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Quality control of *Schizonepeta tenuifolia* Briq by solid-phase microextraction gas chromatography/mass spectrometry and principal component analysis

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ABSTRACT

The chemical composition of *Schizonepeta tenuifolia* Briq. (*Sch.t.*Briq.) is mainly composed of several volatile substances that affect multiple pharmacological targets and provide clinical efficacy. In this work, a headspace/solid-phase microextraction gas chromatography/mass spectrometry (HS-SPME-GC/MS) method was developed to evaluate the profiles of volatile compounds in *Sch.t.*Briq. The optimization of SPME conditions was carried out using four kinds of fiber, extraction time and temperature, desorption temperature and time, and sample amount. The GC/MS analysis allowed the tentative identification of 21 compounds, with similarities higher than 85%, in accordance with the NIST/Wiley mass spectral library. Major components such as (+)-menthone (14.32%), (−)-pulegone (47.73%), 2-hydroxy-2-isopropenyl-5-methylcyclohexane (5.97%), *cis*-pulegone oxide (4.12%), and schizonal (5.36%) were identified by comparison of retention time and mass spectral data of standards isolated from *Sch.t.*Briq. The contents of these compounds were about above 78% against total amounts of volatile compounds extracted from *Sch.t.* Briq. Based on optimized SPME method, 19 different *Sch.t.*Briq. samples collected from markets in Korea and China were analyzed to obtain the profiling data of volatile compounds. In addition, principal component analysis (PCA) was performed on the profiling data in order to classify the samples collected from the different regions. PCA could possibly visualize the grouping tendencies of the studied varieties of herbal samples, as well as the identification of the volatiles responsible for discriminating the groups.

1. Introduction

Herbal medicines have a long history in South Korea and have been widely used worldwide to prevent and treat human disease or maintain health. Quality control is an important issue to ensure the efficacy of herbal medicines and is closely related with the concentrations of their chemical constituents. However, the chemical composition of herbal medicines is complex and active components are rarely identified. In addition, the quantities of active compounds and/or marker compounds in herbal medicines are dependent on intraspecies variability, environmental conditions, harvest period, storage time, and processing method [1,2]. Besides these factors, the extraction methods used to process the herbal plants can also affect the quantities of biologically active compounds in the extract. Thus, the quality control of active constituents or marker compounds in the herbal extract is of great importance in medicinal and dietary applications.

Schizonepeta tenuifolia Briq. (*Sch.t.*Briq.) is one of the Korean medicines, prepared from the dried *Schizonepeta* spike of aerial parts. Generally the volatile constituents of *Sch.t.*Briq. have been known to be the main pharmacological activity such as analgesic, anti-inflammatory, anti-febrile, and anti-spasmodic purpose [3]. However, the volatile compositions of *Sch.t.*Briq. have been subject of only few investigations [4–6]. This herbal medicine contains several active components including essential oils (menthone, pulegone, *d*-limonene, etc.) and flavonoids (diosmetin, hesperidin, luteolin, etc.), monoterpenoids (schizonodiol, schizonol, schizonepetosides D and E, etc.) and cinnamic acid and ursolic acid [7–9]. Among these components, volatile components such as essential oils are known to be the major components and can be selected as marker compounds.

For the analysis of volatile compounds, gas chromatography (GC) and GC-mass spectrometry (GC/MS) have been widely used [10,11]. Several extraction methods including steam distillation [12], liquid–liquid extraction [13] and solid-phase extraction [14], have been used for the quantitative extraction of volatile compounds from sample matrix. However, these methods are laborious, time consuming, and could result in losses of some important volatiles during sample workup due to their high-volatility. Recently, solid-phase microextraction (SPME) and purge-and-trap methods using various adsorbents have been widely used for rapid extraction of volatile compounds from aromatic and medicinal plants. [15] For the rapid

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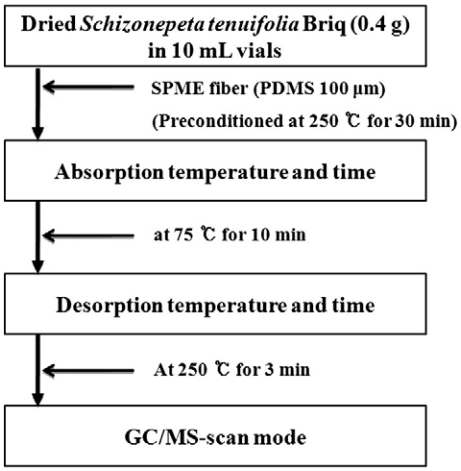

Fig. 1. Overall analytical procedure for the extraction of volatile compounds from *Sch.t.* Briq.

extraction of volatiles, the several kinds of fibers for SPME have been used polymeric materials such as a liquid [polydimethylsiloxane (PDMS)] for the less polar components and two solids [divinylbenzene (DVB) and carboxen (CAR)] for the more polar components [16–18]. However, several parameters in SPME such as adsorption time and temperature, desorption time and temperature should be optimized prior to sample analysis. Recently, headspace (HS) SPME method has been introduced for the determination of volatile fraction from *Atractylodes* species [19], *Capsicum* Chinese species [20] and *Frucus amomi* [21].

To evaluate the quality control of *Sch.t.*Briq., the development of an SPME-GC/MS analytical method to simultaneously determine volatile compounds in herbal plant is preferred. There are a few studies previously published on chemical composition of essential oils from herbal medicines. To our knowledge, there has been no analytical method reported which allows the simultaneous determination of volatile compound in *Sch.t.*Briq. by HS-SPME-GC/MS. The quality of *Sch.t.*Briq. can be predicted by the analysis of these compounds. Based on chromatographic data of selected marker compounds obtained by the developed SPME-GC/MS method, the quality control of herbal extracts can be evaluated. In addition, principal component analysis (PCA) can be used to provide a convenient visual aid for identification of inhomogeneity in the data sets [22–27]. Due to its usefulness in the differentiation of samples, PCA has recently been applied to the classification of traditional herbal plants from different origins [24–27]. The PCA technique combined with chromatographic data of selected marker compounds can be successfully applied in the quality control of herbal extracts [28,29].

The objective of this work was to demonstrate that the HS-SPME was rapid and effective methods for the characterization of volatile components and for quality control of *Sch.t.*Briq. samples. On the present work, the volatile compounds extracted by HS-SPME method were identified and quantified by GC/MS-scan mode. This method could then be used ensure the quality of herbal medicines and the chemical standardization of *Sch.t.*Briq. samples. In addition, the classification of 19 *Sch.t.*Briq. samples collected from various regions in Korea and China was performed using PCA.

2. Experimentals

2.1. Reagents and apparatus

All reagents used in this study were of analytical grade. Diethyl ether and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). Some of volatile components such as limonene and menthone were obtained from Wako (Osaka, Japan, purity ≥95.0% and 90.0%). Major compounds such as 2-hydroxy-2-isopropenyl-5-methylcyclohexanone, (+)-menthone, (−)-pulegone, and *cis*-pulegone oxide and schizonal (8-hydroxy-*p*-menthen-3-one) were isolated by methods outlined in our previous reports. [30] Their purities were determined by GC/MS-scan mode (purity ≥91%).

Analytical samples of the herbal medicine, *Sch.t.*Briq. were purchased from various market places in Korea and China, respectively, and were identified by expert discriminator of herbal medicine. Prior to use, dried *Sch.t.*Briq. was pulverized and then collected by standard sieve with mesh size 18.

2.2. HS-SPME procedure

SPME parameters including fiber type, extraction temperature and time, and desorption temperature and time were optimized. The fibers were conditioned prior to use according to supplier's prescriptions, 0.5 h at 250 °C for PDMS and PDMS/DVB, 2 h at 300 °C for CAR/PDMS, 1 h at 270 °C for DVB/CAR/PDMS. Before sampling each fiber was reconditioned for 5 min in the GC injector port at 250 °C to eliminate the possible remains on the coated fiber. Optimization of SPME conditions was based on the sum of total peak areas with the fiber presenting the most complete profile of volatile compounds. For the optimization of the fiber, extraction temperature and time, and

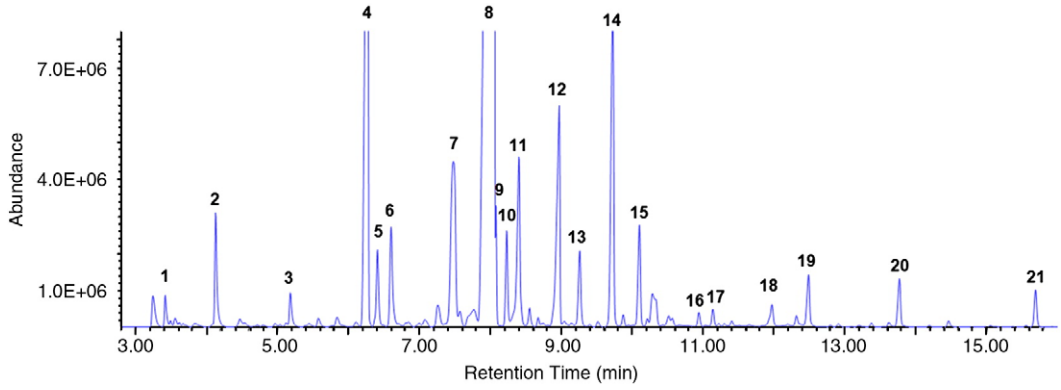

Fig. 2. Total ion chromatograms of volatile extracted from *Sch.t.Briq.* by HS-SPME-GC/MS. SPME experimental conditions: extraction temperature and time (60 °C, 15 min), desorption temperature and time (250 °C, 3 min) and PDMS 100 μm fibers. Peak identities as follows ; 1-octen-3-ol, 2: limonene, 3: octen-1-ol, 4: (+)-menthone, 5: *d*-isomenthone, 6: *cis*-isopulegone, 7: 2-hydroxy-2-isopropenyl-5-methylcyclohexanone, 8: (−)-pulegone, 9: carvone, 10: 8-hydroxy-*p*-menthan-3-one, 11: *cis*-pulegone oxide, 12: schizonal, 13: *trans*-pulegone oxide, 14: endo-4,8-dimethyl-2-oxabicyclo(3,3,1)-non-3-ene, 15: piperitenone, 16: α-copaene, 17: β-boubonene, 18: β-caryophyllene, 19: cyclopentane, 1-isobutyliden-3-methyl-, 20: mint furanone 1, and 21: caryophyllene oxide.

Table 1
Retention times (min) and characteristic ions of volatile compounds extracted by HS-SPME-GC/MS.

Peak no.	RT	Compounds	EI-characteristic ion value (m/z)	Relative content (%)
1	3.42	1-octen-3-ol	57 (100), 43 (21), 55 (21), 72 (15), 41 (14), 70 (11)	0.50
2	4.13	Limonene	68 (100), 93 (70), 79 (35), 121 (23), 52 (22), 107 (21), 136 (20), 41 (19)	1.95
3	5.18	Octen-1-ol	43 (100), 99 (32), 54 (19), 72 (12), 67 (11), 81 (11)	0.64
4	6.27	(+)-menthone	112 (100), 69 (67), 139 (51), 41 (50), 55(49), 154 (35), 97 (32), 83 (24)	14.32
5	6.41	*d*-isomenthone	112 (100), 69 (67), 41 (48), 55 (48), 139 (38), 97 (28), 154 (24), 83 (21)	1.40
6	6.60	*cis*-pulegone	109 (100), 123 (83), 93 (80), 67 (78), 41 (43), 81 (36), 152 (25), 53 (25), 137 (20)	2.19
7	7.48	2-hydroxy-2-isopropenyl-5-methycyclohexanone	97 (100), 69 (99), 41 (84), 125 (53), 83 (44), 55 (43), 111 (40), 79 (21), 153 (17)	5.97
8	8.05	(−)-pulegone	81 (100), 152 (77), 67 (72), 109 (50), 137 (27), 41 (31), 95 (17), 53 (16)	47.73
9	8.08	carvone	82 (100), 54 (41), 41 (37), 93 (34), 108 (34), 69 (28), 97 (25), 125 (13), 150 (8)	0.95
10	8.23	8-hydroxy-*p*-menthan-3-one	112 (100), 43 (70), 70 (66), 59 (58), 97 (44), 55 (25), 84 (19), 155 (17)	1.53
11	8.40	*cis*-pulegone oxide	153 (100), 43 (58), 55 (37), 111 (37), 86 (33), 83 (31), 67 (25), 125 (22), 139 (21), 168 (21)	4.12
12	8.97	8-hydroxy-*p*-menthen-3-one (schizonal)	153 (100), 43 (31), 97 (26), 55 (13), 69 (12), 59 (11), 111 (11), 150 (8)	5.36
13	9.26	*trans*-pulegone oxide	153 (100), 43 (46), 86 (35), 111 (31), 55 (30), 70 (27), 83 (26), 67 (20), 168 (20), 125 (15)	1.51
14	9.73	Unknown-1	84 (100), 43 (28), 152 (26), 134 (22), 119 (20), 91 (19), 105 (7)	6.02
15	10.10	piperitone	150 (100), 107 (64), 135 (33), 91 (31), 79 (22), 41 (17), 82 (17), 53 (13), 67 (11), 121 (11)	1.86
16	10.94	α-copaene	119 (100), 161 (98), 105 (96), 93 (46), 41 (24), 81 (24), 77 (20), 204 (17), 55 (16)	0.25
17	11.14	β-bourbonene	81 (100), 123 (65), 161 (29), 41 (15), 91 (15), 77 (14), 105 (13)	0.31
18	11.97	β-caryophyllene	93 (100), 69 (82), 79 (73), 133 (85), 41 (84), 105 (55), 55 (39), 120 (39), 161 (31), 147 (26), 109 (21)	0.59
19	12.49	Unknown-2	81 (100), 95 (71), 67 (43), 166 (36), 123 (30), 41 (28), 55 (24), 138 (19), 110 (18)	1.09
20	13.77	mint furanone 1	166 (100), 137 (65), 67 (64), 81 (63), 95 (51), 109 (49), 41 (32), 53 (26), 123 (22)	1.00
21	15.69	caryophyllene oxide	79 (100), 41 (93), 93 (79), 69 (67), 55 (48), 109 (48), 83 (25), 105 (34), 121 (33), 135 (16), 149 (13), 131 (12), 161 (12)	0.70

% figure are their relative proportions as percent of total area, % (RSD), $n = 3$.

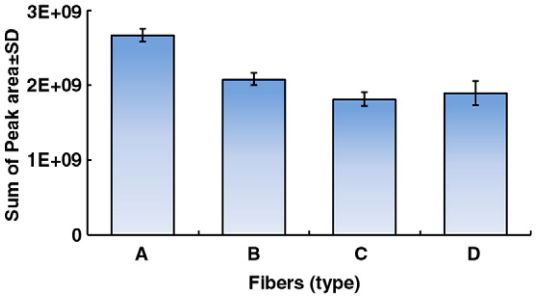

Fig. 3. Fiber adsorption yield of volatile compounds from *Sch.t.*Briq. according to fibers with different coating phases by HS-SPME-GC/MS; (A) PDMS 100 μm, (B) CAR/PDMS 75 μm, (C) DVB/CAR/PDMS 50/30 μm, and (D) PDMS/DVB 65 μm.

desorption temperature and time, we kept outline of the parameters invariable and we changed another parameters. This way, final conditions were: 0.4 g of powdered sample was placed in a 10 mL screw-top vial and Teflon/silicon septum and stored in a hot plate. And then, the SPME device was inserted into the sealed vial by manually penetrating the septum and the fiber was exposed to the headspace of plant material during 75 °C (10 min). After extraction, the needle on the SPME manual holder was set at its 1.8 cm in the GC injector and then the fiber was directly exposed to the hot injector at 250 °C for 3 min in split mode (30:1). Each sampling and analysis was performed in triplicate. The overall analytical procedure of volatiles in herbal medicine is depicted in Fig. 1.

2.3. Gas chromatography/mass spectrometry

GC/MS analyses were carried out with an Agilent 6890N gas chromatography instrument combined with an Agilent-5973 mass selective detector equipped with an electron ionization and quadrupole analyzer. The electron ionization energy was set at 70 eV, and the temperatures of ion source and interface were set at 230 °C and 280 °C, respectively. The mass scan range was from 40 to 400 amu. The separation of volatile compounds was carried out with a 5% phenyl dimethylpolysiloxane fused-silica capillary column (DB-5MS 30 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA). A split injection (split ratio, 1:30) and injector temperature at 250 °C were employed. The oven temperature was programmed from 80 °C to 50 °C at a rate 5 °C/min then at a rate 10 °C to 100 °C and at a rate 5 °C to 185 °C.

For the manual sample injection, the silica fibers and the manual SPME holder were purchased from Supelco (Bellefonte, PA, USA). For SPME fiber, four kinds of polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm), carboxen–polydimethylsiloxane (CAR–PDMS, 75 μm), and StableFlex divinylbenzene–carboxen–PDMS (DVB–CAR–PDMS, 50/30 μm) were purchased from Supelco (Bellefonte, PA, USA).

2.4. Qualitative and quantitative analysis

Data was acquired by MS Chemstation software, version G1701DA. Major compounds were identified by comparison of authentic standards isolated from *Sch.t.*Briq. and reference standard purchased commercial. Some of components were tentatively identified by matching mass spectra with those in the NIST/Wiley database because most of its reference standards are not commercially available. The relative amounts of individual components observed in total ion chromatogram were expressed as percent peak areas against total peak area. Here, it is assumption that the response factors of individual components are same.

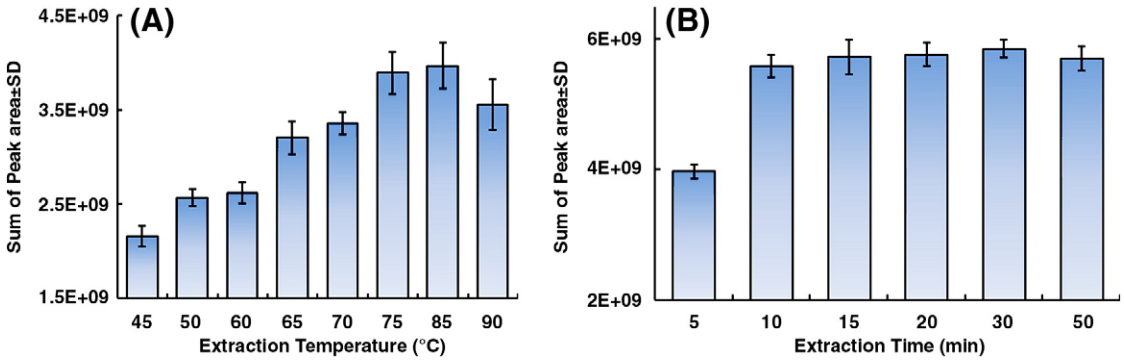

Fig. 4. Effect of extraction temperature and time on extraction of volatiles from *Sch.t.*Briq. according to sum of total peak area.

2.5. Principal component analysis (PCA)

PCA using the singular value decomposition method was performed by the Multivariate Statistical Package program (MVSP, Kovach Computing Service, Anglesey, Wales). For the classification of herbal medicines, PCA was performed by applying the total peak area of detected volatile compounds obtained from the SPME-GC/MS analysis. For pattern recognition analysis, 11 common peaks of total 21 peaks were selected and calculated their peak areas.

3. Results and discussion

3.1. Identification of volatiles in Schizonepeta tenuifolia Briq. by SPME-GC/MS

Total ion chromatogram of volatiles extracted from *Sch.t.*Briq. by SPME-GC/MS is shown in Fig. 2. Table 1 summarizes the retention times, mass spectral characteristic ions, and their peak area percent of detected compounds relative to total peak area. Twenty-one compounds were identified, occupying about 97% of the total peak areas detected by SPME-GC/MS. Most of major compounds were identified by comparison of retention times and mass spectra of authentic standards such as limonene, menthone, 2-hydroxy-2-isopropenyl-5-methylcyclohexanone, pulegone, *cis*-pulegone oxide, schizonal, and 8-hydroxy-*p*-menthen-3-one. Some of standard compounds such as 2-hydroxy-2-isopropenyl-5-methylcyclohexanone, pulegone, *cis*-pulegone oxide, and schizonal were isolated from *Sch.t.*Briq. by a previously reported method [30]. Their peak area was above 78% to total peak area. Some of minor compounds were tentatively identified by matching with NIST/Wiley library provided by the software of GC/MS system because these reference standards are not commercially available.

On the basis of identified volatile compounds, the chemical compositions of volatiles observed in *Sch.t.*Briq. were mainly consisted of monoterpene-ketones (about 70%), monoterpene-alcohols (about 12%), and others (8%). In addition, sesquiterpenes were also found as minor constituents, taking about 1.5% relative to total amount. Typical monoterpene-ketones of *Sch.t.*Briq. were found as (−)-pulegone (45.5%), and (+)-menthone (14.5%). The amounts of monoterpene-alcohols (hydroxy-*p*-menthen-3-one isomers) and oxides (*cis*- and *trans*-pulegoneoxide isomers) were about 12 and 5.6%, respectively. The amount of monoterpene-hydrocarbon (typical limonene) was about 2.0%. Generally, monoterpene hydrocarbons have found as an important meaning of the active pharmacological properties [31]. Besides these compounds, sesquiterpene hydrocarbons such as α-copanene (0.29%), β-bourbonene (0.36%), and β-caryophyllene (0.54%) were constituted 1.19% and sesquiterpene oxides such as caryophyllene oxide (0.66%) were also found. However, the chemical composition of essential oils in *Sch.t.*Briq. could be characterized by the presence of multi-predominant components. To evaluate the quality control of herbal medicines, the concentration level of multi-components should be measured. However, the contents of these components extracted by SPME could be affected by several parameters such as kinds of fibers, extraction time and temperature, and desorption time and temperature.

3.2. Selection of extraction fiber

To optimize the HS-SPME parameters, the selection of fiber, extraction temperature and time, sample amount, and desorption temperature and time was examined. The effective SPME fibers were generally those consisting of three polymers: a liquid [polydimethylsiloxane (PDMS)] for the less polar components and two solids [divinylbenzene (DVB) and carboxen (CAR)] for the more polar components. To find the effective SPME fiber, four types of fiber (PDMS 100 μm, CAR/PDMS 75 μm, DVB/CAR/PDMS 50/30 μm, and PDMS/DVB 65 μm) were tested for the extraction of volatiles from herbal medicines. Each fiber in headspace can absorb volatile

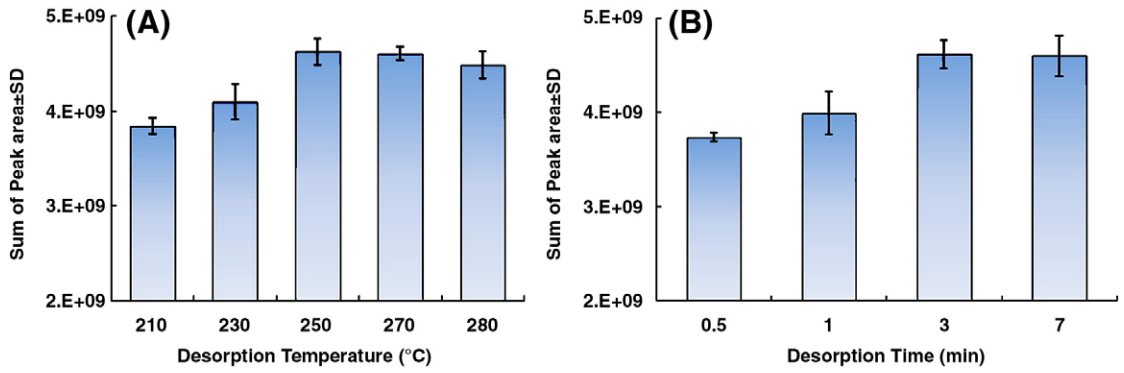

Fig. 5. Effect of desorption temperature and time on extraction of volatiles from *Sch.t.*Briq. according to sum of total peak area.

Table 2
The amount of volatiles compounds in *Schizonepeta tenuifolia* Briq. collected from different regions ($n=3$).

Sample number	(Compounds of area/sum of total compounds)*100						
	1-octen-3-ol	limonene	octen-3-ol	(+)-menthone	*d*-isomenthone	*cis*-pulegone	2-hydroxy-2-isopropenyl-5-methylcyclohexanone
K-1[a]	0.64±3.16	0.93±4.38	0.93±5.85	32.95±1.35	3.05±2.11	1.27±2.14	3.46±1.84
K-2	0.86±7.91	1.51±3.66	0.95±4.37	36.49±0.83	3.61±2.48	1.23±2.34	2.87±2.42
K-3	1.73±2.14	6.65±1.93	1.95±1.16	36.56±2.37	4.10±1.32	1.27±5.98	2.82±3.05
K-4	1.04±6.50	4.32±6.03	1.47±5.65	29.09±1.77	3.10±3.04	1.87±3.04	3.24±2.11
K-5	1.37±0.74	10.22±1.39	2.15±2.32	43.90±1.92	4.81±0.42	0.85±2.57	2.13±4.76
K-6	0.32±3.32	4.93±4.89	0.29±8.49	28.49±3.92	1.91±6.73	1.32±4.05	2.35±2.76
K-7	0.53±1.84	7.00±2.46	0.85±3.76	44.53±2.72	3.91±2.65	1.48±4.04	2.03±3.12
K-8	0.50±2.62	8.26±2.16	0.81±6.73	29.35±2.18	3.62±4.43	1.42±3.90	1.62±4.26
K-9	1.17±5.53	9.28±3.23	1.74±5.66	37.11±1.14	4.29±2.92	1.47±7.59	2.14±5.08
K-10	1.31±9.28	11.17±6.77	2.04±8.73	40.67±1.52	3.50±6.53	1.40±7.09	1.62±4.05
K-11	0.63±4.43	8.39±4.24	1.26±4.80	40.36±3.35	3.51±1.29	1.25±3.05	1.01±1.84
K-12	0.24±4.57	6.63±1.22	0.82±0.37	37.94±0.83	3.59±1.03	1.24±0.78	0.67±3.50
C-1[b]	0.77±3.71	7.12±4.40	0.91±0.54	14.97±5.60	1.38±6.08	2.06±2.09	5.08±1.49
C-2	0.40±5.80	2.83±8.63	0.54±5.70	14.20±3.28	1.25±1.36	2.13±3.00	4.73±3.42
C-3	0.49±3.24	5.38±1.86	0.56±2.59	23.73±2.50	1.93±2.33	1.38±3.92	3.97±1.67
C-4	0.48±3.93	3.68±9.03	0.39±2.73	16.65±2.00	1.70±1.93	2.06±1.37	3.85±0.68
C-5	0.64±5.69	4.68±1.14	0.62±1.71	19.26±0.40	2.39±0.64	2.26±0.77	4.74±1.87
C-6	0.85±5.33	9.70±0.82	0.92±5.13	42.94±2.03	4.26±1.51	1.37±4.74	1.79±3.83
C-7	0.50±0.33	4.22±5.16	0.48±2.06	18.93±0.62	1.85±2.01	2.11±1.61	3.73±1.27
	(−)-pulegone	carvone	8-hydroxy-*p*-menthan-3-one	*cis*-pulegone oxide	schizonal	*trans*-pulegoneoxide	Unknown-1
K-1[a]	39.23±0.65	0.62±5.12	0.32±4.73	0.51±6.69	2.04±4.38	0.35±2.84	6.82±1.08
K-2	34.86±0.58	0.61±1.78	0.33±2.84	0.39±1.16	1.79±0.77	0.32±0.71	6.53±1.78
K-3	33.45±0.27	0.83±13.23	0.28±6.50	0.62±8.97	1.67±10.52	0.35±10.67	3.87±6.10
K-4	41.87±0.54	0.69±10.76	0.26±2.01	0.70±3.78	2.09±4.71	0.41±5.27	5.21±3.46
K-5	24.94±1.93	–	0.38±6.07	0.42±8.55	1.28±6.28	0.23±6.39	3.28±2.07
K-6	45.18±1.85	0.55±3.72	0.55±6.29	0.65±11.55	2.01±15.01	0.33±13.52	4.73±2.39
K-7	28.99±1.66	–	0.61±7.00	0.45±6.38	0.90±9.03	0.22±8.78	4.05±7.23
K-8	40.78±0.78	0.45±5.75	0.32±6.46	0.45±8.95	1.81±8.43	0.29±6.32	4.36±0.74
K-9	34.36±1.61	–	0.31±2.40	0.26±4.67	0.82±3.97	0.17±3.77	3.55±0.85
K-10	30.92±2.07	–	0.25±8.68	0.24±7.08	0.75±7.61	0.14±8.34	2.70±10.21
K-11	35.79±2.62	–	0.12±10.74	0.21±3.65	1.24±1.91	0.16±6.29	2.81±5.01
K-12	38.76±0.96	–	–	–	0.77±7.92	0.17±3.41	4.46±2.68
C-1[b]	45.33±2.29	0.74±15.91	1.79±1.92	3.64±2.45	4.34±4.66	1.18±5.87	5.61±0.94
C-2	53.72±2.33	0.63±4.46	1.44±2.40	1.41±1.13	3.64±3.16	0.75±3.21	6.32±0.95
C-3	40.75±1.85	0.49±1.72	1.36±4.05	1.56±5.87	3.99±5.16	0.81±6.29	6.04±0.34
C-4	52.00±0.68	0.84±13.23	1.32±2.00	1.38±1.44	3.61±2.34	0.71±2.59	6.41±2.65
C-5	46.96±1.80	0.70±19.81	2.08±4.31	1.62±4.21	2.76±5.05	0.59±6.30	5.76±2.15
C-6	28.04±2.59	–	0.55±4.72	0.39±3.79	0.94±8.05	0.21±4.65	3.46±2.53
C-7	49.84±2.19	0.80±4.81	1.50±3.76	1.50±5.73	3.43±7.39	0.70±9.01	5.75±0.79
	piperitenone	α-copanene	β-bourbonene	β-caryophyllene	Unknown-2	mintfuranone	caryophyllene oxide
K-1[a]	1.34±4.90	0.29±2.57	0.52±1.25	1.94±5.38	0.36±14.36	0.49±13.58	1.95±12.13
K-2	1.24±1.05	0.35±3.15	0.67±2.09	2.65±3.98	0.25±5.98	0.52±7.94	1.98±7.39
K-3	1.06±9.40	0.24±4.38	0.33±2.85	1.08±8.11	0.13±29.07	0.43±15.63	0.57±21.94
K-4	1.22±6.14	0.23±2.06	0.31±2.98	1.05±5.50	0.13±20.01	0.78±15.20	0.91±20.51
K-5	0.56±6.28	0.30±2.48	0.82±3.28	1.44±1.43	0.12±17.67	0.37±19.14	0.43±6.81
K-6	1.65±15.48	0.22±12.49	0.31±11.49	1.19±17.42	0.16±24.53	1.94±36.54	0.92±12.72
K-7	0.48±11.03	0.36±7.28	0.83±7.63	1.82±9.62	0.09±17.36	0.18±6.53	0.68±10.02
K-8	1.05±6.21	0.33±5.82	0.52±1.40	2.02±3.84	0.11±8.92	1.26±10.60	0.69±14.24
K-9	0.55±1.13	0.35±4.23	0.40±5.19	1.29±7.48	0.07±19.21	0.19±4.85	0.47±7.04
K-10	0.58±14.62	0.20±10.86	0.32±12.92	0.90±14.08	0.05±29.93	0.87±18.12	0.36±28.06
K-11	0.44±4.14	0.22±7.24	0.66±1.90	1.15±12.44	0.04±24.86	0.53±14.73	0.24±8.07
K-12	0.19±9.23	0.38±3.80	0.81±6.98	2.35±5.26	–	–	0.98±11.34
C-1[b]	1.45±4.92	0.29±3.82	0.36±3.06	0.54±9.27	0.96±11.32	0.81±10.82	0.67±12.21
C-2	2.00±4.26	0.31±2.48	0.24±2.77	0.42±6.89	0.67±13.60	1.25±9.99	1.11±11.84
C-3	1.82±5.26	0.28±8.14	0.25±2.75	1.02±11.40	0.89±7.96	2.12±5.49	1.17±9.14
C-4	1.59±4.39	0.24±2.16	0.21±2.15	0.56±4.49	0.52±2.16	0.84±1.06	0.98±4.40
C-5	1.48±5.27	0.26±9.04	0.31±7.16	0.47±11.20	0.44±9.79	1.21±5.32	0.77±8.94
C-6	0.66±4.53	0.41±2.53	0.39±1.79	2.30±4.88	0.09±8.52	0.19±12.82	0.54±3.85
C-7	1.53±4.39	0.21±7.80	0.19±4.81	0.72±8.53	0.61±18.45	0.65±8.61	0.74±4.04

[a]K-1–12 : Korea sample; K-1: Youngchun (aerial stem), K-2: Ahndong (aerial stem), K-3: Ahndong (aerial stem), K-4: Ahndong (aerial etem), K-5: Uiseong (aerial stem), K-6: Namwon (aerial stem), K-7: Ahndong yongsang (aerial stem), K-8: Namwon qeumdong (aerial stem), K-9: Ahndong okdong (aerial stem), K-10: Young cheon (flower stalk), K-11: Namwon (aerial stem), K-12: Ahndong (aerial stem).
[b]C-1–7 : China sample; C-1: Gangsoseong (flower stalk), C-2-7 (flower stalk, and collected region was not specified).

compounds under the sample temperature and time. The fiber adsorption yield of volatiles extracted from herbal medicine according to fibers with different phases is shown in Fig. 3. The orders of adsorption yield in fibers were as follows: PDMS 100 μm, CAR/PDMS 75 μm, DVB/CAR/PDMS 50/30 μm, and PDMS/DVB 65 μm. Although the SPME fiber could significantly affect the amounts of volatile

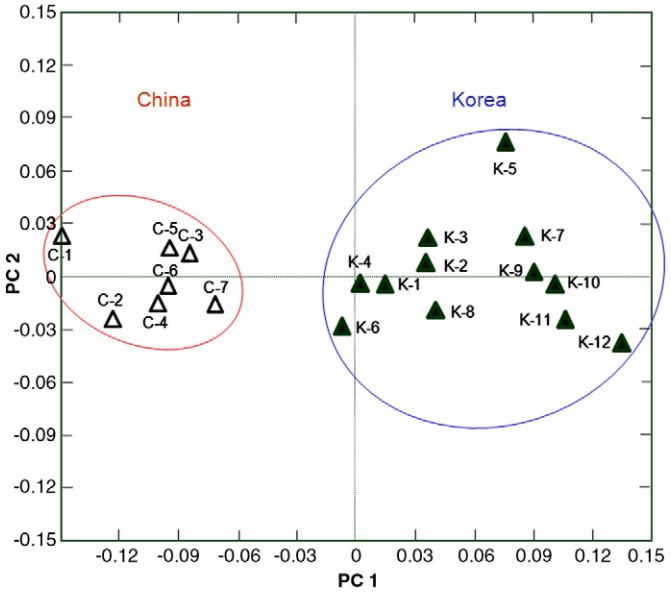

Fig. 6. Representation of 19 chromatographic samples of *Sch.t.*Briq. on PC 1 and PC 2 data (87.1% variance explained).

compounds from herbal medicine, the number of compounds detected in total ion chromatogram was not influenced. The monoterpene-ketones as major volatile compounds in *Sch.t.*Briq. were more effectively adsorbed in PDMS fiber than any other fibers, while as monoterpene-alcohols and oxides were similarly adsorbed on four fibers used. The highest extracted amount of volatiles in PDMS is probably attributed to the film thickness. Although some of polar volatiles were effectively adsorbed in DVB or CAR containing fibers [32], total extraction amounts could not be significantly influenced due to their minor components in *Sch.t.*Briq. Thus, in this study, PDMS fiber was selected for the analysis of the volatile compounds in *Sch.t.* Briq.

3.3. Selection of extraction temperature and time and desorption temperature and time

The effect of adsorption time and temperature on the amount of volatile compounds extracted by HS-SPME was investigated by varying time and temperature in the range from 5 to 50 min (with 6 stepwise) and 45 to 90 °C (with 8 stepwise), respectively. The adsorption time necessary to reach equilibrium is closely related with the extraction efficiency. The adsorption temperature can be strongly affected on the mass transfer and diffusion rate of volatile compounds from solid sample to headspace. Fig. 3 illustrates the sum of total peak area in the adsorption time and temperature ranges studied. Each sample was performed in triplicate, and the results of the peak area were the average value with the variation coefficient less than 6.88%. As shown in Fig. 4A, the amount of total peak area was gradually increased from 45 to 75 °C but slightly decreased above 85 °C. For extraction time, extraction longer than 10 min did not increase extraction efficiency (Fig. 4B). Thus, in this study, extraction temperature and time were selected at 75 °C and 10 min, respectively.

Desorption temperature is controlled with hot GC injection port, to use emitting the analytes adsorbed in fiber surface at high temperature. As shown in Fig. 5A, desorption temperature at 250 °C was shown the highest peak amount, indicating sufficient desorption of volatile compounds. However, some of compounds could be remained in fiber surface below at 250 °C. As can be seen in Fig. 5B, desorption time for 3 min gave the highest peak area. Thus, desorption temperature and time were selected at 250 °C and for 30 min, respectively.

3.4. Application of the method

The optimized HS-SPME method was applied to 19 different samples collected from market in near Seoul, Korea. The chemical compositions of volatiles in *Sch.t.*Briq. were determined in samples collected from 12 regions of Korea (K-1 to K-12) and from seven regions of China (C-1 to C-7). The quantity of each compound present in sample was determined and the results are summarized in Table 2. Each sample was analyzed with five replicate to ensure the reproducibility of quantitative result. The overall relative standard deviation (RSD) for main compounds by HS-SPME was ranged from 0.34 to 9.86%. Thus, it is applicable as a semiquantitative analysis method for volatile compounds from *Sch.t.*Briq. sample. The 19 *Sch.t.* Briq. samples showed apparently similar chromatographic pattern (data not shown here) but some differences on their amounts were observed, as indicated in Table 2. Especially such difference on the quantity of components is closely related with various factors such as origins, cultivation conditions and location, harvesting season, drying, preservation procedures, handling, transportation, and storage conditions.

3.5. Quality assessment by PCA

To classify the herbal plants collected from different regions, PCA were carried out using MVSP 3.1 version in order to perform on the analytical data of all 19 samples. To display the points on two principal components, PC 1 and PC 2 (first and second principal components) were chosen to represent the information. In the evaluation of the discrimination ability, PCA analysis was employed using relative peak areas of 11 common peaks as input data instead of the total peaks detected in TIC. Fig. 6 shows the principal component projection plot of the PC 1 and PC 2 of 19 *Sch.t.*Briq. samples. The score plot of two principal components showed the clear differentiation of cultivation location. From the scatter points, the samples could be classified into two groups, indicating a clear differentiation between *Sch.t.*Briq. collected from different regions.

4. Conclusion

An HS-SPME-GC/MS method for simultaneous quantitative and qualitative analyses of volatile compounds in *Sch.t.*Briq. has been described in this study. The HS-SPME method could greatly simplify the sample preparation procedure compared with any other extraction methods such as solvent extraction and steam distillation. In addition, the HS-SPME combined with GC/MS could provide appropriate sensitivity under optimization conditions and easily identify a number of volatile compounds in herbal medicine. An HS-SPME-GC/MS method has also shown to be effective to determine several volatile compounds in herbal medicine and suitable for the chemical standardization of herbal medicines obtained from *Sch.t.*Briq. regardless of geographic origin. In addition, PCA can provide important information on the differentiation of herbal plants from different regions.

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