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Comparison of different extraction methods for the analysis of fragrances from Lavandula species by gas chromatography–mass spectrometry

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Abstract

Various sampling techniques including solid-phase trapping solvent extraction (SPTE), headspace solid-phase microextraction (HS-SPME), reduced pressure steam distillation (RPSD) and simultaneous steam distillation–solvent extraction (SDE) were compared for the gas chromatography–mass spectrometry of the fragrances from the Lavandula species. Linalyl acetate (35.44%) and linalool (18.70%) were predominant components of Hidcote lavender samples obtained by SPTE whereas those levels were 2.63–4.04 and 36.80–43.47% in the same samples by RPSD and SDE, respectively. The partition coefficients between the headspace gaseous phase and HS-SPME fiber, and the relative concentration factors of the four characteristic components of the lavender were measured for relative evaluation of the fiber efficiency. Five different coatings were evaluated and 100-μm poly(dimethylsiloxane) was the most efficient for the successful extraction of lavender fragrances. A total of 43 compounds were identified by SPTE and gas chromatography–mass spectrometry from four Lavandula species. *Lavandula angustifolia* Hidcote species, which contains a higher level of linalyl acetate and linalool but little camphor, was evaluated as the highest quality among the four different Lavandula species.

Keywords: *Lavandula*; Solid-phase trapping solvent extraction; Solid-phase microextraction; Simultaneous steam distillation–solvent extraction; Reduced pressure steam distillation; Linalyl acetate; Linalool

1. Introduction

Lavender is one of the most useful medicinal plants. Commercially, the lavender provides several important essential oils to the fragrance industry including soaps, colognes, perfumes, skin lotions and other cosmetics. In food manufacturing, lavender essential oil is employed in flavoring beverages, ice cream, candy, baked goods, and chewing gum. Recently, aromatherapy is becoming increasingly popular, and lavender is used in aromatherapy as a relaxant. Several therapeutic effects of lavender such as sedative, spasmolytic, antiviral, and antibacterial activities have been reported [1–5]. The composition of its essential oils has been extensively investigated by using gas chromatography–mass spectrometry (GC–MS) [6–25]. However, little has been reported concerning the fragrance compositions of the various Lavandula species [16,17].

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Traditionally, methods of enfleurage and cold pressing have been used to extract numerous volatile components of floral scent, while alternative techniques include extraction by steam distillation, reduced pressure steam distillation (RPSD), petroleum-based solvent extraction, simultaneous steam distillation solvent extraction (SDE), and trapping using headspace [26,27]. Solid-phase microextraction (SPME) developed by Pawliszyn and coworkers in 1989, is a solventless extraction technique widely used in application of extraction from plants, food, biological and environmental samples [6,27–31].

An et al. recently reported SPME–GC–MS for the analysis of fragrances from lavender flowers [6]. Previously, we have studied the volatile fragrances emitted from rose, thyme, rosemary by GC–MS with solid-phase trapping solvent extraction (SPTE) or SPME [32–34]. SPTE and SPME techniques offer a useful alternative to conventional techniques. SPTE and SPME have diminished decomposition of plant compounds and cells, minimized activity of enzyme, and decreasing loss of those constituents.

In the present study, we compared four different extraction techniques including SPTE, headspace (HS)-SPME, RPSD, and SDE for GC–MS of the fragrance emitted from *Lavandula angustifolia* Hidcote species. The partition coefficients (K_{fg}) between the headspace gaseous phase and HS-SPME fiber, and the relative concentration factors (CF) of the four characteristic components of the lavender were measured for relative evaluation of the fiber efficiency. Five different coatings have been evaluated to select suitable fiber for the successful extraction of lavender fragrances. The fragrance compositions of four different Lavandula species cultivated in Korea were also investigated by SPTE and GC–MS. We evaluated the quality of Lavandula species according to the level of linalyl acetate. The final goal of the fragrance research is to be able to separate and identify the individual mixture components and to characterize the quantitative as well as qualitative aspects of each isolated compound [35].

2. Experimental

2.1. Plant material and reagents

Dried samples of leaves, flowers, and buds of Lavandula species including *Lavandula stochas* French, *Lavandula dentate* Fringed, *Lavandula angustifolia* Hidcote and *Lavandula heterophylla* Sweet harvested in 1999 were supplied from a local herb garden in Korea. All reference standards were of analytical grade and were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Tokyo Kasei (Nihonbashi, Tokyo, Japan). A reference standards mixture was prepared using petroleum ether as a solvent with a concentration of 500 μg/ml for each compound. Organic solvents of chromatographic grade were obtained from Sigma–Aldrich.

2.2. Solid-phase trapping solvent extraction

Fragrance compounds were collected from the Lavandula samples by using a SPTE apparatus designed in our laboratory. The schematic diagram of a SPTE apparatus is illustrated in Fig. 1A. About 20 g of Lavandula samples were filled in a clean, dry barrel of the hypodermic glass syringe (50 ml, 14 cm long×3 cm I.D.) with its plunger and needle removed. And then, two barrels were fitted together with a polytetrafluoroethylene (PTFE, Teflon) spacer gasket and held by a joint clip, as described in our previous report [32–34]. A Pasteur pipet (15 cm long×0.565 cm I.D.) was used as a trap-housing which was packed with 500 mg of ethylvinylbenzene–divinylbenzene copolymer adsorbent (Porapak-Q, Supelco, 149–125 μm) and glass wool plugs. Before use, Porapak-Q particles were prerinsed with organic solvent in order to remove impurities. The inlet of the Pasteur pipet was attached to the luer taper tip of the barrel containing the Lavandula samples. An oil-free electric vacuum pump (Vacuubrand, Wertheim, Germany, diaphragm ME2 model, 2.4 m^3/h) and a PTFE valve restrictor were connected with Tygon tubing to the outlet end of the trap via glass-manifold. A purified nitrogen gas (purity, 99.99%) flow at 400 ml/min was passed into a couple of barrels and out through the adsorbent trap under reduced pressure. The collection was continued for 3 h at ambient temperature. After a run, the fragrance compounds were eluted by two extractions with 2 ml of petroleum ether Aliquots were analyzed by GC–MS.

2.3. Headspace solid-phase microextraction

All SPME holders and coating fibers were ob-

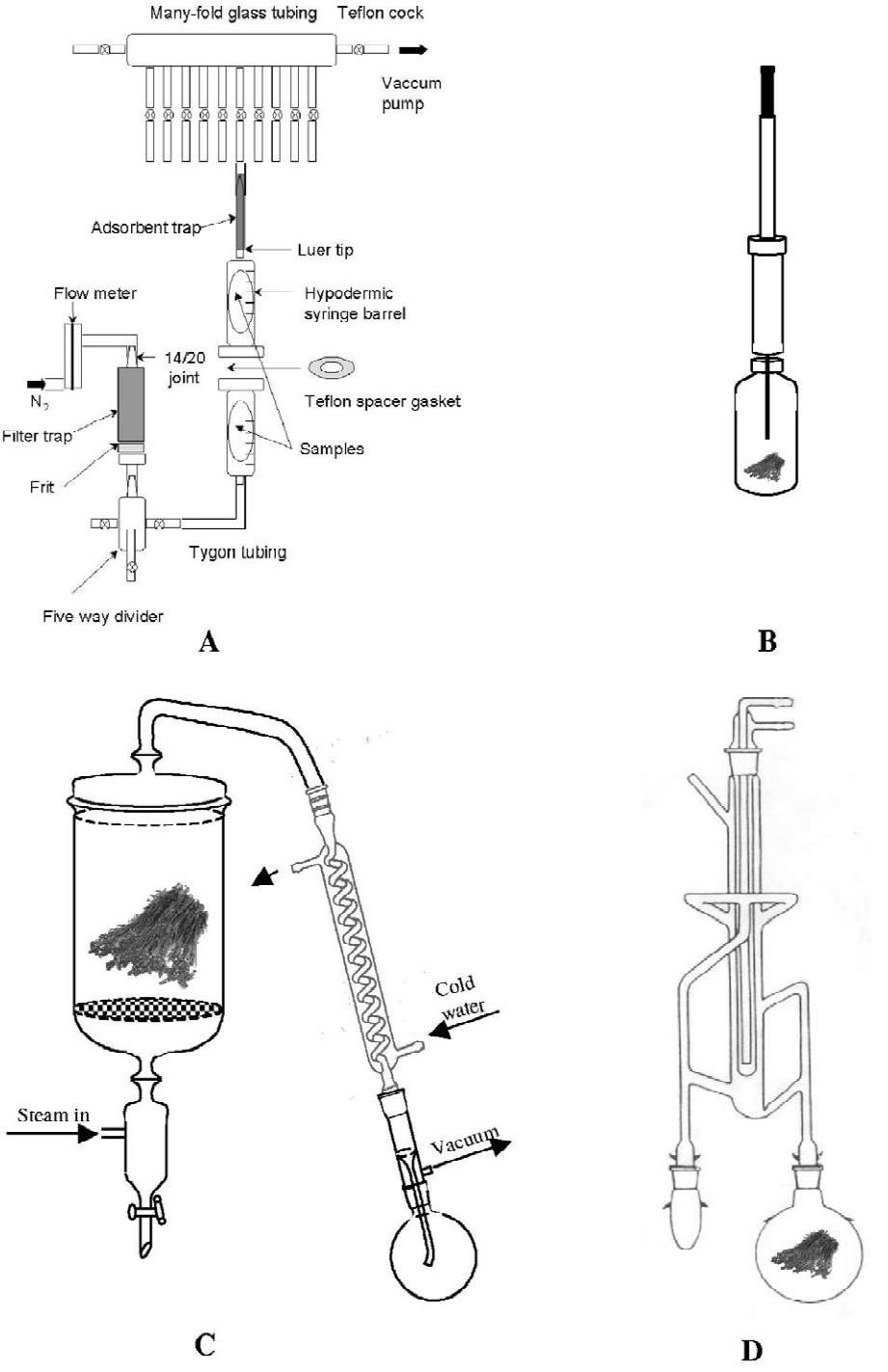

Fig. 1. Schematic representation of solid-phase trapping solvent extraction apparatus (A), solid-phase microextraction (B), reduced pressure steam distillation (C), and simultaneous distillation and extraction (D).

tained from Supelco (Bellefonte, PA, USA). For the HS-SPME sampling, five SPME devices including the 100-μm poly(dimethylsiloxane) (PDMS), 30-μm PDMS, 7-μm PDMS, 85-μm polyacrylate (PA), and 65-μm Carbowax–divinylbenzene (CW–DVB) were used. Before use, SPME fibers were reconditioned by heating them in the hot injection port of a gas chromatograph at 250–320 °C for 30–240 min in order to remove contaminants. Prior to analysis, a fiber blank was run to confirm no contamination peaks. Sampling conditions including SPME fiber exposure time, extraction temperature, desorption time and temperature were investigated. About 1 g of the Lavandula samples were placed in a 25-ml vial and the vial was capped with an aluminum cap. And then, as shown in Fig. 1B, the SPME fiber was exposed to the headspace above the sample for 60 min at 40 °C. After adsorption, the SPME fiber was removed from the sample vial and immediately inserted into the injection port of the GC–flame ionisation detection (FID) or GC–MS system where the thermal desorption occurs at 250 °C for 60 s.

For the static headspace (S-HS) analysis, the sealed vial (100 ml) containing 7 g of Lavandula sample in the form of solid was kept at 40 °C for 60 min to diffuse volatile fragrance components into gaseous headspace until the headspace has reached a state of equilibrium. An aliquot of the vapor in the headspace was then taken by using a 5000 μl gastight syringe (Hamilton no. 1005, Reno, NV, USA) instead of SPME fiber to inject into the injection port of GC.

2.4. Reduced-pressure steam distillation

About 300 g of Lavandula samples were placed in a 1000-ml distillation flask connected to the RPSD apparatus, as shown in Fig. 1C. The RPSD was continued for 3 h 100 °C with reduced pressure (100 mmHg). The distillates (25 ml) were extracted with 5 ml of petroleum ether. Then extracts were dried with anhydrous sodium sulfate.

2.5. Simultaneous steam distillation and solvent extraction

About 10 g of Lavandula samples and 100 ml of distilled water were placed in a 250 ml round flask and 10 ml of petroleum ether were placed in a 30-ml round flask connected to the modified Likens–Nickerson micro SDE apparatus illustrated in Fig. 1D. Chilled water was circulated through the cold finger condenser, as described elsewhere [26,27]. The extraction was continued for 2 h. After this reflux time the petroleum ether fractions of the solvent flask and the return loop were collected. Then the petroleum ether extracts were dried with anhydrous sodium sulfate.

2.6. Gas chromatography–mass spectrometry

GC–MS analyses were carried out by using a Trace GC 2000 and a GC-Q Plus ion trap MS^n (Thermoquest-Finnigan, Austin, TX, USA) with electron impact ionization mode. Chromatographic separations were performed on a crosslinked 5% phenylpoly(dimethylsiloxane) (SPB-5, Supelco, 60 m × 0.25 mm × 0.25 μm film thickness) column and a poly(ethyleneglycol) (Supelcowax-10, Supelco, 60 m × 0.25 mm × 0.25 μm film thickness) column. The oven temperature program of the SPB-5 column was 50 °C (3 min)–5 °C/min–240 °C (10 min). Injector and transfer line temperature were 250 and 275 °C, respectively. The flow-rate of the carrier gas (He, 99.9995%) was 1.0 ml/min. A split injection with a ratio of 1:30 was used. The sample volume injected was 2 μl. The electron impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200 °C; scan mode, 30.0–400.0 (mass range). The oven temperature program of the Supelcowax-10 column was 40 °C (7 min)–4 °C/min–150 °C–8 °C/min–240 °C (10 min); injector, 200 °C; transfer line, 230 °C; the other conditions were the same as those of the SPB-5 column. Volatile compounds were identified by comparing the Kovats retention indices (I) and comparison of the obtained mass spectra of the relevant chromatographic peaks with those of authentic standards and with spectra of the NIST and Wiley libraries. Kovats retention indices were determined by inserting a solution containing the homologous series of normal alkanes (C_8–C_{20}).

For the determination of partition coefficients (K_{fg}) between SPME fiber and lavender headspace, a GC-14B gas chromatograph with FID (Shimadzu, Kyoto, Japan) was also used. Injector and detector

temperatures were set at 250 °C. Gas flow-rates were kept as follows: nitrogen carrier gas, 1 ml/min; hydrogen, 35 ml/min; air, 500 ml/min. GC peak areas were integrated with C-R6A integrator (Shimadzu). The other conditions were the same as those of the GC–MS with SPB-5 column.

3. Result and discussion

3.1. Comparison of extraction techniques from Hidcote lavender by SPTE with RPSD and SDE

Fig. 2 shows total ion chromatograms (TIC) on a

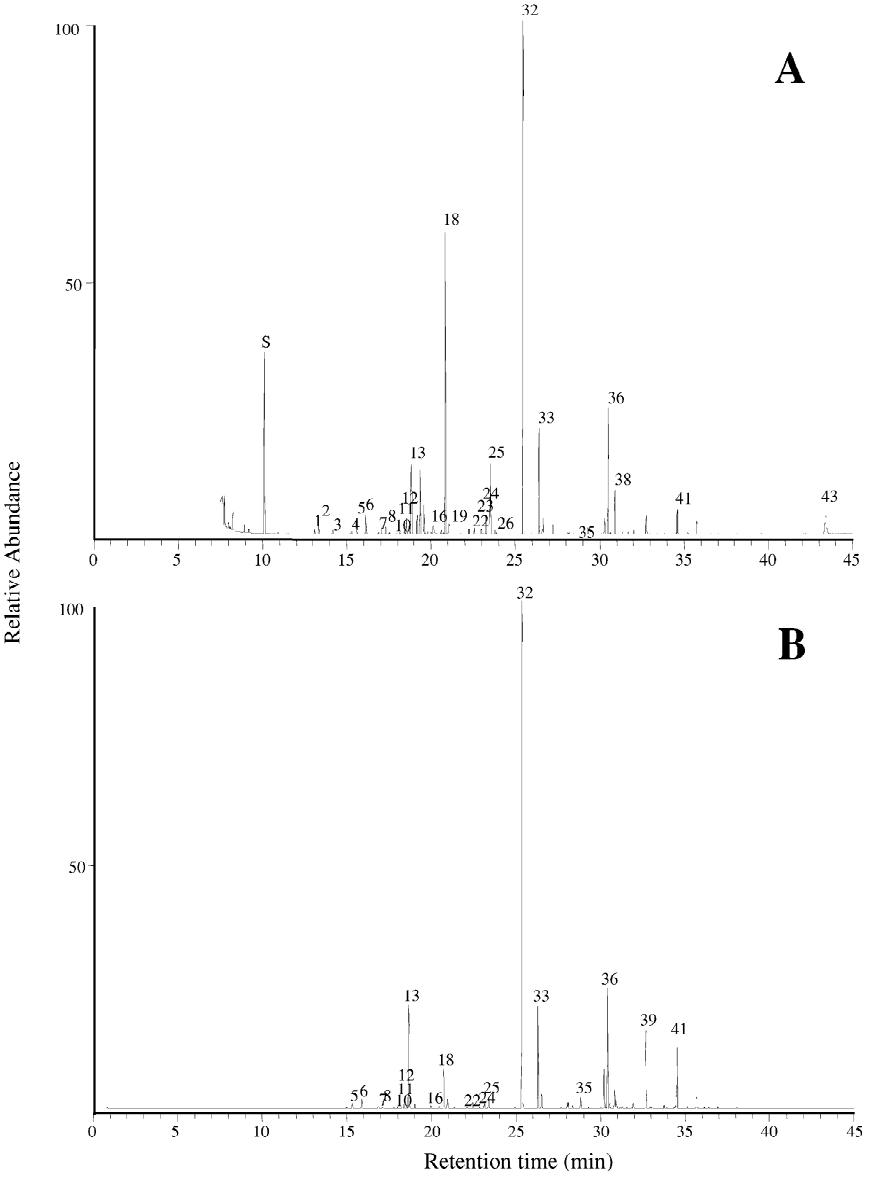

Fig. 2. Total ion chromatograms on a SPB-5 column of fragrances from dried *Lavandula angustifolia* Hidcote collected by different extraction methods. (A) SPTE with Porapak Q; (B) HS-SPME with 100 μm PDMS; (C) RPSD; (D) SDE. Peak numbers correspond to the numbers indicated in Table 1. For analytical conditions, see Experimental.

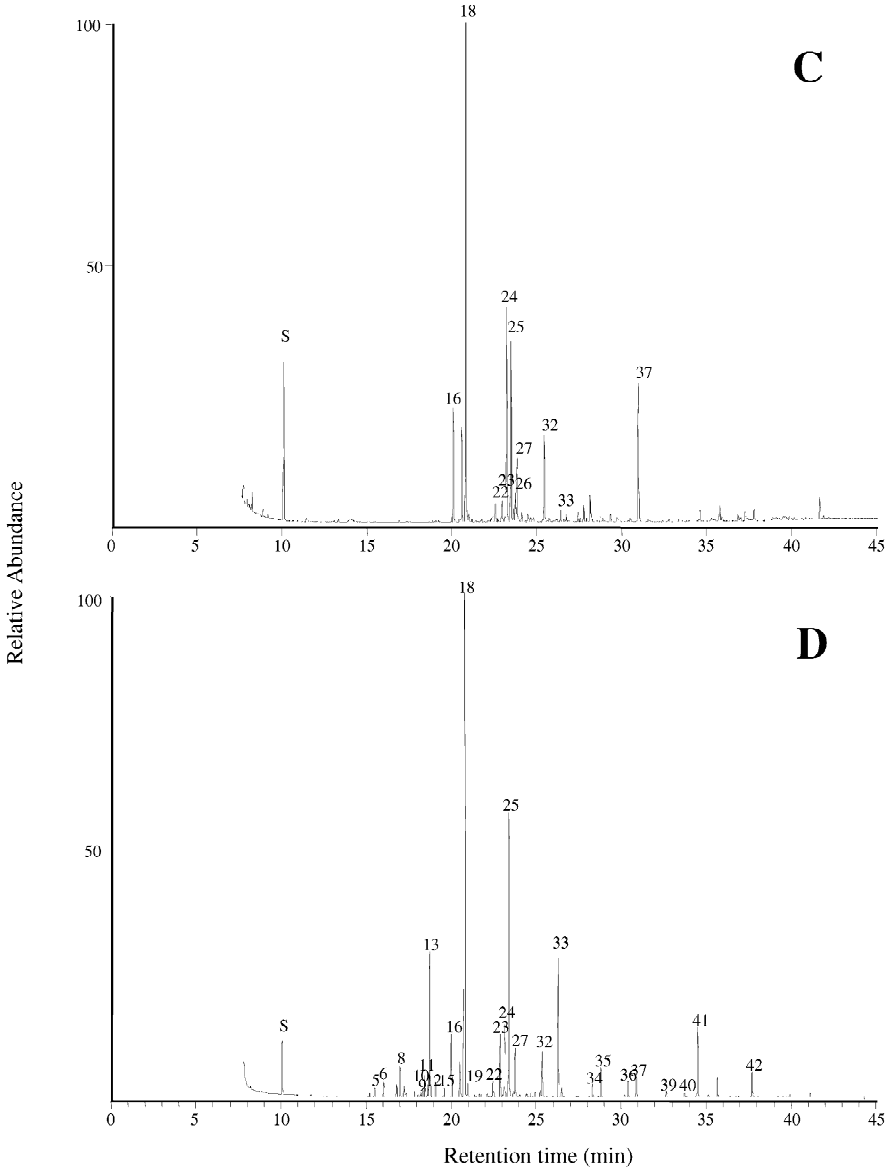

Fig. 2. (*continued*)

SPB-5 column of the fragrant constituents from *Lavandula angustifolia* Hidcote extracted by SPTE, HS-SPME using a 100-μm PDMS fiber, RPSD and SDE techniques. The peak numbers shown in Fig. 2 correspond to the numbers indicated in Table 1, which provides a list of fragrance compounds identified by GC–MS, along with Kovats retention indices (*I*) on a SPB-5 and a Supelcowax 10 columns for reference mixture. The characteristic mass spectral ions of each peak from Lavandula species are given. Components without a Kovats index were identified tentatively by comparison with GC–MS results because their reference reagents were not available.

SPTE as well as SDE was advantageous on the basis of the amount required for sample treatment,

Table 1
Retention indices and identification of fragrance components from Lavandula species

Peak	Compound	Retention index (I)[a]		M_r	Base peak m/z	Characteristic mass spectral ions (EI) m/z (relative abundance, %)
		Supelco SPB-5	Supelco Wax-10			
1	Ethyl benzene	866	1129	106	91	106 (38), 65 (9), 51 (8)
2	*m*-Xylene or *p*-Xylene[b]	875	1137	106	91	106 (60), 77 (10), 107 (8), 65 (7)
3	*o*-Xylene	899	1188	106	91	106 (57), 77 (10), 51 (7)
4	Thujene	931	1041	136	91	93 (73.6), 77 (45), 105 (43.5), 136 (6)
5	α-Pinene	942	1016	136	91	77 (42), 93 (58), 39 (12), 105 (8), 136 (6)
6	Camphene	961	1061	136	93	91 (85), 79 (47), 77 (39), 121 (35), 67 (26), 106 (20), 39 (15), 137 (6)
7	β-Pinene	987	1103	136	91	93 (70), 77(50), 39(21), 41 (20), 121 (12), 67 (10), 51 (8), 107 (8), 136 (9)
8	β-Myrcene	993	1165	136	91	41 (52), 77 (42), 93 (30), 65 (9), 136 (0.58), 137 (0.47)
9	α-Terpinene	1024	1179	136	136	91 (72), 77 (32), 105 (19.1), 39 (8), 51 (7)
10	*m*-Cymene or *o*-Cymene[b]	1029	1275	134	119	134 (50), 91 (23.2), 117 (23), 135 (10), 65 (1)
11	*p*-Cymene	1032	1273	134	119	134 (32), 91 (42), 117 (28),77 (6), 65 (4), 133(4), 51 (3), 135(3.5)
12	Limonene	1036	1198	136	67	91 (72), 93 (60), 79 (50), 94 (34), 39 (22), 53 (14), 65 (20), 107 (21), 121(15), 136 (12), 137 (5)
13	Cineol	1041	1210	154	43	81 (57), 154(1.47), 155 (4.19)
14	*trans*-1-Methyl-4(1-methylethyl)-2-Cyclohexen-1-ol[c]	–	–	154	43	91 (95.9), 79 (86), 77(54),121 (42), 39 (38.4), 67 (30), 136 (11.5)
15	γ-Terpinene	1073	1248	136	91	93 (62.8), 136 (46), 77 (40.5), 121 (22.1), 137 (21), 105 (12), 39 (9), 51 (8), 65 (5.2)
16	Linalyl oxide[b] (mixture of isomer)	1080 1092	1470 1489	170	79	93 (61), 67 (57), 59 (55), 92 (53), 43 (40), 135 (6), 171 (5), 153 (4.1), 170 (1)
17	Fenchone	1099	1439	152	81	79 (64), 41 (23), 153 (22), 39 (21), 67 (14), 152 (6), 53 (6), 137 (4)
18	Linalool	1103	1555	154	43	91 (71), 81 (58), 93 (44), 55 (37), 80 (31), 136 (7)
19	Octen-1-ol, acetate	1107	1420	170	43	67 (35), 39 (25.7), 99 (23), 81 (21.4), 27 (3.6)
20	*cis*-Verbenol	1117	1592	152	67	79 (46.5), 109 (40.5), 91 (38.3), 41 (25), 55 (10.9), 137 (5.2)
21	*cis*-Sabinol[c]	–	–	152	91	55 (33), 119 (31), 55 (33), 105 (12.2), 134 (8.3), 152 (0.1)
22	Camphor	1159	1538	152	95	67 (68), 81 (58), 108 (57), 39 (38), 41 (32), 55 (26), 153 (22), 152 (19), 137 (4)
23	Lavendulol[c]	–	–	154	69	41 (70), 111 (31.1), 55 (25.5), 29 (25), 123 (14.2), 136 (2), 154 (0.4)
24	Borneol	1182	1734	154	95	43 (73.5), 67 (29), 137 (16),121 (7.5), 154 (3),155 (3)
25	α-Terpinen-4-ol	1191	1623	154	93	91 (96.1), 137 (89.1), 43 (74), 77 (45), 67 (37), 39 (32), 154 (18.5), 121 (8)
26	*p*-Cymen-8-ol			150	43	135 (38), 117 (17), 65 (10), 150 (1)
27	α-Terpineol	1203	1730	154	93	91 (88), 136 (70.0), 121 (68), 79 (55.9), 59 (41.9), 67 (35), 43 (22.5), 31 (8.6), 154 (0.5)

Table 1. Continued

Peak	Compound	Retention index (I)[a]		M_r	Base peak m/z	Characteristic mass spectral ions (EI) m/z (relative abundance, %)
		Supelco SPB-5	Supelco Wax-10			
28	Myrtenal	1208	1667	150	79	107 (74), 41 (49),91 (37), 135 (7), 150 (0.5)
29	Verbenone	1225	1592	150	107	91 (96), 79 (84), 151 (70), 135 (60), 39 (36), 122 (30), 150 (28), 65.1 (26), 51 (14)
30	*p*-Cumicaldehyde	1255	1805	148	105	133 (88), 148 (66), 149 (40),119 (50), 77 (47)
31	Carvone	1256	1690	150	82	54 (60), 27 (34), 39 (45), 150 (9), 135 (5)
32	Linalyl acetate	1259	1566	196	91	93 (85), 79 (56), 77 (37), 43 (30), 137 (28), 121 (23), 67 (22), 39 (22), 105 (14), 55 (8), 196 (1)
33	Bornyl acetate	1299	1596	196	95	93 (74), 137 (69), 121 (52), 43 (33), 79 (33), 67 (27), 154 (7), 196 (0.5)
34	Terpineol acetate	1335	1727	196	93	91 (76), 136 (48), 79 (33), 43 (30.8), 67 (22), 107 (17.8)
35	Geranyl acetate	1349	1772	196	41	67 (93), 39 (66), 93 (54), 121 (37), 196 (0.01)
36	Caryophyllene	1384	1613	204	91	105 (58), 79 (50), 77 (43), 39 (25.5), 67 (22), 204 (9)
37	Coumarin[c]	-	–	146	146	90 (37, M-C_2O_2), 118 (M-CO)
38	Farnesene[b]	1379	1647	204	91	41 (84), 39 (61), 77 (47),
	(mixture of isomers)	1393	1684			93 (44), 204 (0.2)
		1476	1738			
		1512	1746			
		1518	1762			
		1544	1785			
39	γ-Cadinene	1529	1780	204	161	189 (75.4), 204 (68), 41 (55), 105 (53), 91 (49), 119 (30), 55 (25), 81 (21), 67 (14)
40	Calamenene	1538	1823	202	159	202 (10), 105 (8), 41 (5)
41	Caryophyllene oxide	1663	1892	220	41	55 (28), 69 (18), 220 (2)
42	7-Methoxy coumarin[c]	–	–	176	133	148 (71), 105 (17), 89 (11)
43	Bis (2-ethylhexyl) phthalate[c]	–	–	390	149	41 (94), 55 (52), 77 (24), 57 (22), 390 (0.01)

–, Its reference substance are not available.
[a] Identified on basis of retention behavior compared with authentic compounds.
[b] Correct isomeric form not identified.
[c] Tentatively identified by comparison of MS data with those of library.

which in the case of RPSD was as high as 300 g. Experimentally, only 1.3 ml equivalent to 1.1 g of lavender oil could be obtained from 300 g of dried lavender samples by RPSD. However, sample amount could be reduced to as low as 10 g by SDE, 20 g by SPTE, and 1–7 g by HS-SPME. When compared with SDE, RPSD and HS-SPME using a 100-μm PDMS fiber, the use of SPTE provides several differences of fragrance compositions. The most obvious differences are in the amounts of linalyl acetate and linalool, and in the detection of minor components with low molecular mass. Linalyl acetate (35.44%) and linalool (18.70%) were predominant components of Hidcote lavender samples obtained by SPTE whereas these components were present at levels 4.04 and 36.80% in the same samples by RPSD and 2.63 and 43.47% in those by SDE (Fig. 2 and Table 2). Ethyl benzene, xylene, and α-thujene with low molecular masses were observed between 13 and 25 min by SPTE but not in any samples by RPSD and SDE. Therefore, regarding lavender fragrance it can be seen that SPTE is a

Table 2
Fragrances composition identified by GC–MS of Hidcote lavender collected by different extraction methods and peak area percentages

Peak no.	Compound	SPTE (Porapak Q)	RPSD	S.D.E.
1	Ethyl benzene	0.34±0.75	–	–
2	*m*-Xylene or *p*-Xylene	0.85±0.26	–	–
3	*o*-Xylene	0.40±1.14	–	–
4	Thujene	0.36±13.15	–	–
5	α-Pinene	0.97±0.21	–	0.23±0.04
6	Camphene	1.57±2.44	–	0.72±0.37
7	β-Pinene	0.63±20.03	–	–
8	β-Myrcene	0.91±0.56	–	0.84±2.40
9	α-Terpinene	–	–	0.23±2.89
10	*m*-Cymene or *o*-Cymene	–	–	0.23±2.75
11	*p*-Cymene	0.11±1.60	–	1.28±2.92
12	Limonene	1.23±2.13	–	0.29±20.44
13	Cineol	5.94±6.25	–	8.25±5.38
15	γ-Terpinene	–	–	0.41±1.44
16	Linalyl oxide	0.21±4.10	5.27±0.58	2.61±1.60
17	Fenchone	–	4.67±0.88	–
18	Linalool	18.70±2.09	36.8±11.23	43.47±3.49
19	Octen-1-ol, acetate	0.45±2.22	–	0.43±12.17
22	Camphor	0.45±5.32	1.18±1.45	0.89±5.06
23	Lavandulol	0.25±4.08	1.20±6.55	2.62±4.30
24	Borneol	1.88±10.12	11.37±1.06	3.64±3.14
25	α-Terpinen-4-ol	4.63±0.98	9.53±12.92	14.01±5.19
26	*p*-Cymen-8-ol	0.53±1.13	1.30±1.17	–
27	α-Terpineol	–	4.61±21.60	2.58±1.94
28	Myrtenal	–	0.10±0.43	–
32	Linalyl acetate	35.44±3.66	4.04±0.96	2.63±2.67
33	Bornyl acetate	5.88±6.43	0.53±21.00	5.73±2.81
34	Terpineol acetate	–	2.08±2.74	0.56±1.46
35	Geranyl acetate	0.27±2.18	–	1.28±4.80
36	Caryophyllene	9.39±3.01	0.16±1.09	0.76±14.78
37	Coumarin	–	7.42±1.07	1.16±21.67
38	Farnesene	2.60±2.97	–	–
	(mixture of isomers)	–	–	0.29±1.66
39	γ-Cadinene	–	–	0.29±0.40
40	Calamenene	1.21±5.96	–	0.17±0.89
41	Caryophyllene oxide	1.80±13.60	–	3.08±1.58
42	7-Methoxy coumarin	–	–	1.32±5.96
43	Bis(2-ethylhexyl)phthalate	3.00±2.76	–	–

Unit, normalized peak area (%)±RSD, $n=3$.
Components without a peak number were not found as separate peaks in the analysis of Hidcote lavender sample.
Operating conditions: SPB-5 column; oven, 50 °C (3 min)–5 °C/min–240 °C (10 min); injector, 240 °C; ion source, 200 °C; transfer line, 275 °C; EI, 70 eV; carrier (He) flow, 1 ml/min; split ratio, 1:30; injection volume, 1 μl; instrument, Thermoquest-Finnigan Trace GC with GCQ plus ion trap MS^n.
–, Not detected.

superior technique compared with RPSD and SDE because the quality of the lavender oil is evaluated by the level of linalyl acetate [16–19]. Another difference is the detection of trace components. Higher molecules such as geranyl acetate, farnesene, γ-cardinene, calamenene, caryophyllene oxide were not detected by RPSD. Fenchon, α-terpineol, myrtenal and terpineol acetate were observed by RPSD but not by SPTE. Interestingly, coumarin known as component of leaf tissues was observed by RPSD

and SDE. These results are possibly caused by hydrolysis, thermal degradation, molecular rearrangements and loss of components due to harsh conditions during the distillation process [16].

3.2. Comparative analysis of Hidcote lavender by different SPME fiber coatings

The effect of the extraction temperature on the HS-SPME efficiency of Lavandula Hidcote was tested at 20 and 40 °C with three different fibers. At this evaluation stage, the extraction time was set at 60 min in order to obtain equilibrium. GC–FID peak areas for five components such as cineol, linalool, linalyl acetate, geranyl acetate, and linalyl oxide are shown in Fig. 3. The peak areas of four components obtained at 40 °C were a little higher than those at 20 °C, however, those of linalyl oxide were almost the same at two extraction temperature. Linalyl acetate using the 100-μm PDMS at 40 °C showed relatively the highest peak area. The relatively lower temperature is preferred for the prevention of degradation of thermally labile components.

HS-SPME is considered complete when the analyte concentration has reached equilibrium between the sample matrix and the fiber coating. Determination of adsorption equilibrium times for α-pinene, cineol, linalool, and linalyl acetate as the test references was carried out using the 100-μm PDMS and 85-μm PA fibers. In this investigation, adsorption times were varied every 10 min from 10 to 60 min at 40 °C. The extraction time profile is shown in Fig. 4. For the 100-μm PDMS fiber, the equilibrium condition for the adsorption of reference compounds was almost reached after 40 min. In the case of 85-μm PA fiber peak areas for three reference compounds except α-pinene increased steadily through a 60-min

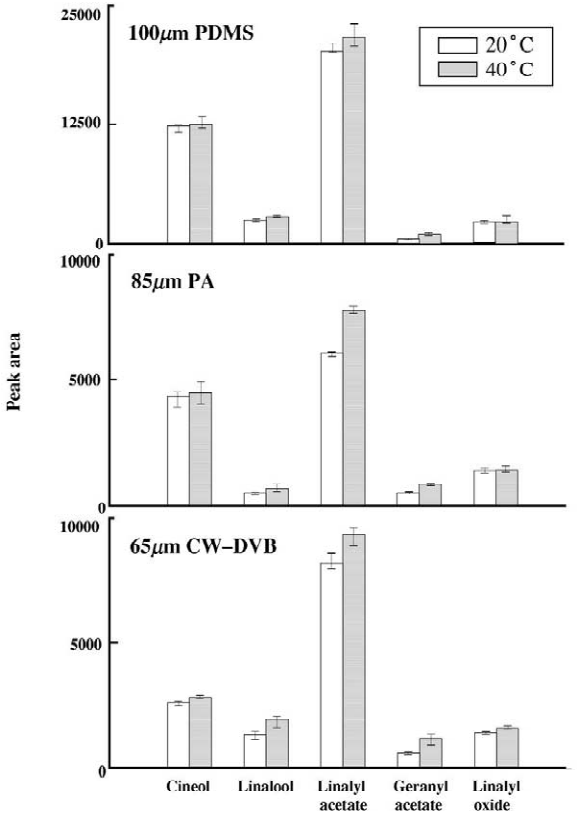

Fig. 3. Effect of extraction temperature on the HS-SPME–GC of the selected components of Lavandula Hidcote using 100-μm PDMS, 85-μm PA and 65-μm CW–DVB fibers.

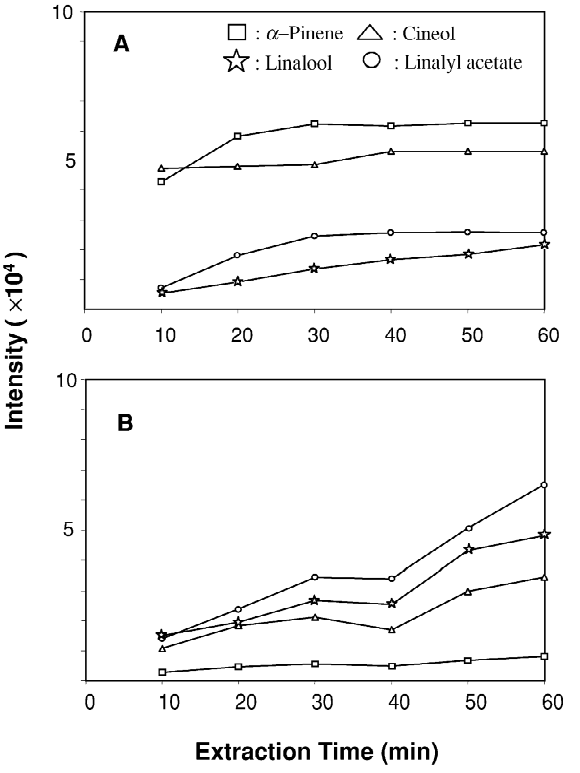

Fig. 4. Effects of extraction time on the HS-SPME–GC–MS for the four fragrance standards obtained with (A) 100-μm PDMS; (B) 85-μm PA.

period, indicating that equilibrium had not been reached. Response of α-pinene resulted to be not influenced by extraction time when using PA fiber, since this fiber is not suitable at all for the adsorption of this low-polar analyte. For application of sample, 40 °C extraction temperature and 1 h extraction time were finally used.

The desorption temperatures were compared at 200 and 250 °C. Stabilization of the chromatograms was observed and reproducible peak areas were obtained for the references mixture using desorption time of 60 s at 250 °C.

In HS-SPME, the transfer of an analyte from a liquid or solid matrix onto the coating of a fiber is related to the two partition equilibria steps [36–41]:

K_{gs} = (Concentration in gas phase)/
(Concentration in liquid or solid matrix phase)

K_{fg} = (Concentration in fiber coating)/
(Concentration in gas phase)

$$K = K_{gs} \cdot K_{fg}$$

where K_{gs} is defined as the analyte partition coefficient between the headspace gaseous phase and sample matrix; K_{fg} is the analyte partition coefficient between the SPME fiber coating and the headspace gaseous phase; and K is the overall partition coefficient.

The K_{fg} values can be calculated by the following forms [39–43]:

$$K_{fg} = (A_f V_g)/(A_g V_f)$$

where A_f refers to the peak area of analyte on the SPME fiber, V_g is the volume of the gas sample injected by S-HS–GC; A_g is the peak area of analyte in the headspace; and V_f is the volume of the fiber coating.

Generally, compounds that have high K_{fg} values will tend to partition more readily into the fiber coating from headspace gaseous phase, and have relatively high responses and low limits of detection. It must be noted that above equations can be used for the determination of K_{fg} only in systems under equilibrium conditions, and produce reliable results when the volume of the vial used during the measurement of the K_{fg} is relatively large [42–44]. Some authors have already systematically compared the performance of several SPME fibers for specific applications by using the K_{fg} values [37–39]. Recently, Zabaras and Wyllie reported on the quantitative analysis of terpenoids in the gas phase of sage (*Salvia officinallis* L.) samples using HS-SPME and determined the experimental K_{fg} values [41].

In this study the K_{fg} values and the relative concentration factors (CF) of the four characteristic components of the lavender were measured for relative evaluation of the fiber efficiency of HS-SPME. The matrices encountered in the extraction and analysis of herb samples were usually living or dried plants. The determination of the experimental K_{fg} values was carried out using solid Lavandula samples instead of standards to ensure that matrix effects are identical to those encountered during actual sampling modified to the previous report [41]. HS-SPME followed by S-HS was applied successively to the same sample. The relatively large amount (7 g) of Lavandula samples were placed in a relatively large volume (100 ml) of capping vial to ensure that the depletion of the headspace by HS-SPME sampling prior to S-HS was negligible and therefore the S-HS was not affected [41]. And the CF of an analyte achieved by an SPME fiber versus the corresponding S-HS sampling is the ratio between the analyte peak area obtained by HS-SPME–GC with that fiber and the corresponding area obtained by S-HS–GC [39].

The results of the experimental K_{fg} values and the CF values for characteristic compounds of lavender samples are given in Table 3. Our data of the experimental K_{fg} values and the CF values for cineol and linalool were in fairly good agreement with data of Bicchi et al. [39] and Zabaras and Wyllie [41].

The peak areas of each characteristic compound were percent normalized, taking the corresponding peak areas obtained with 100-μm PDMS fiber as equal to 100 according to the report by Bicchi et al. [39]. Fig. 5 shows the HS-SPME–GC–FID normalized intensity of characteristic compounds of Lavandula Hidcote obtained with different fibers versus 100-μm PDMS fiber. The 100-μm PDMS was most efficient among the five fiber types.

From the fragrance compositional point of view, the comparative GC–MS responses were also in-

Table 3
Partition coefficient (K_{fg})[a] between fiber coating and headspace gas phase, and concentration factors (CF)[a] of characteristic components of Lavandula Hidcote samples

Fiber coatings	V_f[b] (μl)	Cineol		Linalool		Linalyl aceate		Linalyl oxide	
		K_{fg}	CF	K_{fg}	CF	K_{fg}	CF	K_{fg}	CF
100-μm PDMS	0.612	$1.3\cdot10^4$	1.55	$3.2\cdot10^4$	3.92	$6.6\cdot10^5$	16.24	$9.1\cdot10^3$	1.11
30-μm PDMS	0.132	$9.6\cdot10^3$	0.25	$3.1\cdot10^4$	0.82	$1.8\cdot10^5$	4.88	$4.3\cdot10^4$	1.13
7-μm PDMS	0.026	$1.2\cdot10^4$	0.06	$4.6\cdot10^4$	0.24	$2.0\cdot10^5$	1.07	$2.5\cdot10^4$	0.13
85-μm PA	0.521	$3.9\cdot10^3$	0.40	$2.1\cdot10^4$	2.16	$1.1\cdot10^5$	11.54	$4.7\cdot10^4$	3.37
65-μm CW–DVB	0.436	$5.0\cdot10^3$	0.36	$5.6\cdot10^4$	4.00	$2.4\cdot10^5$	16.85	$6.2\cdot10^4$	4.40

[a] Mean value of duplicate determinations.
[b] The volume of the fiber coating.

vestigated to choose the suitable fiber coating for HS-SPME. Comparison of the extraction efficiencies of five different SPME fibers for the reference standards of α-pinene, cineol, linalool, linalyl acetate is illustrated in Fig. 6. In the all fiber types, linalyl acetate along with cineol, linalool, and caryophyllene was detected as predominant components. Ethyl benzene, xylene, and α-thujene were not detected by any types of SPME fiber. Normalized peak areas (%) of identified components from Hidcote lavender by five different fibers are summarized in Table 4. In the HS-SPME technique using 100 μm PDMS fiber, the most predominant component was linalyl acetate (47.41%) while the levels of linalool and lavandulol were much lower or not detected comparing with those by SPTE technique. This result suggests that polar alcohols and low-molecular-mass terpenes are not well adsorbed by PDMS fiber. When polar or bipolar fibers such as PA or CW–DVB were used for HS-SPME, higher levels of linalool were observed. In cases of the PDMS fibers with smaller thickness and 65 μm CW–DVB fiber, several minor components were not observed. This result is possibly related to the various effects including polarity, phase thickness and analyte matrix etc. According to the results previously obtained by An et al. [6] linalool, lavandulol and α-thujene were identified

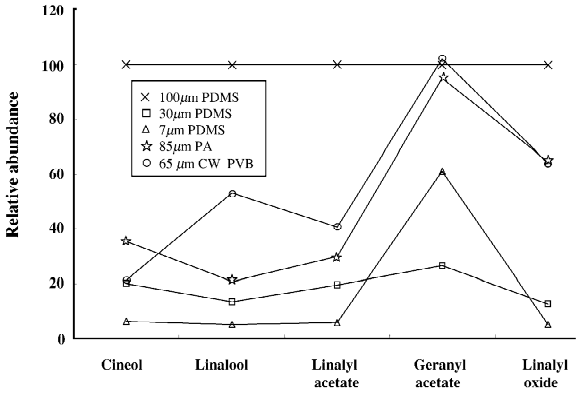

Fig. 5. HS-SPME–GC normalized peak areas of characteristic components of Lavandula Hidcote obtained with 100-μm PDMS fiber versus different fibers.

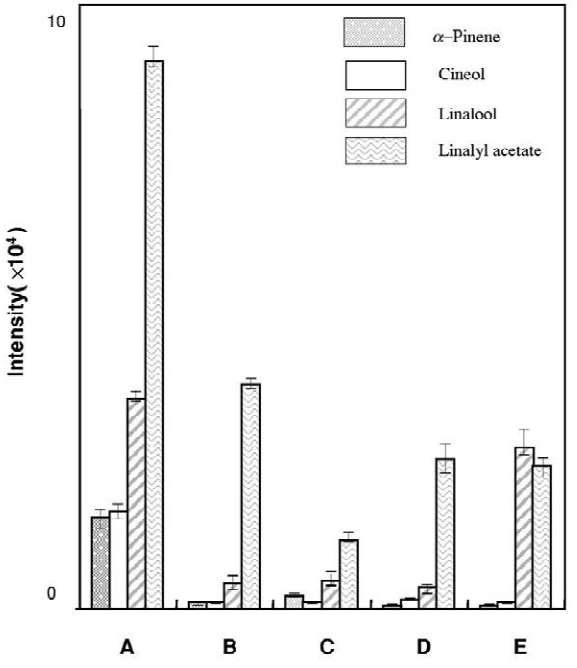

Fig. 6. Comparison of the extraction efficiencies of different SPME fibers for the fragrance standards obtained by HS-SPME–GC–MS: (A) 100-μm PDMS; (B) 30-μm PDMS; (C) 7-μm PDMS; (D) 85-μm PA; (E) 65-μm CW–DVB.

Table 4
Fragrances composition of Hidcote lavender collected by SPME with different fiber type

Peak no.	Compound	SPME				
		100-μm PDMS (Red)	30-μm PDMS (Yellow)	7-μm PDMS (Green)	85-μm PA (White)	65 μm CW–DVB (Orange)
1	Ethyl benzene	–	–	–	–	–
2	m-Xylene or p-Xylene	–	–	–	–	–
3	o-Xylene	–	–	–	–	–
4	Thujene	–	–	–	–	–
5	α-Pinene	0.51±0.06	0.63±2.62	–	–	–
6	Camphene	0.99±0.76	1.30±3.41	1.64±5.96	0.45±10.21	–
7	β-Pinene	0.09±0.59	0.60±42.64	–	–	–
8	β-Myrcene	0.90±3.96	0.21±1.36	–	–	–
9	α-Terpinene	–	–	–	–	–
10	m-Cymene or o-Cymene	0.01±2.10	–	–	–	–
11	p-Cymene	0.48±3.93	–	–	0.69±1.12	–
12	Limonene	0.23±3.81	–	–	0.45±23.20	–
13	Cineol	8.63±3.10	8.96±3.52	10.31±2.99	4.77±42.06	2.87±3.21
15	γ-Terpinene	0.05±23.51	–	–	–	–
16	Linalyl oxide	0.35±23.23	–	–	0.90±0.93	0.46±43.65
17	Fenchone	–	–	–	–	–
18	Linalool	3.29±4.63	4.71±1.63	8.34±23.97	26.69±0.90	19.29±4.15
19	Octen-1-ol, acetate	–	1.51±2.16	1.48±4.00	0.80±0.98	0.38±0.95
20	Camphor	0.55±4.54	0.56±42.74	–	0.79±0.66	0.43±0.07
23	Lavandulol	–	–	–	–	–
24	Borneol	0.85±3.29	0.49±96.73	–	3.08±1.03	2.01±2.15
25	α-Terpinen-4-ol	1.30±3.49	1.41±2.23	–	5.56±0.25	4.03±44.96
26	p-Cymen-8-ol	–	–	–	–	–
27	α-Terpineol	–	–	–	–	–
28	Myrtenal	–	–	–	–	–
32	Linalyl acetate	47.41±3.63	64.00±1.04	30.82±6.12	48.06±14.10	62.08±5.43
33	Bornyl acetate	7.25±83.62	0.99±10.41	–	–	0.66±4.49
34	Terpineol acetate	–	–	–	–	–
35	Geranyl acetate	1.06±3.09	–	–	0.89±0.65	0.55±4.47
36	Caryophyllene	12.45±10.38	5.32±6.76	12.91±2.42	1.58±0.35	2.47±7.26
37	Coumarin	–	2.17±0.18	17.98±1.35	–	–
38	Farnesene (mixture of isomers)	2.61±4.25	–	–	–	–
39	γ-Cadinene	–	3.61±9.13	8.24±0.40	1.76±5.23	2.31±4.76
40	Calamenene	5.64±9.31	–	–	–	–
41	Caryophyllene oxide	–	3.53±10.27	8.29±22.99	3.53±18.02	2.46±3.93
42	7-Methoxy coumarin	5.35±8.19	–	–	–	–
43	Bis(2-ethylhexyl) phthalate	–	–	–	–	–

Unit: normalized peak area (%)±RSD, n=3.
Components without a peak number were not found as separate peaks in the analysis of Hidcote lavender sample.
Operating conditions: SP-5 column; oven, 50 °C (3 min)–5 °C/min–240 °C (10 min); injector, 240 °C; ion source, 200 °C; transfer line, 275 °C; EI, 70 eV; carrier (He) flow-rate, 1 ml/min; split ratio, 1:30; injection volume, 1 μl; instrument, Thermoquest-Finnigan Trace GC with GCQ plus ion trap MS^n.
–, Not detected

from living flower sample of Lavandula angustifolia by HS-SPME–GC–MS.

3.3. Analysis of fragrance compositions of Lavandula species by SPTE and GC–MS

A total 43 compounds were identified from the fragrances of four Lavandula species. These fragrance compounds could be classified into several chemical groups: esters, alcohols, aldehydes, terpenes, and others. Comparative TIC of components identified from four species collected by SPTE technique and GC–MS on a apolar SPB-5 column are shown in Fig. 2A and Fig. 7. Four different Lavandula species showed distinct differences in fragrance compositions. Normalized peak areas of

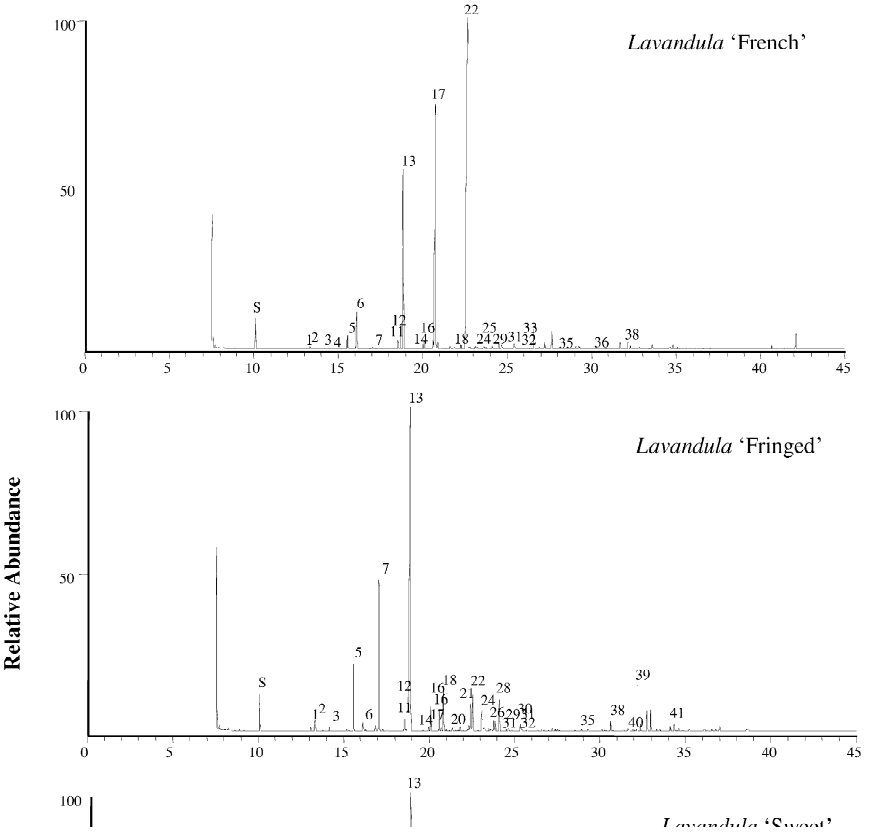

Fig. 7. Total ion chromatograms on a SPB-5 column of fragrances collected by SPTE from different Lavandula species.