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Hydrodistillation-headspace solvent microextraction, a new method for analysis of the essential oil components of *Lavandula angustifolia* Mill.

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Abstract

A new method involving concurrent headspace solvent microextraction combined with continuous hydrodistillation (HD-HSME) for the extraction and pre-concentration of the essential oil of *Lavandula angustifolia* Mill. into a microdrop is developed. A microdrop of *n*-hexadecane containing *n*-heptadecane (as internal standard) extruded from the needle tip of a gas chromatographic syringe was inserted into the headspace above the plant sample. After extraction for an optimized time, the microdrop was retracted into the syringe and injected directly into a GC injection port. The effects of the type of extracting solvent, sample mass, microdrop volume and extraction time on HD-HSME efficiency were investigated and optimized. Using this method, thirty-six compounds were extracted and identified. Linalool (32.8%), linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%) and geranyl acetate (5.0%) were found to be the major constituents. To the best of our knowledge this is the first report on the use of continuous headspace solvent microextraction coupled with hydrodistillation for investigation of essential oil components.

Keywords: Headspace solvent microextraction (HSME); Hydrodistillation; *Lavandula angustifolia* Mill.; Extraction method; Essential oils

1. Introduction

Lavender is one of the most useful medicinal plants. Commercially, the lavender is an important source of essential oil that is widely used in fragrance industry including soaps, colognes, perfumes, skin lotions and other cosmetics [1]. In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods, and chewing gum [2]. Recently, aromatherapy is becoming increasingly popular, and lavender is used in aromatherapy as a relaxant [3,4]. Several therapeutic effects of lavender, such as sedative, spasmolytic, antiviral, and antibacterial activities have been reported [5,6]. The composition of the essential oil of *Lavandula angustifolia* Mill. has been extensively investigated by using gas chromatography–mass spectrometry (GC–MS) [7–10].

A wide variety of analytical methods is used to extract the volatile compounds from plant material. Techniques commonly used to extract the essential oils include steam distillation, hydrodistillation, dynamic and static headspace, supercritical-fluid extraction and solvent extraction [2,11–16]. Headspace sampling for gas chromatographic analysis has many advantages, the most important of which is the elimination of much of the interferences arising from the sample matrix. In addition to the standard methods for headspace sampling, the technique of solid-phase microextraction (SPME) has found acceptance because it is simple and inexpensive [2,7,16]. Tellez et al. developed a new method involving concurrent solid-phase microextraction combined with continuous hydrodistillation of essential oil [17].

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More recently, single drop microextraction (SDME) has been evaluated as an alternative to SPME [18]. In this technique, a microdrop of solvent is suspended from the tip of a conventional microsyringe and then is immersed in a sample solution in which it is immiscible or suspended in the headspace (HSME) above the sample [19,20]. HSME has a number of advantages including: choice of a wide variety of solvents, minimal solvent use, low cost, renewability of the drop (thereby eliminating sample carry-over), high precision, simplicity and ease of use, high sensitivity and low detection limit, short pre-concentration time, possibility of automation, and no requirement for conditioning procedures (as is the case with the fiber in solid-phase microextraction). HSME has a high potential in many areas of analytical chemistry including environmental, pharmaceutical, forensic and food analysis in which volatile compounds are frequently determined [21].

In this paper, a new method involving concurrent headspace solvent microextraction coupled with continuous hydrodistillation (HD-HSME) for analysis of the essential oil of lavender is reported and results are compared with those obtained by the routine hydrodistillation method. To the best our knowledge, this is the first report of a HD-HSME method for investigation of essential oils.

2. Experimental

2.1. Reagents and material

The aerial parts (including flowers, leaves and stems) of *L. angustifolia* Mill., were collected in August 2004, from the research field of Medicinal Plants and Drugs Research Institute (MPDRI) of Shahid Beheshti University, Tehran, Islamic Republic of Iran. A voucher specimen has been lodged at MPDRI Herbarium (MP-882).

Solvents, such as *n*-pentadecane (>99%), *n*-hexadecane (>99%), *n*-heptadecane (>99%), 1-octanol (99.5%) and benzyl alcohol (99.8%) were purchased from Merck and Fluka chemical companies and were used without any further purification.

2.2. Instrumentation

The extraction and injection procedures were carried out using a 10 μl Hamilton gas tight syringe Model 1701N with a fixed bevelled-point needle. GC analysis of the oil was conducted using a Thermoquest-Finnigan Trace instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm I.D., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow-rate of 1.1 ml/min. The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min. The injector and flame ionization detector (FID) temperatures were kept at 250 °C and 280 °C, respectively. GC–MS analysis was carried out on a Thermoquest-Finnigan Trace GC–MS instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm I.D., film thickness 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min and the transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow-rate of 1.1 ml/min and the split ratio was adjusted at 1/50. The quadrupole mass spectrometer was scanned over the 45–465 amu range with an ionizing voltage of 70 eV and an ionization current of 150 μA. The constituents of the volatile oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6–C_{24}) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [22]. Quantitative data were obtained from FID area percentages without the use of correction factors.

2.3. Essential oil isolation

Air-dried aerial parts of *L. angustifolia* Mill. (50 g) were ground and subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus as recommended by British Pharmacopeia [23]. Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4 °C until analysis by GC and GC–MS.

2.4. HD-HSME of essential oil

HD-HSME was performed by using the apparatus shown in Fig. 1. A 100 ml round-bottom flask containing 2 g of the dried plant and 50 ml of water was heated at 100 °C by a mantle. The Hamilton syringe was rinsed and primed at least seven times with the solvent/standard solution. After uptake of 3 μl of *n*-hexadecane containing *n*-heptadecane (as internal standard, 200 ppm), the needle of the syringe was then inserted into the headspace of plant sample. Five minutes after the refluxing was commenced, the extraction was started. The syringe plunger was depressed and a microdrop of extracting solvent was suspended from the needle tip. After an optimized period of time, the plunger was withdrawn and the microdrop was retracted back into the syringe. The needle was removed from the headspace and its contents were injected into the GC system. Finally, the analytical signal was calculated as the peak areas of the analytes relative to the internal standard.

3. Results and discussion

3.1. Optimization of experimental conditions

Hydrodistillation is a time consuming method and needs large amounts of plant material. Here, good results were obtained by a combination of continuous hydrodistillation with concurrent headspace solvent microextraction (HD-HSME) in a short period of time and by using a few grams of the plant. The present study was commenced by optimization of experimental parameters, such as the nature of the extracting solvent, sample

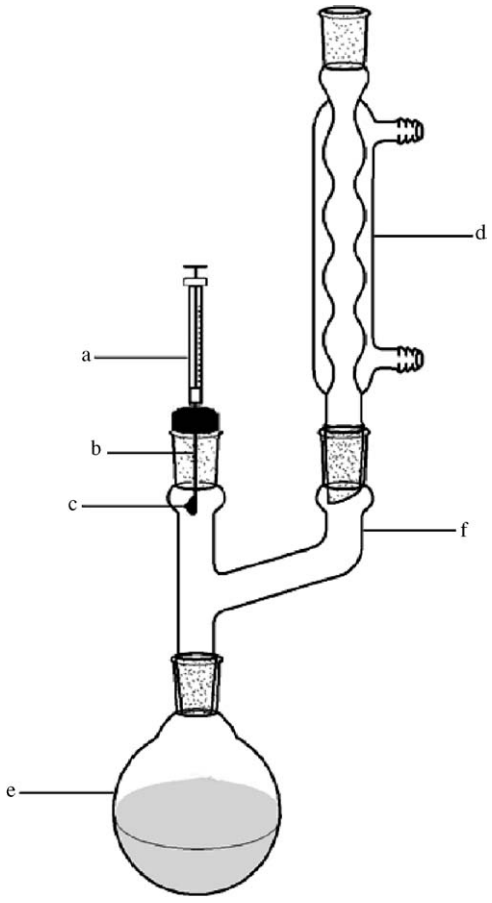

Fig. 1. HD-SPME apparatus: (a) syringe; (b) needle; (c) microdrop; (d) condenser; (e) round bottom flask; (f) Claisen distillation head.

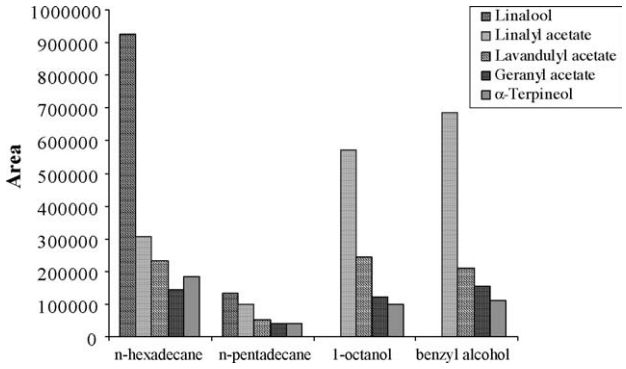

Fig. 2. Effect of various solvents on the extraction efficiency. Extraction condition: sample weight, 2 g; extraction time, 4 min; microdrop volume, 3 μl.

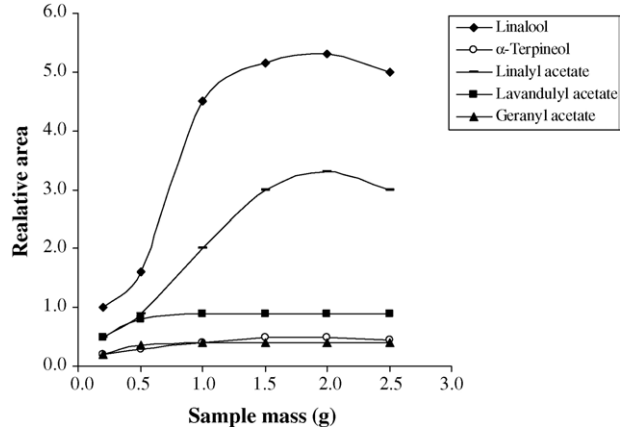

Fig. 3. Effect of sample weight on the extraction efficiency. Extraction condition: extraction time, 4 min; microdrop volume, 3 μl.

mass, microdrop volume and extraction time for the HD-HSME method.

The choice of an appropriate extracting solvent is critical. This solvent should fulfill three requirements: it should not evaporate under the extraction condition (in order to be stable at the extraction period) [24], it should have the ability to extract the analytes efficiently, and finally, the peak due to the solvent should not overlap with the analyte peaks in the chromatogram. In order to find the solvent of choice for extraction of the essential oil of *L. angustifolia* Mill., several solvents including 1-octanol, benzyl alcohol, *n*-pentadecane and *n*-hexadecane were examined. *n*-Pentadecane showed low efficiency. In other hand, 1-octanol and benzyl alcohol peaks interfered with the peak of linalool. However, *n*-hexadecane gave the best extraction efficiency and its peak was well separated from those of the essential oil components, except for caryophyllene oxide and γ-cadinene. Therefore, *n*-hexadecane was chosen as extracting solvent (Fig. 2).

The influence of sample weight on the composition of the extracted compounds was also studied. The results are shown in Fig. 3. The extracted amounts of linalool, linalyl acetate as the major constituents of the oil were increased continuously with the increased sample weight, and then showed a decrease. The reason for this behaviour might be that after saturation of the microdrop with volatile analyte compounds, increasing the sample had no further effect on the mass transfer into the extracting solvent. The observed extraction behaviour might also be related to solubility of the volatile compounds in water. On the basis of these experimental observations, the optimum sample weight was chosen to be 2 g.

The effect of the volume of the microdrop on the analytical signal is shown in Fig. 4. This figure shows that the extrac-

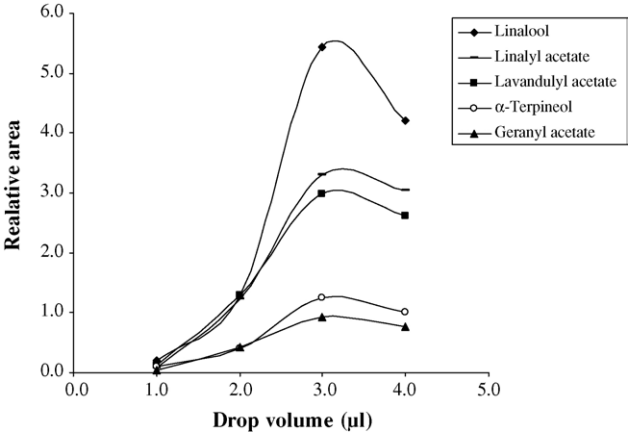

Fig. 4. Effect of drop volume on the extraction efficiency. Extraction condition: sample weight, 2 g; extraction time, 4 min.

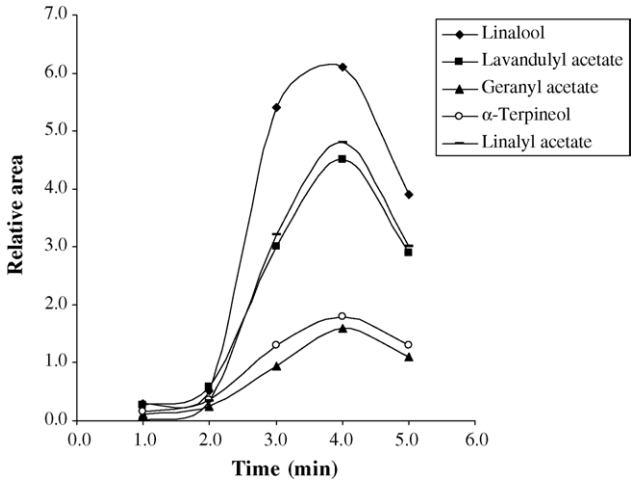

Fig. 5. Effect of extraction time on the extraction efficiency. Extraction condition: sample weight, 2 g; microdrop volume, 3 μl.

tion efficiency increased up to a microdrop volume of 3 μl. A decrease in the analytical signal was observed for all analytes at volume of 4 μl and this can be attributed to insufficient equilibration time, as reported previously [22].

In the HD-HSME method, the amount of extracted analyte is expected to increase with increasing the microdrop exposure time in the headspace of the sample. A plot of relative peak area versus extraction time (Fig. 5) showed that the best results were obtained for an extraction time of 4 min. The observed decrease after 5 min might be attributed to solvent evaporation and also to back-extraction from the microdrop into the headspace [25]. Therefore, an extraction time of 4 min was chosen for further studies.

3.2. HD-HSME of L. angustifolia Mill. oil

The components identified from *L. angustifolia* Mill. oil and their percentages obtained by calculation of the peak area relative to total peak area for conventional hydrodistillation and the proposed HD-HSME methods are presented in Table 1, where compounds are listed in order of their elution from the DB-1 column. Table 1 shows good correlation between the levels of the essential oil constituent determined by the two methods. Thirty-six compounds were extracted and identified. Linalool (32.8%) was found to be the major constituent of the oil extracted by HD-HSME method followed by linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%), geranyl acetate (5.0%), and lavandulol (4.3%). A similar pattern was observed by hydrodistillation with a few exceptions.

The precision of the method performed under optimized conditions (i.e., sample weight, 2 g; extraction time, 4 min; drop volume, 3 μl) was determined by analyzing the samples in triplicate. The precision, expressed as the percentage relative standard deviation (RSD), is included in Table 1. It was observed that RSD values for the main compounds were less than 10%, except for geranyl acetate. The chromatograms obtained for extraction by hydrodistillation and using HD-HSME are shown in Fig. 6.

Table 1
Constituents of the oil of *Lavandula angustifolia* Mill.

Compound	RI[a]	HD[b]	HD-HSME[c]	RSD[d]
Tricyclene	926	tr	tr[e]	–
α-Pinene	936	0.1	tr	–
Camphene	950	0.4	0.1	12.0
1-Octen-3-ol	962	0.4	0.4	18.0
3-Octanone	966	1.0	0.3	13.0
β-Pinene	977	1.4	0.2	21.0
Myrcene	982	0.2	tr	–
Hexyl acetate	995	tr	0.1	–
α-Phellanderene	1003	0.2	0.9	23.0
α-Terpinene	1014	tr	0.3	3.5
p-Cymene	1016	0.3	tr	–
1,8-Cineole	1026	6.7	0.8	4.0
cis-Ocimene	1038	1.3	tr	–
γ-Terpinene	1046	0.5	tr	–
cis-Linalool oxide	1062	0.4	0.7	3.9
trans-Linalool oxide	1077	tr	tr	–
α-Terpinolene	1079	tr	tr	–
Linalool	1090	35.3	32.8	0.2
Chrysanthenone	1110	tr	tr	–
Camphor	1131	1.6	1.9	0.7
Lavandulol	1153	3.0	4.3	8.3
Borneol	1158	3.1	3.8	4.9
Cryptone	1165	tr	tr	–
Terpin-4-ol	1169	tr	tr	–
Hexyl butyrate	1175	tr	nd[f]	–
α-Terpineol	1180	4.2	6.7	4.8
Verbenone	1193	tr	tr	–
trans-Carveol	1204	tr	nd	–
Nerol	1214	0.7	1.0	1.7
Cumin aldehyde	1221	1.6	2.5	10.6
Carvone	1223	0.3	nd	–
Piperitone	1236	2.0	nd	–
Linalyl acetate	1243	13.4	17.6	6.5
Lavandulyl acetate	1273	10.9	15.9	10.0
Bornyl acetate	1276	0.2	tr	–
Neryl acetate	1343	1.2	2.4	17.0
Geranyl acetate	1362	2.5	5.0	17.0
trans-Caryophyllene	1424	1.6	1.5	22.0
α-Santalene	1428	0.3	nd	–
trans-β-Farnesene	1448	1.0	0.5	24.0
γ-Cadinene	1515	tr	nd[g]	–
Caryophyllene oxide	1583	1.9	nd[g]	–
Monoterpene hydrocarbons		4.4	1.5	
Oxygenated monoterpenes		87.5	92.9	
Sesquiterpene hydrocarbons		4.8	2.0	
Oxygenated sesquiterpenes		1.9	–	
Other		3.0	3.3	
Total		99.6	99.7	

[a] Retention indices using a DB-1 column.
[b] Relative area (peak area relative to total peak area) for hydrodistillation method.
[c] Relative area (peak area relative to total peak area except for the solvent peak) for HD-HSME method.
[d] RSD values for HD-HSME method (relative peak area).
[e] Trace (<0.05%).
[f] Compound not detected.
[g] Interfered with the peak of extracting solvent.

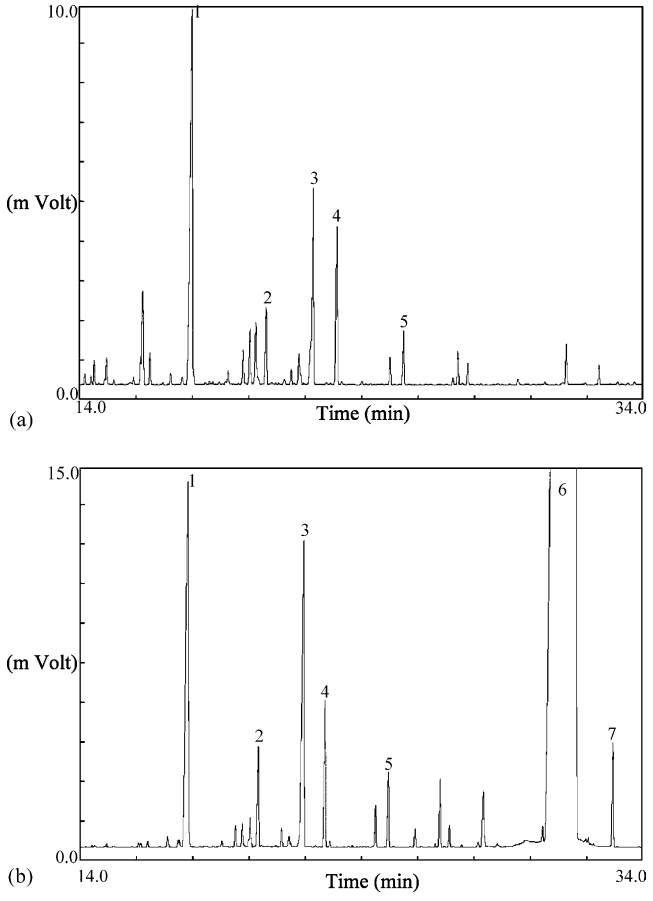

Fig. 6. (a) The FID-chromatogram of *L. angustifolia* extracted by hydrodistillation: (1) linalool, (2) α-terpineol, (3) linalyl acetate, (4) lavandulyl acetate, (5) geranyl acetate. (b) The FID-chromatogram of *L. angustifolia* extracted by HD-HSME at optimum conditions: sample weight, 2 g; extraction time, 4 min; drop volume, 3 μl: (1) linalool, (2) α-terpineol, (3) linalyl acetate, (4) lavandulyl acetate, (5) geranyl acetate, (6) solvent (*n*-hexadecane), (7) internal standard (*n*-heptadecane).

4. Conclusions

This study shows that HD-HSME is a useful technique which complements existing methodologies in the analysis of volatile components in *L. angustifolia* Mill., and for other plant samples in general. The method is environmentally friendly, because only a few microliter of a nontoxic solvent is used. The advantage of this method over HSME applied to dry plant material and without the use of hydrodistillation [20] is that in HSME the more volatile constituents are extracted in preference to less volatile compounds. However, the constituents extracted by HD-HSME are not biased in this way and more truly reflect the real composition of the oil. The general applicability of this new method required further investigation, but it is already apparent that HD-HSME offers some advantages over HS-SPME, such as low cost, absence of a memory effect, good ability for extraction of a wide range of polar and non-polar analytes by changing the choice of the solvent, and reduction of peak tailing [20]. On the other hand, HS-SPME offers the advantage that there is no solvent peak in the chromatogram, therefore splitless injection can be employed and the identification of the volatile compounds in GC–MS chromatogram will be simpler.

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