.36	192.1509	192.1517	906	907	1496	–	3.43	12.00
γ-Selinene			33.43	2.12	204.1873	204.1874	826	882	1498	1487	8.63	3.49
Longifolene-(V4)			33.57	2.11	204.1873	204.1874	865	872	1502	1488	9.08	12.81
α-Farnesene	33.51	0.05	33.83	1.96	204.1873	204.1874	903	916	1511	1496	4.98	7.71
α-Bulnesene	33.80	0.20	33.77	2.10	220.1822	220.1822	882	888	1509	1502	4.56	3.96
β-Cadinene	34.51	0.08	34.57	2.10	204.1873	204.1874	874	877	1536	1522	12.25	14.64
cis-Calamenene	34.54	0.09	34.63	2.25	202.1716	202.1705	875	878	1538	–	9.24	13.36
β-Calacorene			35.23	2.36	200.156	200.1558	836	874	1558	1548	17.28	12.06

[a] The retention time on 1st column in GC × GC.
[b] The retention time on 2nd column in GC × GC.
[c] Retention indices from the present study measured on a HP-5MS column according to n-C8~n-C25 alkanes.
[d] Retention indices from https://webbook.nist.gov/chemistry.

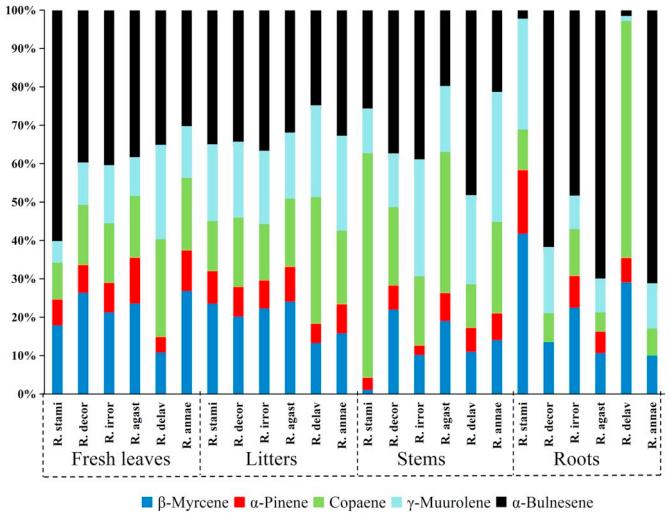

Fig. 6. Distribution (%) of major compounds presented within four parts of different types of *Rhododendron*.

channel. In the contrary, linalool was presented in extremely low concentrations (average 15.6 μg/kg) in root, and was even absent in *R. stamineum*, *R. irroratum* and *R. delavayi*. However, drastically increased concentration of linalool was found in the stems (average 600.50 μg/kg), followed by fresh leaves (average 173.39 μg/kg) and litters (average 39.60 μg/kg). In addition, 6-methyl-5-hepten-2-one was presented in comparable amount with α-terpineol. It has been reported that both of linalool and α-terpineol have the allelopathic potential with algicidal properties [29,30]. Bioactivities of plant extracts have been generally attributed to particular compounds (major constituents), and synergistic phenomenon among corresponding mixtures have been shown to result in a higher bioactivity compared to the isolated individual component [31], reflecting the importance of compositional complexity in conferring bioactivity to natural terpenoid mixtures. Consequently, it can be concluded that the inhibitory effects of allelopathic by *Rhododendron* are mainly attributed to monoterpenes components, which are the predominant volatile terpenoids. The distribution of the five compounds with lower content than the major components in different parts of the species was illustrated in Fig. 6. Based on these five compounds concentration, α-Bulnesene was the main component in fresh leaves (30.22%–60.15%), and took a relatively high percentage in roots of *R. decorum* (61.73%), *R. irroratum* (48.30%), *R. agastum* (69.93%) and *R. annae* (71.11%). β-Myrcene and copaene were found as the dominant compounds in roots of *R. stamineum* (41.77%) and *R. delavayi* (61.77%), respectively. α-Pinene (2.37%–16.51%) and γ-Muurolene (1.30%–33.87%) presented slightly low proportion in most parts of different species.

In view of the great disparities of concentrations performed between

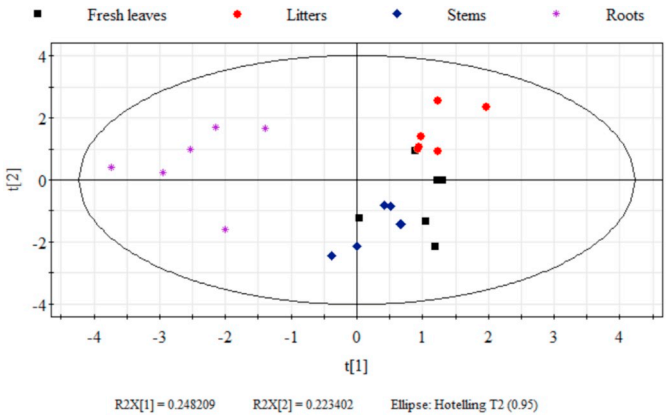

Fig. 7. PCA analysis of 24 *Rhododendron* samples.

different parts of plant samples, the difference of terpenoids in concentration among different *Rhododendron* were studied by PCA method. As can be seen in Fig. 7, three well-defined clusters of samples from different parts of plant were formed in this model. Firstly, significant discrimination between aerial parts (fresh leaves, litters and stems) and underground part (roots) of *Rhododendron* with the regional division was observed. Then fresh leaves and stems were separated with litters (detached from the plant). The partial overlap between fresh leaves and litters was justified by the factor that litters were originally leaves of the plant.

4. Conclusions

In conclusion, an efficient HS-SPME and MDGC system combining simultaneous QTOFMS and FID detection with optimized experimental parameters was established to analyze volatile terpenoids in *Rhododendron*. The qualitative and quantitative analysis of terpenoids in different types of *Rhododendron* were carried out in one single GC injection, with ideal repeatability and reliability. Our present results enrich the basic theory of *Rhododendron* chemistry and lay a theoretical foundation for the volatile terpenoids existing in different *Rhododendron* species. D-limonene, p-cymene, linalool, 6-methyl-5-hepten-2-one and α-terpineol were proved to be the major components of terpenoids in all varieties of *Rhododendron*. Moreover, the PCA results helped to distinguish the terpenoids distribution among the 24 *Rhododendron* samples.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2019.104064.

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