GC-MS analyses of the volatiles of *Houttuynia cordata* Thunb.

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Abstract: GC-MS is the basis of analysis of plant volatiles. Several protocols employed for the assay have resulted in inconsistent results in the literature. We developed a GC-MS method, which were applied to analyze 25 volatiles (*α*-pinene, camphene, *β*-pinene, 2-methyl-2-pentenal, myrcene, (+)-limonene, eucalyptol, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexeneyl-acetate, 1-hexanol, *α*-pinene oxide, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, decanal, linalool, acetyl-borneol, *β*-caryophyllene, 2-undecanone, 4-terpineol, borneol, decanol, eugenol, isophytol and phytol) of *Houttuynia cordata* Thunb. Linear behaviors for all analytes were observed with a linear regression relationship (r^2>0.9991) at the concentrations tested. Recoveries of the 25 analytes were 98.56-103.77% with RSDs <3.0%. Solution extraction (SE), which involved addition of an internal standard, could avoid errors for factors in sample preparation by steam distillation (SD) and solid-phase micro extraction (SPME). Less sample material (≈0.05g fresh leaves of *H. cordata*) could be used to determine the contents of 25 analytes by our proposed method and, after collection, did not affect the normal physiological activity or growth of *H. cordata*. This method can be used to monitor the metabolic accumulation of *H. cordata* volatiles.

Keywords: Analysis, Gas chromatography-mass spectrometry (GC-MS), *Houttuynia cordata* Thunb., Solid-phase micro extraction (SPME), Steam distillation (SD), Volatiles

INTRODUCTION

A search through Google Scholar in December 2013 using the keywords "volatiles" and "GC-MS" returned 16,900 hits, whereas "*Houttuynia cordata*" and "GC-MS" returned 380 hits. Employing "*Houttuynia cordata* volatiles" and "*Houttuynia cordata* volatiles" combined with "GC-MS" returned 260 and 16 hits, respectively. In the same way, "*Houttuynia cordata* volatiles" and "determination" returned 29 hits, and "*Houttuynia cordata* volatiles and determination" combined with "GC-MS" returned 6 hits. Using "volatiles", "steam distillation" and "GC-MS" returned 13,100 hits. Employing "volatiles", "SPME" and "GC-MS" returned 19,700 hits. Using "*Houttunia cordata*", "SPME" and "GC-MS" returned 243 hits. Employing "*Houttunia cordata*", "steam distillation" and "GC-MS" returned 123 hits. Using "volatiles", "normalization" and "GC-MS" returned 17,400 hits. Employing "volatiles", "quantitation" and "GC-MS" returned 2 hits. Employing "*Houttunia cordata*", "normalization" and "GC-MS" returned 231 hits. Using "*Houttunia cordata* volatiles", "quantitation" and "GC-MS" returned zero hits.

These searches showed that considerable research has been done on plant volatiles (including *H. cordata* volatiles) obtained by steam distillation (SD) and headspace solid-phase micro extraction (HS-SPME) and analyzed using gas chromatography-mass spectrometry (GC-MS), followed by more studies on analyses of peak areas based on normalization. However, much less work has focused on analyses of volatiles according to the internal standard method and selective ion monitoring (SIM) using GC-MS.

Analytical methods for plant volatiles have been applied to traditional Chinese medicines (TCMs) (Zeng *et al.,* 2007; Raina *et al.,* 2014; Qi *et al.,* 2004; Lu *et al.,* 2006; Saha *et al.,* 2012). However, monitoring plant volatiles during growth cannot be done accurately and reliably using normalization based on peak areas. In addition, the original plant volatiles may be affected by the heating of volatiles obtained by SD (which consumes large amounts of samples) and HS-SPME (Xu *et al.,* 2005; Chiang *et al.,* 2003). If metabolic accumulative processes or various volatiles in plants need to be monitored, the normal physiological activity or growth of the plant should not be affected after sample collection. Therefore, fewer samples should be collected and then the analytical method must not make changes to the original volatiles.

Houttunia cordata Thunb. is a TCM. It possesses antiviral (Chiang *et al.,* 2003), antioxidant (Styskal *et al.,* 2012), anti-inflammatory (Chang *et al.,* 2001), anti-SARS activity (Lau *et al.,* 2008) and antimutagenic effects (Tian *et al.,* 2011). Its medicinal properties have been attributed to its main volatile: 2-undecanone (Shimura *et al.,* 1999; Tsui and Brown *et al.,* 1996). However, because of the instability and ready oxidization of 2-undecanone and other aldehydes and/or ketones (Ceng *et al.,* 2003) during distillation, temperature increases, and storage, *H. cordata* volatiles must be prepared fresh before analyses. Presently, *H. cordata* volatiles extracted by SD and HS-SPME (Yang *et al.,* 2009; Liu *et al.,* 2007) and analyzed by GC

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or GC-MS are quantified according to normalization of their chromatographic peak areas (Liang *et al.,* 2005; Xu *et al.,* 2005). However, metabolic accumulative processes or various *H. cordata* volatiles (including 2-undecanone and decanal) cannot be analyzed accurately and reliably by normalization-based methods (which is only preliminary semi-quantitation because each volatile has a different response to the instrument) (Yao *et al.,* 2014; Wang *et al.,* 2013). In addition, some aldehydes and ketones of *H. cordata* volatiles may be changed in the heating processes of SD and SPME. Dynamic monitoring or distribution of *H. cordata* volatiles must not involve collection of many samples or damage to the normal physiological activities of the plant. Therefore, it is necessary to establish a validated analytical method that not only reflects accurately the dynamic processes of *H. cordata* volatiles but also does not destroy their physiological activities and growth.

We attempted to establish an accurate, reliable and validated method of 25 *H. cordata* volatiles analyzed simultaneously using GC-MS and to monitor the metabolic accumulative processes of *H. cordata* volatiles. using this method.

MATERIALS AND METHODS

Chemicals and materials

Standard volatiles (*α*-pinene, camphene, *β*-pinene, 2-methyl-2-pentenal, myrcene, (+)-limonene, eucalyptol, *trans*-2-hexenal, *γ*-terpinene, *cis*-3-hexeneyl-acetate, 1-hexanol, *α*-pinene oxide, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, decanal, linalool, acetyl-borneol, *β*-caryophyllene, 2-undecanone, 4-terpineol, borneol, decanol, eugenol, isophytol and phytol) of purity >95% were purchased from Sigma-Aldrich (St Louis, MO, USA). *n*-tetradecane was obtained from Acros Organics (Morris Plains, NJ, USA). Dichloromethane (AR), anhydrous sodium sulfate (AR), n-hexane (AR) and other reagents were obtained from Shanghai Chemical Reagents Company (Shanghai, P. R. China).

The fresh leaves of *H. cordata* were the samples. A stock solution was prepared. Briefly, 25 standard volatiles and internal standard were weighed (to 0.0001mg) and then dissolved in dichloromethane in two 10mL volumetric flasks, respectively. To prepare working solutions, stock solutions were diluted further with the appropriate amount of dichloromethane. Prepared solutions were at 4°C until GC-MS analyses.

Instrumentation

The GC-MS instrument (GCMS-QP2010) equipped with GCMS solution 2.10 (Shimadzu, Kyoto, Japan) was used in this work. Components were separated on capillary columns (Factor Four™ VF-WAXms, Factor Four™ VF-5ms and Zebron™ phase Zb-1701, 30m × 0.25mm × 0.25 μm, USA).

GC conditions

Injector temperature: 250°C. Column temperature program: 30°C (3 min), 5.6°C/min to 100°C, maintained for 1 min, 3.1°C/min to 125°C, 15°C/min to 230°C (10 min). Carrier gas: helium (99.999%); flow rate: 1.10 mL/min; split ratio: 1:20.

MS conditions

Ion source and ionization voltage: EI and 70 eV, respectively; ion source temperature: 210°C; detection voltage: 1.2 kV; interface temperature: 270°C; solvent delay time: 5 min; scan mode: SIM. The characteristic ions for all analytes are shown in table 1.

SD extraction

SD Extraction was carried out as described (Liu *et al.,* 2007; Liang *et al.,* 2005). Fresh *H. cordata* leaf samples (500g) were weighed in a 2500mL distillation flask into which ≈1200mL deionized water was added, then the mixture was distilled for 4h. The essential oil obtained from the condenser was dried with anhydrous Na_2SO_4. The yield of essential oil was calculated according to the weight of fresh *H. cordata* leaves. Then essential oil (0.1mL) diluted with *n*-hexane (5mL) was stored at -20°C until GC-MS analyses. Relative contents of normalization-based peaks were obtained and calculated using the following equation:

$$Xi\% = [A_i/(A_1 + A_2 + \cdots + An)] \times 100$$

where X and A is the percentage and peak areas, respectively. The contents of volatiles (C) were obtained and calculated for comparative analyses with the developing method using the following equation:

$$C_i\,(mg/kg) = \frac{M_{\text{Yields of oils}}\,(mg) \times X_i\,(\%)}{M_{\text{Sampls}}\,(kg)}$$

HS-SPME extraction

Before use, a 100μm polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, PA, USA) was used with the manual SPME holder assembly. Experiments of extraction were performed according to methods described previously (Liang *et al.,* 2005). *H. cordata* samples (0.5g) were sealed hermetically in a 4mL vial. In a thermostatic bath, the SPME fiber suspended in the vial was equilibrated for 60 min at 80°C. Percentages of volatiles were obtained and calculated according to normalization of peak areas.

Solution extraction (SE)

A *H. cordata* sample (0.05g; accurate to 0.0001g) was weighed into a 4-mL vial with 2mL dichloromethane and 10μL stock solution of internal standard. The vial was then sealed and extracted by sonication (250 W; 50 Hz) at room temperature for 30 min. Extracts were centrifuged and sample solutions stored at 4°C until GC-MS analyses.

RESULTS

SE optimization

The extraction efficiency of *H. cordata* samples was evaluated using ethyl acetate, dichloromethane and *n*-hexane at room temperature. Dichloromethane produced fewer interference peaks and obtained the highest content values of 25 analytes. An orthogonal array design (OAD) was developed so that the extraction could be optimized. This OAD was based on a four-factor-three-level to ensure that power for extraction was subjected to sonication (150, 200 and 250W), dichloromethane volume (1, 2 and 3mL) and extraction duration (20, 30 and 40 min). The results showed that the optimized extraction condition (extraction power =250W, extraction volume = 2mL and extraction duration =20min) was suitable and

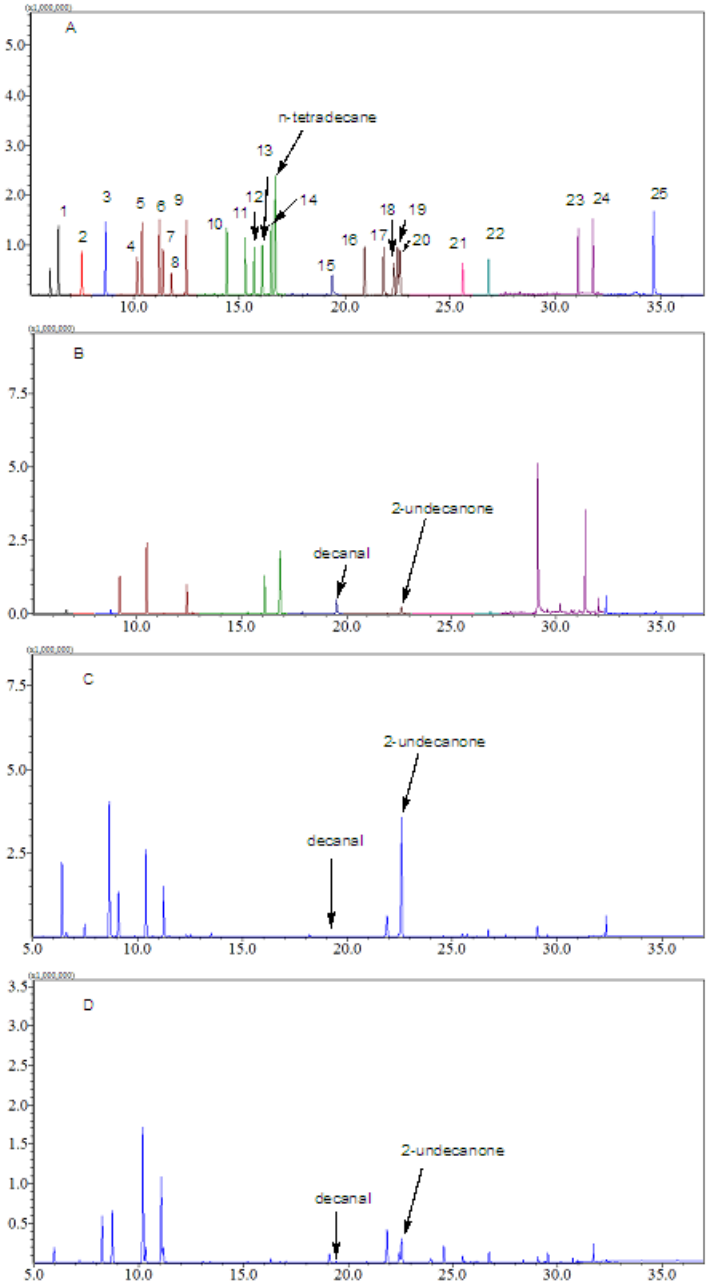

Fig. 1: Representative GC-MS chromatographic profiles of 25 mixed standards (A) and *H. cordata* sample obtained by SE (B), SD (C) and SPME (D) (1, *α*-pinene; 2, camphene; 3, *β*-pinene; 4, 2-methyl-2-pentenal; 5, myrcene; 6, (+)-limonene; 7, eucalyptol; 8, *trans*-2-hexenal; 9, *γ*-terpinene; 10, *cis*-3-hexenyl-acetate; 11, 1-hexanol; 12, *α*-pinene oxide; 13, *cis*-3-hexen-1-ol; 14, *trans*-2-hexen-1-ol; 15, decanal; 16, linalool; 17, acetyl-borneol; 18, *β*-caryophyllene; 19, 2-undecanone; 20, 4-terpineol; 21, borneol; 22, decanol; 23, eugenol; 24, isophytol; 25, Phytol)

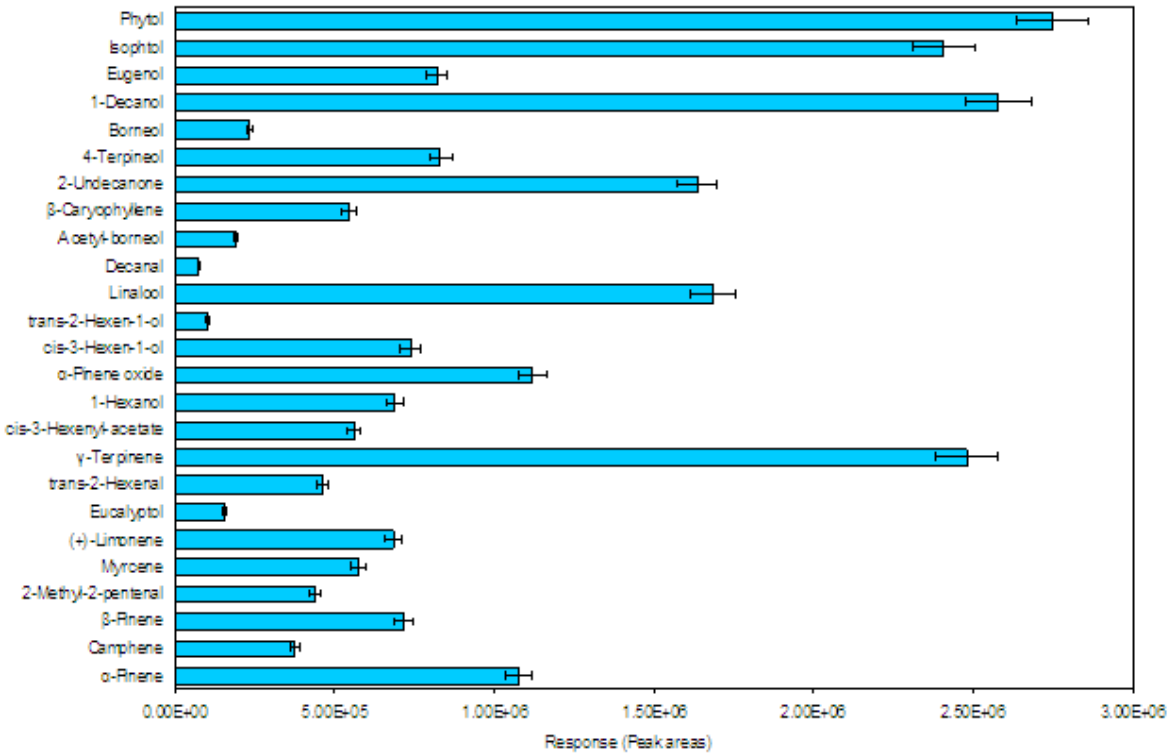

Fig. 2 Response of the 25 analytes (the concentration of the standard mixture solution of the 25 analytes is 0.05 mg/mL)

appropriate for analyses. A small quantity of sample (≈0.05g) obtained by SE was determined using GC-MS. However, SPME and SD required increasing amounts of samples (≈0.5 and ≈500g, respectively). The amount of SE did not influence the normal physiological activity and/or growth of *H. cordata*, thereby contributing to the monitoring of the *H. cordata* volatiles. Sample preparation by SD and SPME (which required heating to boiling temperature and 80°C, respectively) could elicit changes to the original *H. cordata* volatiles. However, sample preparation by SE at room temperature could avoid such changes. Additionally, the time required for SE was much shorter than that for SD.

Chromatographic condition

Optimization of the chromatographic condition was undertaken using *H. cordata* sample solutions. Components were determined and compared using different analytical chromatographic columns (Factor Four™ VF-WAXms, Factor Four™ VF-5ms or Zebron™ phase Zb-1701) at different temperature programs. The results showed that the analytes investigated could be separated efficiently by the Factor Four™ VF-WAXms (fig. 1).

Quantifier and qualifier ions

Table 1 shows the quantifier ions of the 25 analytes and internal standard to be *α*-pinene (93), camphene (93), *β*-pinene (93), 2-methyl-2-pentenal (98), myrcene (93), (+)-limonene (68), eucalyptol (154), *trans*-2-hexenal (55), *γ*-terpinene (93), *cis*-3-hexeneyl-acetate (67), 1-hexanol (56), *α*-pinene oxide (109), *cis*-3-hexen-1-ol (67), *trans*-2-hexen-1-ol (57), decanal (57), linalool (71), acetyl-borneol (95), *β*-caryophyllene (93), 2-undecanone (58), 4-terpineol (71), borneol (95), decanol (55), eugenol (164), isophytol (71), phytol (71) and n-tetradecane (57). The qualifier ions of analytes and internal standard are shown in table 1.

GC-MS method validation

Calibration curve, limit of detection (LOD) and limit of quantitation (LOQ)

Standard solutions of different concentrations were prepared by the stock solution of 25 analytes and the internal standard diluted. The appropriate concentration ranges needed to create the calibration curves using GC-MS were optimized. The respective calibration curves were plotted by linear regression to the mean peak areas of the analytes (Y axis) *versus* their concentrations (X axis). The LODs and LOQs of the analytes were tested at a signal-to-noise ratio (S/N) of 3 and 10, respectively, under the optimized GC-MS condition. The relevant information for the calibration curves, LODs and LOQs of the analytes are shown in table 2. Linear behavior of all analytes was observed with a linear regression relationship (r^2>0.9991) at the concentration ranges investigated. The LODs and LOQs were ≤0.44ng and ≤ 1.51ng, respectively (except that the LOD and LOQ of

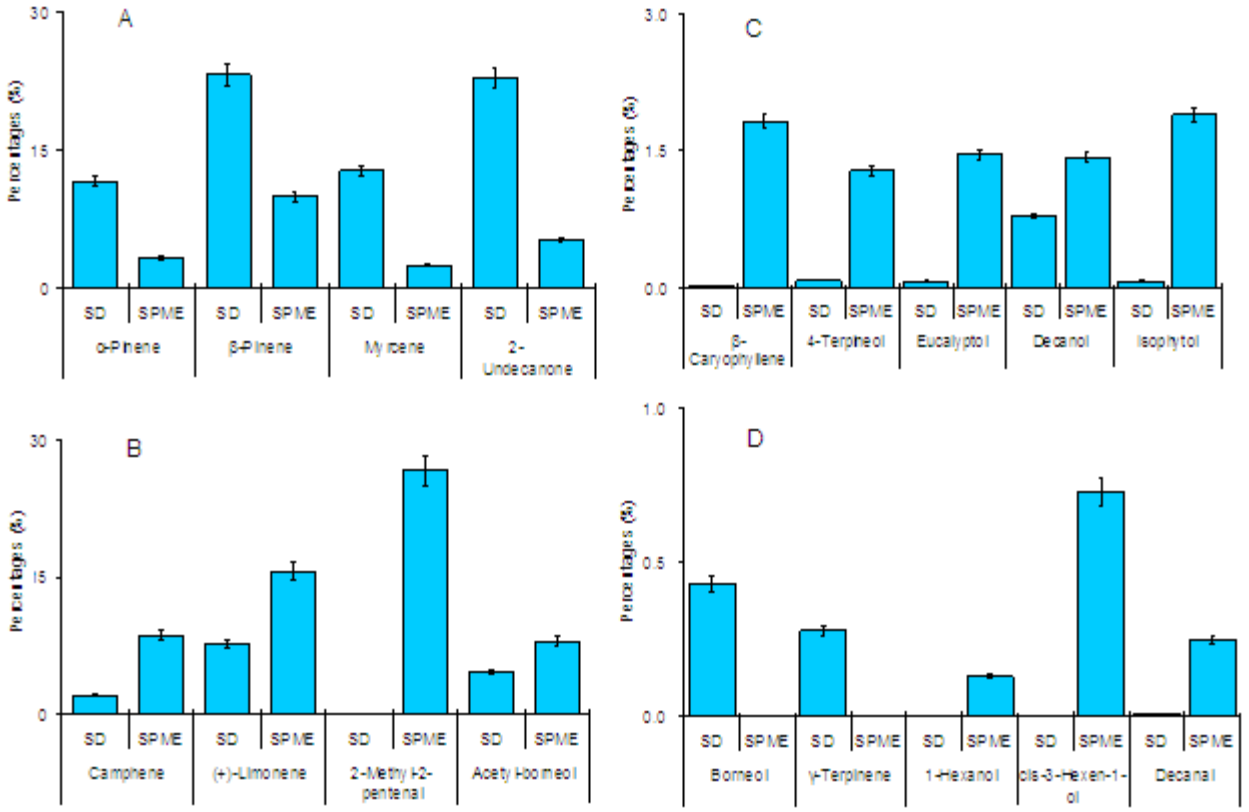

Fig. 3: Comparison of the contents of volatiles obtained by SD and SPME (quantification of volatiles based on normalization of peak areas)

decanal is 2.93 and 9.85ng, respectively). The results obtained showed the method to have good linear regression, LOD and LOQ.

Precision, repeatability and stability

To elucidate precision, inter-day and intra-day variations were examined using the mixed standard solution of appropriate concentration and *H. cordata* sample solution under optimized extraction and GC-MS conditions. Repeat abilities were tested by six working solutions (which were prepared independently from the *H. cordata* sample) and one of them was determined every 4 h for five consecutive injections to examine the stability of the *H. cordata* sample solution. The results are expressed in relative standard deviations (RSDs). The RSDs of precisions, repeat abilities and stabilities (table 3) are ≤4.13, ≤4.07 and ≤4.25%, respectively. Hence, the described method revealed very good precision, repeatability and stability.

Recovery

The recovery experiment was undertaken by adding known amounts of all analytes and internal standard to *H. cordata* samples at three concentrations (80, 100 and 120%). The resultant *H. cordata* samples (which were extracted and processed with the methods proposed) were analyzed using the GC-MS conditions developed. The recoveries of the analytes ranged from 98.56 to 103.77%, and the RSDs of analytes were ≤3.0% (table 4). The results obtained suggested that the *H. cordata* volatiles were accurately determined by the proposed method.

Analyte response

Fig. 1 shows that the response peaks of the *H. cordata* volatiles obtained by SE (fig. 1B), SD (fig. 1C) and SPME (fig. 1D) were significantly different, such as decanal and 2-undecanone. fig. 2 shows that the peak areas of 25 analytes at the same concentration (0.05 mg/mL) were significantly different. For example, the peak area of 2-undecanone was 21.8-times greater than the peak area of decanal. Therefore, the contents of analytes in the essential oil obtained by SD (which were calculated by normalization of their peak areas) did not accurately reflect the contents of the volatiles of the *H. cordata* essential oil. Also, the volatiles obtained by SPME did not accurately reflect the contents of *H. cordata* volatiles. However, the contents of the *H. cordata* volatiles obtained by SPME were not evaluated accurately because the adsorption/desorption of volatiles are in dynamic equilibrium in SPME. Addition of the internal standard and selection of quantifier ions can reduce the uncertainty of normalization-based quantification, so the LOD and LOQ can be higher.

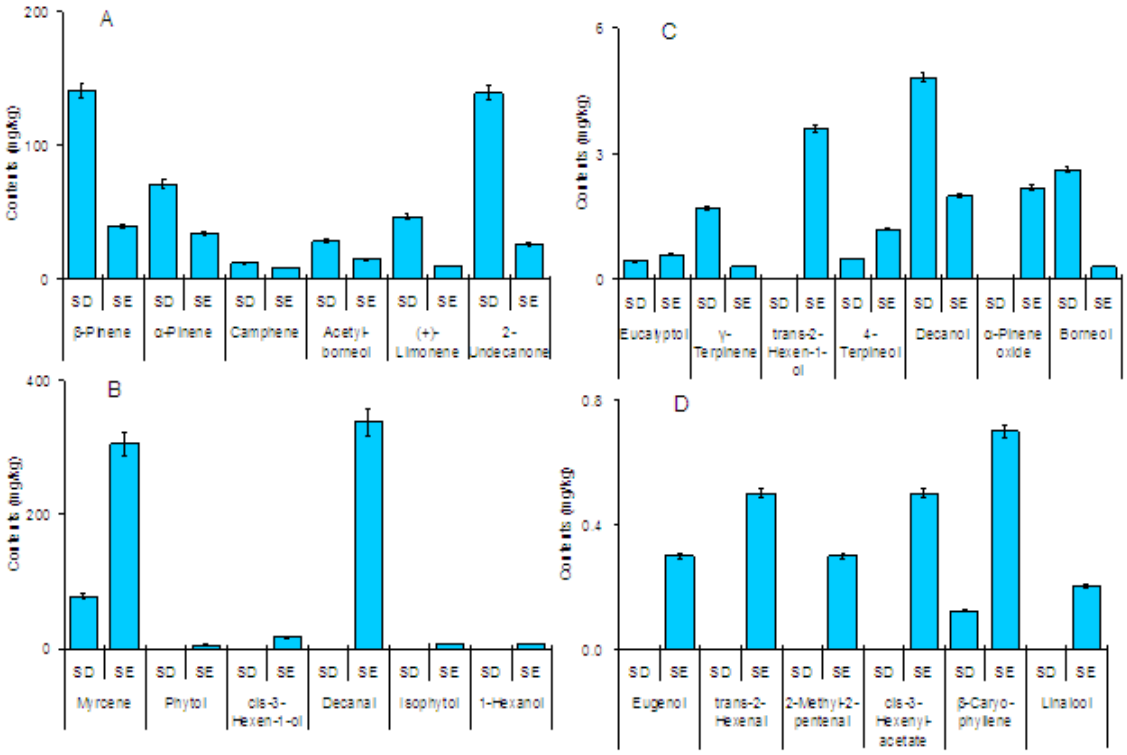

Fig. 4: Comparison of the contents of volatiles obtained by SD and SE. Contents of the volatiles of *H. cordata* were calculated according to the yields of *H. cordata* essential oil obtained by SD. Contents of volatiles obtained by SE were calculated by the proposed method.

Table 1: Basic information of the volatiles

No.	Volatiles	Retention times (min)	Molecular formulas	Molecular masses	Quantifier ions	Qualifier ions		
						Ion 1	Ion 2	Ion 3
1	α-Pinene	6.425	$C_{10}H_{16}$	136	93 (100)	105(11.0)	121(10.5)	136 (6.8)
2	Camphene	7.517	$C_{10}H_{16}$	136	93 (100)	79 (40.5)	121(59.4)	136(12.3)
3	β-Pinene	8.642	$C_{10}H_{16}$	136	93 (100)	107 (5.5)	121(11.2)	136 (7.8)
4	2-Methyl-2-pentenal	10.142	$C_6H_{10}O$	98	98 (100)	55 (35.1)	69 (49.5)	83(20.6)
5	Myrcene	10.375	$C_{10}H_{16}$	136	93 (100)	69 (77.9)	121 (4.6)	136 (2.7)
6	(+)-Limonene	11.192	$C_{10}H_{16}$	136	68 (100)	93 (66.2)	121(18.8)	136(16.0)
7	Eucalyptol	11.358	$C_{10}H_{18}O$	154	154 (100)	81(296.1)	93(178.6)	108(258.2)
8	trans-2-Hexenal	11.767	$C_6H_{10}O$	98	55 (100)	69 (78.8)	83 (60.2)	98 (19.5)
9	γ-Terpinene	12.475	$C_{10}H_{16}$	136	93 (100)	77 (35.6)	121(24.6)	136(27.8)
10	cis-3-Hexenyl-acetate	14.383	$C_8H_{14}O_2$	142	67 (100)	43(113.3)	54 (7.9)	82 (50.3)
11	1-Hexanol	15.275	$C_6H_{14}O$	102	56 (100)	55 (49.1)	69 (30.1)	84 (4.6)
12	α-Pinene oxide	15.692	$C_{10}H_{16}O$	152	109 (100)	67(186.2)	137(39.1)	
13	cis-3-Hexen-1-ol	16.075	$C_6H_{12}O$	100	67 (100)	55 (48.9)	82 (42.3)	100 (2.8)
14	trans-2-Hexen-1-ol	16.517	$C_6H_{12}O$	100	57 (100)	67 (17.0)	71 (10.5)	82 (20.2)
15	Decanal	19.375	$C_{10}H_{20}O$	156	57 (100)	82 (46.4)	95 (31.3)	112 (22.8)
16	Linalool	20.950	$C_{10}H_{18}O$	154	71 (100)	93 (73.2)	121(17.3)	136 (4.9)
17	Acetyl-borneol	21.842	$C_{12}H_{20}O_2$	196	95 (100)	121(34.6)	136(32.5)	154 (6.7)
18	β-Caryophyllene	22.325	$C_{15}H_{24}$	204	93 (100)	105(64.3)	133(60.2)	189 (16.3)
19	2-Undecanone	22.508	$C_{11}H_{24}O$	170	58 (100)	71 (31.6)	112 (4.7)	170 (4.1)
20	4-Terpineol	22.617	$C_{10}H_{18}O$	154	71 (100)	93 (47.6)	111(46.8)	154 (8.4)
21	Borneol	25.608	$C_{10}H_{18}O$	154	95 (100)	110(23.1)	121 (5.1)	139 (4.4)
22	Decanol	26.833	$C_{10}H_{22}O$	158	55 (100)	70 (91.3)	83 (61.1)	112 (18.8)
23	Eugenol	31.083	$C_{10}H_{12}O_2$	164	164 (100)	77 (55.1)	103(55.4)	149 (34.5)
24	Isophytol	31.775	$C_{20}H_{40}O$	296	71 (100)	57 (12.2)	82 (8.9)	123 (4.5)
25	Phytol	34.650	$C_{20}H_{40}O$	296	71 (100)	57 (31.3)	81 (26.4)	123 (20.3)
26	n-Tetradecane	16.692	$C_{14}H_{30}$	198	57 (100)	71 (61.8)	85 (38.8)	198 (3.5)

Table 2 Linear regression equations, regression relationships (r^2), linear ranges, LODs and LOQs of volatiles

No.	Volatiles	Calibration curves			LODs (ng)	LOQs (ng)
		Linear regression equations	r^2	Linear ranges (μg/mL)		
1	*α*-Pinene	Y=0.0477X-0.0244	0.9997	0.42-17.29	0.10	0.31
2	Camphene	Y=0.0652X-0.0190	0.9997	0.78-30.66	0.12	0.39
3	*β*-Pinene	Y=0.0685X-0.0482	0.9991	0.86-34.63	0.16	0.53
4	2-Methyl-2-pentenal	Y=0.0220X-0.0102	0.9995	0.85-34.42	0.14	0.43
5	Myrcene	Y=0.0444X-0.0111	0.9997	0.39-16.11	0.17	0.58
6	(+)-Limonene	Y=0.0550X-0.0141	0.9991	0.44-17.05	0.29	0.89
7	Eucalyptol	Y=0.0211X-0.0051	0.9997	0.42-16.46	0.22	0.65
8	*trans*-2-Hexenal	Y=0.0175X-0.0117	0.9997	0.82-33.43	0.34	1.21
9	*γ*-Terpinene	Y=0.0643X-0.0159	0.9997	0.44-16.87	0.08	0.23
10	*cis*-3-Hexenyl-acetate	Y=0.0644X-0.0327	0.9991	0.74-29.86	0.27	1.11
11	1-Hexanol	Y=0.0650X-0.0327	0.9995	0.61-24.58	0.17	0.53
12	*α*-Pinene oxide	Y=0.0381X-0.0249	0.9994	0.93-37.57	0.26	0.79
13	*cis*-3-Hexen-1-ol	Y=0.0411X-0.0281	0.9991	0.82-32.43	0.25	1.43
14	*trans*-2-Hexen-1-ol	Y=0.0625X-0.0384	0.9997	0.85-33.53	0.18	0.57
15	Decanal	Y=0.0024X+0.0111	0.9994	7.90-316.44	2.93	9.85
16	Linalool	Y=0.0326X-0.0062	0.9991	0.70-27.05	0.36	1.15
17	Acetyl-borneol	Y=0.0376X-0.0072	0.9996	0.74-29.22	0.39	1.20
18	*β*-Caryophyllene	Y=0.0197X-0.0145	0.9991	1.31-52.78	0.23	0.69
19	2-Undecanone	Y=0.0794X-0.0060	0.9991	0.42-16.46	0.29	0.95
20	4-Terpineol	Y=0.0337X-0.0131	0.9996	0.83-33.61	0.17	0.51
21	Borneol	Y=0.0735X+0.0010	0.9993	0.30-12.01	0.22	0.71
22	Decanol	Y=0.0292X+0.0068	0.9993	0.59-24.31	0.28	0.97
23	Eugenol	Y=0.0217X+0.0049	0.9997	1.08-43.59	0.44	1.51
24	Isophytol	Y=0.1357X+0.0219	0.9993	0.20-8.46	0.16	0.51
25	Phytol	Y=0.0414X+0.0227	0.9997	0.31-51.89	0.20	0.71

Y and X refers to the peak area and the concentration, respectively.

DISCUSSION

Comparison of SD and SPME

Thirty-one *H. cordata* volatiles obtained by SD were identified. The main volatiles were *α*-pinene (11.63%), camphene (1.96%), *β*-pinene (23.12%), myrcene (12.76%), (+)-limonene (7.69%), acetyl-borneol (4.46%) and 2-undecanone (22.82%). The percentages of other volatules were ≤1%. Twenty-seven *H. cordata* volatiles obtained by SPME were identified. The main volatules were *α*-pinene (3.37%), camphene (8.58%), *β*-pinene (9.98%), 2-methyl-2-pentenal (26.64%), myrcene (2.50%), (+)-limonene (15.65%), acetyl-borneol (7.94%), 2-undecanone (5.28%), eucalyptol (1.46%), *β*-caryophyllene (1.82%), 4-terpineol (1.28%), decanol (1.42%) and isophytol (1.90%). fig. 3 shows that, even though they originated from the same sample, the percentage of *H. cordata* volatiles obtained by SD and SPME were very different. The percentages of *α*-pinene, *β*-pinene, myrcene and 2-undecanone extracted by SD were higher than those by SPME (fig. 3A). In contrast, the percentages of camphene, (+)-limonene, 2-methyl-2-pentenal, acetyl-borneol, *β*-caryophyllene, 4-terpineol, eucalyptol, decanol and isophytol extracted by SPME were higher than those extracted by SD (figs 3B and C). In addition, other lower-concentration components were also similar (fig. 3D). The results suggested that quality control of *H. cordata* is difficult because the values of volatiles isolated by SD and SPME are extremely different. Hence, we do not know if the contents of volatiles obtained by SD and SPME are credible and accurate. Traditionally, analyses of *H. cordata* volatiles are preceded by extracting the essential oil by SD, which requires more *H. cordata* material and takes longer to complete. Sample preparations (which are complex and time-consuming) can complicate the analytical results because of other influencing factors (e.g., temperature). SPME is a solvent-less method used widely for extraction from plants, food and environmental samples for analyses of volatiles (Zini *et al.,* 2002; Cai *et al.,* 2001). However, the contents of the *H. cordata* volatiles could not be quantified accurately and reliably by SPME due to the absorption capacity of the solid-phase fiber coating, absorption temperature, and the partition coefficient of the vapor phase/absorbent fiber coating (Liang and Pawliszyn *et al.,* 1995). Another factor influencing the data was normalization of peak areas.

Table 4: Recoveries of the volatiles in *H. cordata* samples

No.	Volatiles	Simples	Contents			Recoveries (%)	Average recoveries (%) ± RSDs
			Original contents (μg)	Added contents (μg)	Determined contents (μg)		
1	α-pinene	S1	34.601	28.027	62.387	99.64	100.42±2.03
		S2	34.601	34.671	70.759	102.17	
		S3	34.601	41.487	75.672	99.44	
2	Camphene	S1	7.905	6.404	14.302	99.92	100.84±1.27
		S2	7.905	7.921	16.088	101.69	
		S3	7.905	9.478	17.534	100.89	
3	β-Pinene	S1	39.483	31.981	72.690	101.82	101.73±2.41
		S2	39.483	39.562	81.012	102.59	
		S3	39.483	47.340	87.710	101.22	
4	2-Methyl-2-pentenal	S1	0.265	0.214	0.474	98.64	99.75±1.64
		S2	0.265	0.266	0.530	99.57	
		S3	0.265	0.318	0.589	100.78	
5	Myrcene	S1	305.427	247.395	553.865	100.89	100.79±1.90
		S2	305.427	306.037	609.114	99.68	
		S3	305.427	366.207	688.560	102.55	
6	(+)-Limonene	S1	9.651	7.817	17.714	101.44	101.07±1.82
		S2	9.651	9.670	19.632	101.65	
		S3	9.651	11.571	21.220	100.08	
7	Eucalyptol	S1	0.584	0.473	1.046	99.01	100.12±2.03
		S2	0.584	0.585	1.182	101.51	
		S3	0.584	0.700	1.281	99.71	
8	trans-2-Hexenal6	S1	0.489	0.396	0.895	101.52	100.06±1.86
		S2	0.489	0.490	0.974	99.45	
		S3	0.489	0.586	1.071	99.66	
9	γ-Terpinene	S1	0.288	0.234	0.526	100.92	100.04±1.50
		S2	0.288	0.289	0.575	99.70	
		S3	0.288	0.346	0.629	99.35	
10	cis-3-Hexenyl-acetate	S1	0.505	0.409	0.918	100.65	101.71±1.55
		S2	0.505	0.505	1.030	102.04	
		S3	0.505	0.605	1.137	102.64	
11	1-Hexanol	S1	6.627	5.367	11.826	98.60	99.46±1.17
		S2	6.627	6.640	13.229	99.78	
		S3	6.627	7.946	14.569	99.98	
12	α-Pinene oxide	S1	2.208	1.789	4.083	102.86	101.37±2.62
		S2	2.208	2.213	4.540	102.78	
		S3	2.208	2.648	4.803	98.97	
13	cis-3-Hexen-1-ol	S1	16.548	13.404	29.858	99.59	100.91±1.25
		S2	16.548	16.581	33.382	100.96	
		S3	16.548	19.841	37.091	101.90	
14	trans-2-Hexen-1-ol	S1	3.574	2.895	6.478	100.01	100.98±1.74
		S2	3.574	3.582	7.347	102.60	
		S3	3.574	4.286	7.858	99.65	
15	Decanal	S1	337.790	273.610	614.178	100.95	101.35±1.90
		S2	337.790	338.465	690.871	102.86	
		S3	337.790	405.010	753.002	101.34	
16	Linalool	S1	0.210	0.171	0.388	102.26	102.45±1.38
		S2	0.210	0.211	0.433	103.10	
		S3	0.210	0.252	0.469	101.50	
17	Acetyl-borneol	S1	14.904	12.072	26.770	99.26	100.29±1.41
		S2	14.904	14.934	29.890	100.57	
		S3	14.904	17.870	33.228	101.38	
18	β-Caryophyllene	S1	0.731	0.593	1.332	100.71	101.38±1.77
		S2	0.731	0.733	1.465	100.21	
		S3	0.731	0.877	1.656	103.07	

No.	Volatiles	Simples	Contents			Recoveries (%)	Average recoveries (%) ± RSDs
			Original contents (μg)	Added contents (μg)	Determined contents (μg)		
19	2-Undecanone	S1	25.846	20.935	47.679	101.99	101.55±1.28
		S2	25.846	25.897	52.841	102.14	
		S3	25.846	30.991	57.117	100.56	
20	4-Terpineol	S1	1.162	0.941	2.093	99.54	100.59±1.46
		S2	1.162	1.164	2.365	101.52	
		S3	1.162	1.393	2.562	100.36	
21	Borneol	S1	0.327	0.265	0.600	101.66	100.51±1.26
		S2	0.327	0.327	0.653	99.98	
		S3	0.327	0.392	0.718	100.49	
22	Decanol	S1	2.033	1.646	3.731	101.45	100.31± 1.46
		S2	2.033	2.037	4.080	99.24	
		S3	2.033	2.437	4.465	99.07	
23	Eugenol	S1	0.308	0.250	0.565	100.55	100.91±1.28
		S2	0.308	0.309	0.619	98.56	
		S3	0.308	0.369	0.670	99.10	
24	Isophytol	S1	7.566	6.129	13.750	102.41	101.37±1.12
		S2	7.566	7.581	15.371	100.48	
		S3	7.566	9.071	17.009	102.94	
25	Phytol	S1	5.498	4.454	10.306	103.77	101.79±2.34
		S2	5.498	5.509	11.140	101.41	
		S3	5.498	6.592	12.197	98.89	

Comparison of SD and SE

The contents of the *H. cordata* volatiles were calculated according to the yields of essential oil of *H. cordata* obtained by SD, which was 0.061%. fig. 4 shows that the contents of the analytes of the same *H. cordata* sample obtained by SD and SE were significantly different. The main volatiles of 25 analytes of the *H. cordata* essential oil obtained by SD were (in mg/kg) *α*-pinene (70.9), camphene (12.0), *β*-pinene (141.0), myrcene (77.8), (+)-limonene (46.9), acetyl-borneol (28.4) and 2-undecanone (139.2). However, the main volatiles of 25 analytes of *H. cordata* obtained by SE were analyzed by the method described here. *α*-pinene (34.6 mg/kg), camphene (7.9), *β*-pinene (39.4), myrcene (305.4), (+)-limonene (9.7), 1-hexanol (6.6), *cis*-3-hexen-1-ol (16.5), decanal (337.7), acetyl-borneol (14.9), 2-undecanone (25.8), isophytol (7.5) and phytol (5.5) were the main volatiles and their contents were >5 mg/kg. The results suggested that the contents of *α*-pinene, camphene, *β*-pinene, (+)-limonene, acetyl-borneol and 2-undecanone of *H. cordata* obtained by SD were higher than those obtained by SE (fig. 4A). Conversely, the contents of 1-hexanol, *cis*-3-hexen-1-ol, phytol, myrcene, decanal and isophytol obtained by SE were higher than obtained by SD. Also, 1-hexanol, *cis*-3-hexen-1-ol and phytol in *H. cordata* essential oil obtained were not found using SD (fig. 4B). However, 2-methyl-2-pentenal, *trans*-2-hexenal, *cis*-3-hexenyl-acetate, *α*-pinene oxide, *trans*-2-hexen-1-ol, linalool, eugenol and phytol,

which displayed lower contents by SE, were not found by SD (figs 4C and D). The contents of the *H. cordata* volatiles obtained by SD and SE, and analyzed using GC-MS according to normalization of peaks and a SIM-based internal standard, respectively, were significantly different due to the response of the components to instrumentation and within the effects of these extractions. Different components under the same analytical condition elicit different responses, which reflect differences in the peak areas of the components (17). The contents of the volatiles in *H. cordata* were calculated according to a relative correction factor by SIM *via* a method developed for simultaneous determination of 25 volatiles. Therefore, the credibility and accuracy of the results analyzed by our method will be increased considerably. Also, the response of the components to instrumentation and other factors in the sample preparation could be avoided. Also, the LOD and LOQ of the analytes in our method are much higher.

CONCLUSION

GC-MS analyses assisted by SIM and an internal standard can be used to determine 25 volatiles of *H. cordata* accurately and reliably. It can also resolve the inaccuracy when contents are calculated by normalization of the peak areas of volatiles, which elicits different responses to instrumentation. SE can avoid errors because of heating and other factors in sample preparation using SD and SPME. Less sample is needed by the proposed method, and a fresh *H. cordata* sample of ≈0.05g can be used to determine the contents of 25 analytes. The normal physiological activity or growth of *H. cordata* was not affected by sample collection. The proposed method was shown to monitor various metabolic accumulations of the *H. cordata* volatiles.

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