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On-site field sampling and analysis of fragrance from living Lavender (*Lavandula angustifolia* L.) flowers by solid-phase microextraction coupled to gas chromatography and ion-trap mass spectrometry

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Abstract

Solid-phase microextraction coupled to gas chromatography and mass spectrometry has been applied as a simple alternative method for the analysis of essential oil directly from lavender intact flowering spikes and genuine oils. All recognised major oil constituents were detected by this procedure, with results comparable to those given by a conventional method (organic solvent extraction). Distinctive chromatographic profiles were found for various species.

Keywords: *Lavandula angustifolia*; Plant materials; Solid-phase microextraction; Fragrances; Essential oil

1. Introduction

Essential oil from lavender is of great interest owing to its broad commercial usage. It is important to the perfume, cosmetic, flavouring and pharmaceutical industries. Interest in growing lavender for oil production is increasing in Australia.

The essential oils of *Lavandula* species are obtained by steam distillation of the fresh flowering spikes. Oil quality is assessed by oil chemical composition and by the organoleptic opinion of the flavourists. Conventional analytical techniques for oil composition are often time consuming, limited in aroma correlation, and have to be carried out on the finished products. Over the past decade a number of new analytical methods have been developed for the analysis of essential oils, such as supercritical CO_2 extraction [1–4], but they are still time consuming and limited in correlation with natural flower fragrance. The continuing search for a fast-screening, sensitive, analytical technique is always warranted given that oil quality varies, being regulated endogenously and by the growing environment of the lavender plant, as well as by production methods. Solid-phase microextraction (SPME) offers a useful alternative to conventional analytical techniques.

Solid-phase microextraction (SPME) is a simple, solventless extraction technique in which a phase-

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coated fused-silica fibre is immersed in a liquid sample or exposed to the headspace above a liquid or solid sample. Analytes adsorb or absorb to the phase, and then can be thermally desorbed in the injection port of a gas chromatograph with subsequent transferral to a capillary column [5,6]. This technique eliminates most drawbacks in conventional analysis techniques and has shown adaptability to a variety of applications, including volatile organic compounds in air, fatty acids and flavours in foods and beverages, drugs in urine and blood, and pesticides in environmental samples [7,8]. The simplicity and the high sensitivity of the procedure render it a desirable technique for the analysis of essential oils in lavender. Although the application of SPME to the analysis of volatiles from plants has been reported [9–11], no literature reports appear on the use of SPME to assess essential oils from lavender, nor on-site field sampling of living flower fragrance.

The objective of this study was to develop a SPME-enclosed chamber method to analyse essential oil directly from intact living lavender flowers, and to examine the feasibility of using SPME–GC–MS flower fragrance profiles for characterising various lavender species.

2. Experimental

2.1. Materials

Lavender (*Lavandula angustifolia*) plants grown in Charles Sturt University (Australia) gardens were analysed in this study by daylight field sampling and by conventional solvent extraction of essential oil. An authentic Tasmania oil specimen, regarded as perfumery grade, produced from *L. angustifolia* [12], was obtained from Bridestowe Estate of Tasmania through David Ingram.

2.2. SPME apparatus

The SPME holder for manual sampling, and the fibre assembly with 100 μm polydimethylsiloxane coating were purchased from Supelco Australia (Sydney, Australia). The fibre was conditioned prior to use in a hot GC injector according to instructions provided by the supplier.

2.3. On-site field sampling

A single living flowerhead at top one peduncle of a healthy angustifolia plant in the University garden during daylight was completely surrounded by a clear and empty round-bottom Pyrex glass flask (100 ml) before sealing the flask neck with non-absorbent cotton. The aroma-trapping flask was kept in place for 1.0 h prior to SPME fibre sampling. The preconditioned fibre assembly was inserted through the cotton seal and the fibre exposed to the flask internal space for various times in order to absorb headspace vapours. After exposure, the fibre was retracted and then thermally desorbed and the absorbate analysed by GC–MS. A desorption time of 10 min was found to be sufficient to desorb all volatiles onto the GC. For analysis of the genuine industrial oil sample, 10 μl of oil was pipetted into a 10 ml headspace vial crimped with a PTFE-rubber disc and aluminium cap. The vial headspace was equilibrated for 1 h prior to analysis. A SPME fibre absorption time of 1 s was used to collect oil vapour samples.

2.4. Absorption time profile study

A selection of different SPME fibre absorption times was employed for sample collection from lavender flower headspace volatiles. The absorption times used were 30 s, 2.5 min, 5, 15 and 30 min. For each time interval, one GC–MS determination was carried out. The chromatographic peak area counts of identified volatile were plotted against the fibre absorption time (Fig. 1).

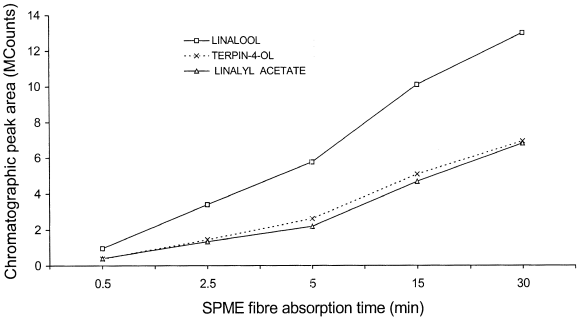

Fig. 1. Representative SPME absorption time profiles.

2.5. Instrumentation and constituents identification

All analyses were done with a Varian 3400 gas chromatograph coupled to a Varian Saturn ion-trap mass spectrometer. A non-polar DB-5 fused-silica column (J & W Scientific, Australia), 30 m×0.25 mm I.D. with a phase thickness of 0.25 μm was used. The carrier gas was helium with a linear velocity of 36 cm/s at 60°C. The split/splitless injection port was heated at 240°C. The syringe needle containing the fibre was inserted into the GC injector port for 10 min to thermally desorb the analytes into the GC–MS system. After 3 min desorption, the split vent was opened with a He flow of 100 ml/min, and the oven temperature program started as follows: 60°C, then ramped at 3°C/min to 150°C, then at 30°C/min to 250°C and held for 5 min. All mass spectra were acquired in the electron impact (EI) mode at 70 eV. The mass spectrometer scanned in the range of m/z 41–300 at a rate of 1.0 scans/s. The mass spectrometer was tuned using FC-43.

Constituents of essential oil vapour were identified by comparing their retention times as well as their mass spectra with those in a commercial library (Newterp) for essential oils [13], and in some cases by using standards. Retention times were adjusted by using a 'retention time locking' technique to compare those in the Newterp library determined on the same stationary phase.

2.6. Analysis of sample extracted by organic solvent

A flowering flowerhead was cut from the same plant as above (2.3) and soaked in 10 ml hexane for 1 h. One μl of this hexane extract was then injected into the GC–MS system. All conditions were the same as above (Section 2.5) except that the MS was turned off (solvent delay) for the first 300 s of the analysis.

3. Results and discussion

3.1. Optimization of SPME conditions

SPME, unlike most conventional sampling techniques, is not based on an exhaustive extraction of the sample, but on an equilibrium between the analyte concentration in the sample or sample headspace and that in the solid-phase fibre coating. The time required to reach the equilibrium is the optimal sampling time. As indicated by the absorption profile shown in Fig. 1, the area counts of the analyte were rising steadily through the 30-min period, indicating that equilibrium had not been reached. For practical purposes it is not essential for equilibrium to be reached in the SPME sampling process [11]. In addition, the depletion of analytes in the headspace, which is a concern in SPME [5] is negligible in this study since the tiny quantity taken away by the fibre is constantly replenished by living flowers. Therefore, a shorter sampling time can be used as long as the extraction conditions are kept constant. A 10-min period was determined to be sufficient for desorbing the extracted analytes in the GC injector port. Memory effects on the fibre were excluded by desorbing the same fibre for a second time after the initial desorption, and through monitoring blank values.

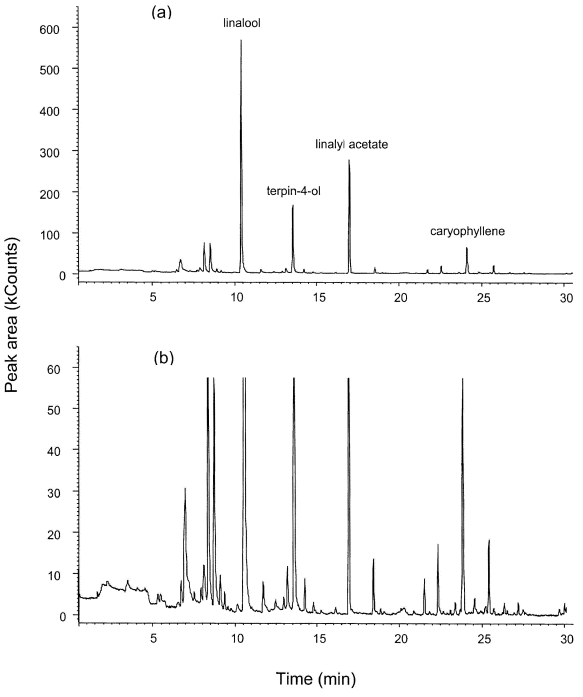

Fig. 2. SPME–GC–MS chromatogram of field sampled *L. angustifolia* fragrance. (a) Full scale chromatogram; (b) amplified chromatogram showing all analytes including the minor components.

3.2. Determination of the identified analytes

Fig. 2 illustrates a GC–MS chromatogram resulting from the SPME sampling of lavender fragrance from living flowers during daylight. All major components for the essential oil of *L. angustifolia* defined by ISO and AFNOR standards [14] were detected. Table 1 shows the components and their relative proportions (% total ion current) detected around the *L. angustifolia* flowering spike by SPME–GC–MS. They were identified as mostly terpene hydrocarbons, oxygenated terpenes, and ses-

Table 1
Identification and quantification of compounds detected in lavender flowerhead fragrance by SPME, and by solvent extraction of flowers

No.	Retention time (min)	Compound	SPME				Solvent extract (%)	RSD (%)
			Living flower fragrance (%)[a]	RSD (%)	Tasmania oil vapour (%)	RSD (%)		
1	3.09	(*E*)-2-Octene	0.2	61	0.7	1	–	–
2	4.99	α-Thujene	0.1	37	0.9	3	0.3	22
3	5.17	α-Pinene	0.1	40	3.2	4	0.3	11
4	6.46	3-Octanone	0.4	7	12.1	2	1.1	2
5	6.72	Myrcene	3.6	8	0.5	13	0.1	11
6	7.70	*p*-Cymene	0.2	8	3.4	1	–	–
7	7.87	Sylvestrene	0.9	4	2.6	4	1.4	7
8	**8.14**	**(Z)-β-Ocimene**	**5.4**	**3**	**1.0**	**2**	**3.6**	**6**
9	**8.51**	**(*E*)-β-Ocimene**	**5.7**	**5**	**1.4**	**2**	**3.0**	**2**
10	8.90	γ-Terpinene	0.5	20	–	–	0.3	6
11	9.17	*trans*-Sabinene hydrate	0.2	61	–	–	0.4	12
12	9.38	*cis*-Linalool oxide	–	–	2.8	1	0.2	28
13	10.00	Terpinolene	0.1	10	2.3	1	0.3	21
14	**10.39**	**Linalool**	**41.2**	**8**	**39.6**	**1**	**41.3**	**3**
15	11.60	*allo*-Ocimene	0.5	3	0.1	20	0.4	13
16	12.17	Camphor	–	–	0.9	2	0.1	72
17	13.12	Lavandulol	0.8	64	0.1	2	0.1	17
18	**13.55**	**Terpin-4-ol**	**12.1**	**4**	**2.5**	**1**	**10.6**	**1**
19	14.22	Hexyl butyrate	0.5	18	0.6	1	1.1	6
20	14.77	*n*-Dodecanol	0.1	17	–	–	<0.1	66
21	15.73	Isobornyl formate	–	–	0.1	2	<0.1	27
22	**16.99**	**Linalyl acetate**	**16.1**	**6**	**20.3**	**2**	**20.4**	**1**
23	18.53	Lavandulyl acetate	0.9	88	1.2	1	4.3	7
24	20.32	Hexyl tiglate	–	–	0.0	4	0.2	35
25	21.05	α-Terpinyl acetate	–	–	0.1	2	0.1	94
26	21.73	Neryl acetate	0.5	8	0.7	2	0.1	43
27	22.25	α-Copaene	–	–	<0.1	10	<0.1	32
28	22.56	Geranyl acetate	1.3	2	1.5	1	0.3	38
29	22.90	7-*epi*-Sesquithujene	<0.1	29	–	–	0.1	16
30	23.63	α-Cedrene	–	–	0.1	6	<0.1	51
31	23.97	α-*cis*-Bergamotene	1.5	1	–	–	<0.1	15
32	**24.11**	**(E)-Caryophyllene**	**5.3**	**1**	**1.1**	**2**	**6.0**	**8**
33	24.83	α-*trans*-Bergamotene	0.2	21	–	–	–	–
34	25.22	α-Himachalene	0.1	41	<0.1	9	0.1	15
35	25.53	α-Humulene	<0.1	22	–	–	0.1	55
36	25.73	(*E*)-β-Farnesene	1.3	16	<0.1	2	2.5	5
37	26.70	Germacrene D	–	–	–	–	0.8	13
38	26.87	β-Selinene	<0.1	109	–	–	<0.1	10
39	27.35	Bicyclogermacrene	–	–	–	–	<0.1	67
40	28.00	γ-Cadinene	–	–	<0.1	1	–	–
41	28.48	δ-Cadinene	–	–	–	–	<0.1	33
42	30.57	Caryophyllene oxide	0.1	7	0.2	1	0.1	12

[a] (%) figures are their relative proportions as percent of total ion current (TIC). The dominant compounds are indicated in bold.

quiterpenes, i.e. the grouping of compounds indicated as responsible for the characteristic smell of lavender [14]. Because this procedure uses a chamber-trapping technique known to stress plants, the relative proportions of compounds detected will be slightly different from the natural untrapped fragrance. Comparison between species fragrance profiles requires therefore, strict reproduction in sampling procedure.

The lavender vapour consisted of terpene hydrocarbons (17.0%), oxygenated terpenes (54.3%), esters of monoterpene alcohols (18.7%), sesquiterpene hydrocarbons (8.4%), oxygenated sesquiterpene (0.1%), and miscellaneous compounds (1.5%). Forty-two compounds were detected, 31 of which were identified. Though the number of components was high, three of them, linalool, terpin-4-ol and linalyl acetate, were dominant and constituted 70% of the total sample. They correspond to the most significant compounds as indicated by extensive studies performed on lavender plants [2,12,15]. Other compounds, in small but significant proportions were myrcene, sylvestrene, *cis*-β-ocimene, *trans*-β-ocimene, lavandulol, lavandulyl acetate, geranyl acetate, *trans*-α-bergamotene, *trans*-caryophyllene and *trans*-β-farnesene.

3.3. Comparison of SPME with solvent extraction and authentic oil

A comparison of the compositions of the organic solvent flower extract and the SPME extract of authentic lavender oil vapour is presented in Table 1. Their GC–MS chromatograms are shown in Fig. 3. The results are comparable to those given by SPME around living plant flowerheads, including the major oil constituents, dominant peaks, and internal peak ratios, with the exception of 3-octanone, which is a dominant compound in authentic oil vapour. Some minor constituents were detected by SPME–GC–MS, which were not detected by the conventional method. This carries great significance because of the importance of oil volatiles to aroma as sensed by organoleptic opinion. The comparability of results between SPME and solvent extraction indicates the applicability and feasibility of SPME as an alternative in the analysis of lavender essential oil.

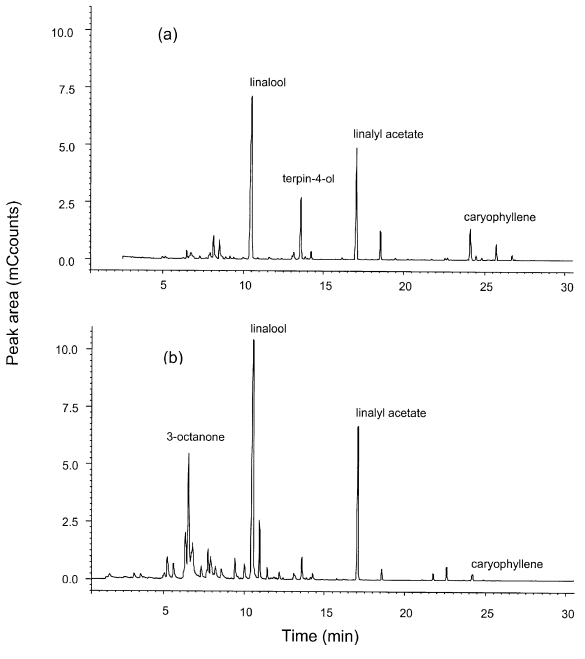

Fig. 3. GC–MS profile comparison of conventional and SPME methods. (a) *L. angustifolia* (flower solvent extraction); (b) Tasmania oil vapour.

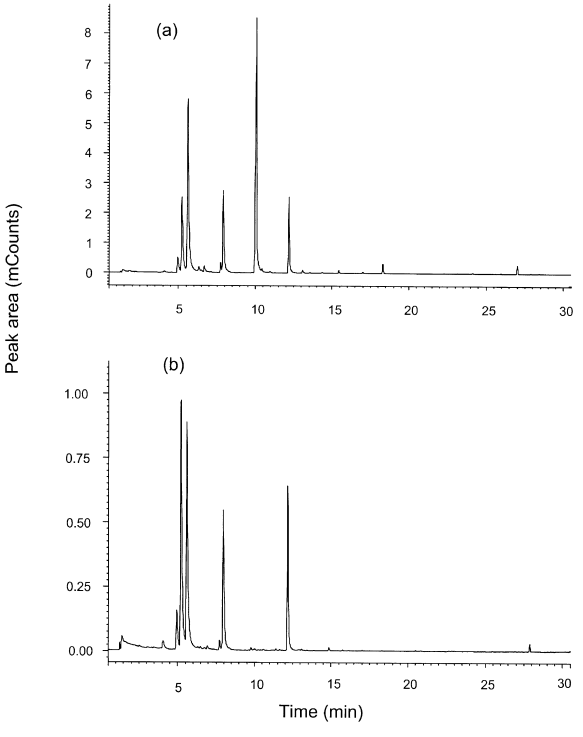

Fig. 4. SPME–GC–MS profiles of two other lavender species. (a) *Lavandula stoechas*; (b) *Lavandula viridis*.

3.4. Examination of various lavender species

Apart from *L. angustifolia*, two other lavender species, which are not known for oil production [16], were examined by this SPME procedure. Fig. 4 shows the chromatograms obtained. Each species has its own characteristic composition pattern which may be conducive to species identification. The comprehensive evaluation of such fingerprints among lavender species and cultivars is currently under investigation by means of this SPME procedure and pattern recognition programs, and will be reported in the future.

4. Conclusions

On the basis of this study, it is concluded that the SPME–GC–MS analysis technique described herein is technically feasible for field sampling and analysis of essential oils. Given the volatile nature of essential oils, their organoleptic testing for oil quality, and the convenient analysis by SPME of intact flowering spikes, SPME coupled to GC–MS represents a simple, time-saving, highly sensitive, solvent-free, and organoleptically correlatable testing method, which has great prospects for future use as an alternative to conventional techniques for the analysis of essential oils from *Lavandula* species.

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