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Comparison of hydrodistillation-headspace liquid phase microextraction techniques with hydrodistillation in determination of essential oils in *Artemisia Haussknechtii* Boiss

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Abstract

A novel method for extraction and analysis of volatile compounds of *Artemisia Haussknechtii* Boiss, using simultaneous hydro-distillation and headspace liquid microextraction followed by gas chromatography–mass spectrometry (HD-HLPME-GCMS) is developed. Headspace liquid phase microextraction was performed in two modes of static and dynamic. Then, effective parameters of the both optimized. Comparison of these methods with hydro-distillation alone showed that HD-HLPME is fast, simple, inexpensive and effective for the analysis of volatile compounds of aromatic plants. Also, dynamic method represented higher efficiency than static mode. Finally, fifty six compounds were extracted and identified for *Artemisia Haussknechtii* Boiss. by dynamic-HLPME. The main constituents of the essential oil, extracted by this method, includes camphor (40.83%), 1,8-cineole (26.84%), cis-davanone (4.77%), linalool (4.44%), 4-terpineol (3.62%), beta-fenchyl alcohol (3.52%), borneol (2.87%) and comphene (1.34%).

Keywords: Hydrodistillation (HD); Hydrodistillation-Static Headspace Liquid Phase Microextraction (HD-SHLPME); Hydrodistillation-Dynamic Headspace Liquid Phase Microextraction (HD-DHLPME); *Artemisia Haussknechtii* Boiss.; Essential oil; Gas Chromatography-Mass Spectrometry (GC-MS)

Introduction

As the sophisticated analytical instruments are not capable of handling sample matrices directly, a sample preparation step is required. Sample preparation is a critical step in an analytical procedure. The main aim of sample preparation, is to transfer the analyte into a form that is prepurified, concentrated, and compatible with the analytical system (Shen & Lee 2003). In analytical chemistry, the trend is toward simplification and miniaturization of the sample preparation step, and a decrease in the quantities of organic solvents used The desire to reduce the time and the quantities of organic solvents needed for the extraction has led to the development of new extraction approaches such as headspace sampling (HSS) (Kolb 1999) and liquid phase microextraction (LPME) (Wood *et al.* 1997). Generally, there are two different modes of LPME: static LPME consisted of a microdrop suspended at the tip of a microsyringe needle and dynamic LPME, in which the microsyringe is used as a separatory funnel and featured the repeated movement of the syringe plunger (Psillakis & Kalogerakis 2002, He & Lee 1997, Pedersen-Bjergaard & Rasmussen 2005, Palit *et al.* 2005, Ho *et al.* 2002, Jeannot & Cantwell 1996). Headspace microextraction (HSME) is a combination of HSS and LPME, and has the high capabilities of them. It is a novel method that can be used for sample preparation in chromatographic analysis (Yamini et al. 2004, Przyjazny & Kokosa 2002, Ouyang *et al.* 2005).

Essential oils (EOs) (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant materials. The greatest use of EOs is in food (as flavourings), perfumes and pharmaceuticals (for their functional properties) (Burt 2004). To achieve the best possible separation performance for essential oil analysis, we used gas chromatography-mass spectrometry (GC–MS) technique along with improved data handling. However, the extraction of essential oils still is a problem from the point of view of duration and also amount of plant material needed. The most common method of extraction, hydro-distillation needs about 3-4 hours and tens to hundreds grams which in some situation is problematic, especially in cases that enough plant material is not accessible.

The genus *Artemisia* (Compositae), with the common Persian name of ‘dermane’, includes 34 species that can be found as wild plant all over Iran of which two are endemic (Mozaffarian 1996,

Ahmadi *et al.* 2002). In the present work chemical composition of *Artemisia Hassknechtii Boiss.*, which was collected from Yazd province in Iran and extracted by hydro-distillation is reported (Table 1).

Experimental

Reagents and material. The plant material of *Artemisia haussknechtii Boiss.*, were collected from Yazd province in the center of Iran, in April 2006. Chemicals, such as *n*-heptadecane, *n*-hexadecane, *n*-hexane, *n*-pentadecane, *n*-dodecane, 1-dodecanol, hexamethylene diisocyanate, *n*-pentane, dichloromethane and sodium sulphate with the purity higher than 99% were purchased from Merck chemical company. Normal alkanes' standards (C-6 to C-18) purchased from ULTRA Scientific, North Kingstown, USA.

Instrumentation and analysis. GC analyses were carried out using a Shimadzu-17A gas chromatograph equipped with a flame ionization detector (FID) and a DBP-5 capillary fused silica column (25 m, 0.25 mm I.D.; 0.22 µm film thickness). The oven temperature was held at 40 ºC for 1 min then programmed at 3 ºC/min to 250 ºC, held for 10 minutes. Other operating conditions were as follows: carrier gas, He (99.999%); inlet pressure, 103 kPa; with a linear velocity of 28.8 cm/s; injector temperature, 250 ºC; detector temperature, 250 ºC; split ratio, 1:50. GC-MS analyses were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (60 m, 0.25 mm I.D., 0.25 µm film thickness). The oven temperature program initiated at 40 ºC, held for 1 minute then raised at 3 ºC/min to 250 ºC, held for 20 minutes. Other operating conditions were as follows: carrier gas, He (99.999%); with a flow rate of 1 mL/min; injector temperature, 250 ºC; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was from 20–500 amu. Both processes of the extraction of essential oil and the injections into GC and GC-MS were carried out using one 10 µl micro-syringe model ITO MICRO SYRINGE MS-E10 with the needle tip of angled cut (to facilitate piercing of the septum in the headspace and also the GC injection port). Hydrodistillation was performed in a full glass Clevenger-type apparatus as recommended by British Pharmacopeia.

Identification of essential oil constituents. The components of the essential oils were identified by comparing of their retention indices and mass spectra fragmentation patterns with those stored on the Wiley7n.l MS computer library built up using pure substances or with authentic compounds and confirmed by the comparison of their retention indices. The Kovats' retention indices of all the constituents were obtained using gas chromatograms by interpolation between bracketing *n*-alkanes (Oprean *et al.* 1998, Oprean *et al.* 2001). The homologous series of *n*-alkanes (C-6 to C-18; ULTRA Scientific, Inc; North Kingstown, USA) were used as standards. An Enhanced ChemStation G1701 DA version D.00.01.27 was used for the data collection and processing.

Isolation of essential oil by Hydro-distillation. Hydro-distillation is the most common method for the extraction and isolation of essential oils from aromatic plants. The botanical materials of *Artemisia haussknechtii Boiss.* were dried under shade at room temperature for 48 hours and 50 g of aerial parts of it i.e. the leaves and fine stems were separated and ground, then fully submerged in water in a 1 liter round bottom flask and hydro-distilled in a full glass Clevenger-type apparatus as recommended by British Pharmacopeia's, giving transparent light yellow oils. The distillation prolonged for 3.5 hours. When the system cooled down, the water and the oils were separated. The oils decanted to be used as essential oils. To improve the recovery and the analysis, the essential oils were taken up in *n*-pentane (Merck), dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a dark glass bottle at 4 °C prior to GC and GC-MS analyses. The extraction yields for the essential oils of *Artemisia haussknechtii Boiss*. was 2.41%.

Hydro-distillation static headspace liquid microextraction-gas chromatography–mass spectrometry. There are three phases involved in the extraction process: aqueous sample mixture, headspace, and organic microdrop acceptor phase. The apparatus that has been designed in our laboratory is a developed one that used by Tellez et al. (Tellez *et al.* 2004) and shown in Figure 1. In this experiment, 2.5 grams of the dried and ground plant was immersed in 50 mL water in a 100 mL round-bottom flask and heated to boil using a heating mantle. A suitable and clean micro-syringe was used

to uptake 1µl of *n*-heptadecane containing 200-ppm *n*-pentadecane as internal standard. The needle of the micro-syringe was then inserted into the headspace of hydro-distilling plant sample through a septum. Waiting 5 min after the reflux began, to reach a steady state, the micro-syringe plunger was depressed and a microdrop of extracting solvent was suspended from the needle tip, and the extraction was started. After an optimized period of time (4 min), the plunger was withdrawn back 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. Then the relative peak areas of components with respect to the internal standard (*n*-pentadecane) were calculated.

Table 1 - Components of *Artemisia Haussknechtii Boiss.* extracted by HD and HD-SHLPME methods.

No.	R. T.	Compound	[a]RI	[b]HD%	[c]HD-SHLPME %	[d]RSD
1	9.359	Methyl isopropenyl ketone	620	0.225	tr	
2	16.406	2-Hexenal	820	0.018	tr	
3	20.117	Tricyclene	895	0.169	0.187	
4	20.293	alpha-Thujene	899	0.116	0.030	
5	20.751	alpha-Pinene	907	2.589	0.648	24.08
6	21.608	Comphene	924	4.232	1.344	16.90
7	21.824	Verbenene	928	0.090	0.010	24.39
8	22.816	Sabinene	946	0.973	0.561	13.54
9	23.067	beta-Pinene	951	0.985	0.499	25.17
10	23.548	beta-Myrcene	960	0.273	0.343	13.19
11	23.694	2,3-Dehydro-1,8-cineole	963	0.323	0.069	
12	25.085	alpha-Terpinene	989	0.691	0.285	27.67
13	25.587	Cymene	998	0.469	0.326	11.70
14	25.836	Limonene	1003	0.133	0.156	11.77
15	26.116	1,8-Cineole	1008	31.401	26.841	10.57
16	26.499	Benzene acetaldehyde	1016	0.050	0.046	18.19
17	27.295	gamma-Terpinene	1031	1.105	0.626	24.97
18	27.752	cis-beta-Terpineol	1040	0.365	1.211	26.74
19	28.844	alpha-Terpinolene	1060	0.282	0.267	15.23
20	29.092	Rosefuran	1065	0.045	0.045	22.38
21	29.399	Linalool	1071	1.556	4.442	20.74
22	30.364	beta-Thujone	1089	0.022	0.054	30.62
23	30.737	1-Terpineol	1096	0.234	0.304	
24	31.867	trans-Pinocarveol	1119	0.710	0.638	9.76
25	32.24	Camphor	1126	32.702	40.830	15.65
26	32.861	Pinocarvone	1139	0.429	0.603	11.81
27	33.034	Borneol	1142	2.417	2.870	11.09
28	33.58	4-Terpineol	1153	3.002	3.622	16.92
29	34.166	beta-Fenchyl alcohol	1165	1.938	3.519	11.75
30	34.453	Myrtenol	1170	0.284	0.297	13.07
31	34.538	Myrtenal	1172	0.219	0.245	13.55
32	34.89	Terpinene-3-ol	1179	0.061	0.133	39.82
33	35.157	Verbernone	1184	0.055	0.108	
34	35.4	trans-Carveol	1189	0.108	0.227	10.33
35	36.363	Z-Citral	1208	0.035	0.063	11.25
36	36.536	alpha-Terpinene	1212	0.115	0.068	
37	36.699	Carvone	1215	0.217	0.327	8.00
38	36.843	Geraniol	1218	0.102	0.148	27.98
39	37.411	Chrysanthenyl acetate	1230	0.067	0.258	12.99
40	37.709	E-Citral	1236	0.033	0.123	
41	38.668	Borneol acetate	1255	0.455	0.636	15.82
42	41.825	Eugenol	1322	0.056	0.072	1.24
43	42.672	Geranyl acetate	1342	0.100	0.292	
44	42.773	Methyl cinnamate	1344	0.068	0.118	
45	43.734	cis-Jasmone	1367	0.188	0.313	10.89
46	44.998	trans-Caryophyllene	1396	0.216	0.135	12.04
47	47.484	Davana oil	1454	0.363	0.140	28.69
48	47.571	Germacrene D	1456	0.319	0.131	36.95
49	48.209	Bicyclogermacrene	1471	0.236	0.078	21.34
50	48.308	Davana ether	1474	0.731	0.214	30.26
51	50.393	Farnesol	1524	0.062	0.114	26.25
52	51.553	cis-Davanone	1554	7.461	4.774	29.66
53	51.852	Caryophyllene oxide	1562	0.122	0.252	26.72
54	52.706	Artemisia ketone	1583	0.438	0.157	20.88
55	53.041	E-sesqui-lavandulol	1592	0.129	0.084	29.21
56	53.791	Methyl jasmonate	1611	0.214	0.102	45.53

[a] Retention indices using a HP-5MS column.
[b] Relative area percent (peak area relative to total peak area) for hydrodistillation method.
[c] Relative area percent (peak area relative to total peak area except for the solvent peak) for HD-SHLPME method.
[d] Relative Standard Deviation values for HD-SHLPME method (relative peak area).
[e] Trace (<0.01).

Hydrodistillation-dynamic headspace liquid phase microextraction of essential oil. The apparatus used to perform HD-DHLPME is shown in Figure 1. The sample weight of 2.5 gr was chosen according to the optimized sample mass in the static LPME.Therefore, 2.5 gr of the dried and ground plant immersed in 50 mL water in a 100 ml round-bottom flask and was heated to boil using a heating mantle. The extraction process begins with withdrawal of 1.0 µl of *n*-hexane containing 200 ppm *n*-heptadecane as internal standard into the clean microsyringe. Then passing the microsyringe needle through the septum into the headspace of hydro-distilling sample flask and keeping the needle suspended over the liquid sample (Fig. 1). Waiting 5 min, after the reflux began, to reach steady state and then withdraw 4µl of gaseous sample at the rate of 1µl/s, then depress the plunger back to the original mark immediately, and hold for 5 s. The same process was repeated 25 times. Finally, the syringe needle from the flask was removed and the essential oils trapped in the organic solvent was injected into the GC for the analysis.

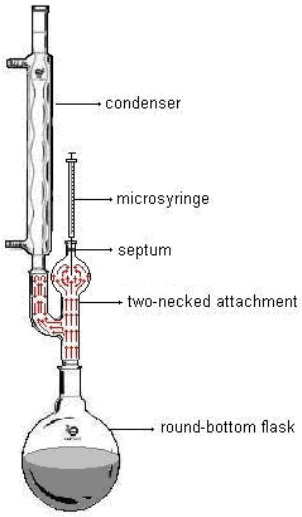

Figure 1. The HD-DHLPME apparatus with schematic diagram of flowing vapor.

Results and Discussion

Optimization of HD-SHLPME: parameters affecting the extraction. There are some parameters such as extracting solvent, extraction time, sample weight and microdrop volume which directly affect the results of the extraction and control the optimum performance. In the present study, we tried to optimize these parameters. All quantifications made were based on the relative peak area of the analytes to the internal standard (*n*-pentadecane).

Nature of extracting solvent. Considering the principle `like dissolves like', several important criteria such as ability to extract the components of essential oil, stability of the drop under the experiment conditions, low volatility, selectivity, extraction efficiency, a favorable partition coefficient for volatile components, low volatile impurities, rate of drop dissolution, non-overlapping peak with sample components in GC, and level of toxicity should be met (Fang *et al.* 2006, Besharati-Seidani *et al.* 2005, Cao *et al.* 2006, Mohammadi & Alizadeh 2006). Taking these into account, several different solvents with a diverse range of polarity such as *n*-heptadecane, *n*-hexadecane, *n*-pentadecane, *n*-dodecane, 1-dodecanol and hexamethylene diisocyanate were examined. According to the results shown in Figure 2, after a detailed comparison, *n*-heptadecane was selected as the extraction solvent.

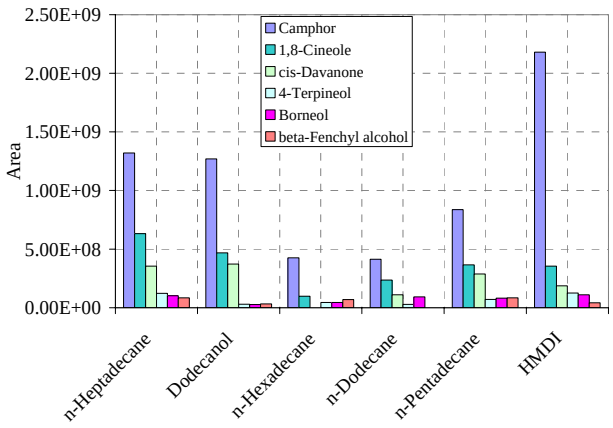

Figure 2. Relative extraction efficiency for different solvents.

Optimization of the extraction time. The SHLPME is not an exhaustive extraction method and the analyte partitions among the bulk sample phase, the headspace and the microdrop.However, complete equilibrium is not needed for accurate and precise analysis. Only when sufficient mass is transfered into the microdrop in an exact reproducible extraction time is adequate. Figure 3 shows an increase in extraction with sampling time in the range of 2-4 min, and decreasing after 4 min.This decrease can be attributed to the solvent evaporation and to the back-extraction from the microdrop into the headspace (Fakhari *et al.* 2005). As prolonged

extraction times, may result in drop dissolution and drop loss. However, when an extraction time is chosen in the rising portion of the profile, choosing precise timing becomes essential for a good precision. Based on Figure 3, an exposure time of 4 min was selected in this work.

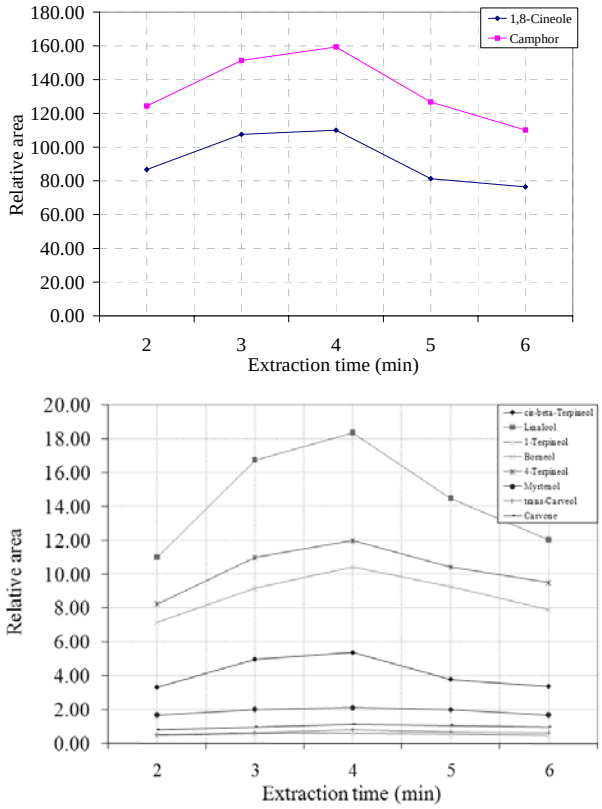

Figure 3. Effect of extraction time on the efficiency. Extraction conditions: sample weight, 2.5 g; microdrop volume, 1.0 µl.

Optimization of the sample weight. Increasing sample weight should enhance the extracted analytes in the drop (Psillakis & Kalogerakis 2001). The effect of sample weight on the extraction efficiency is shown in Figure 4. It can be seen from the figure that the extracted analytes has increased continuously with increasing the sample size from 0.5 to 2.5 g, and then is decreased. This is due to the fact that after saturation the microdrop with volatile analyte compounds, increasing the sample has no further effect on the mass transfer into the extracting solvent. This behavior might also be due to the solubility of the volatile compounds in water and the microdrop vaporization. As a result, the sample weight of 2.5 g was used for the extraction in this work.

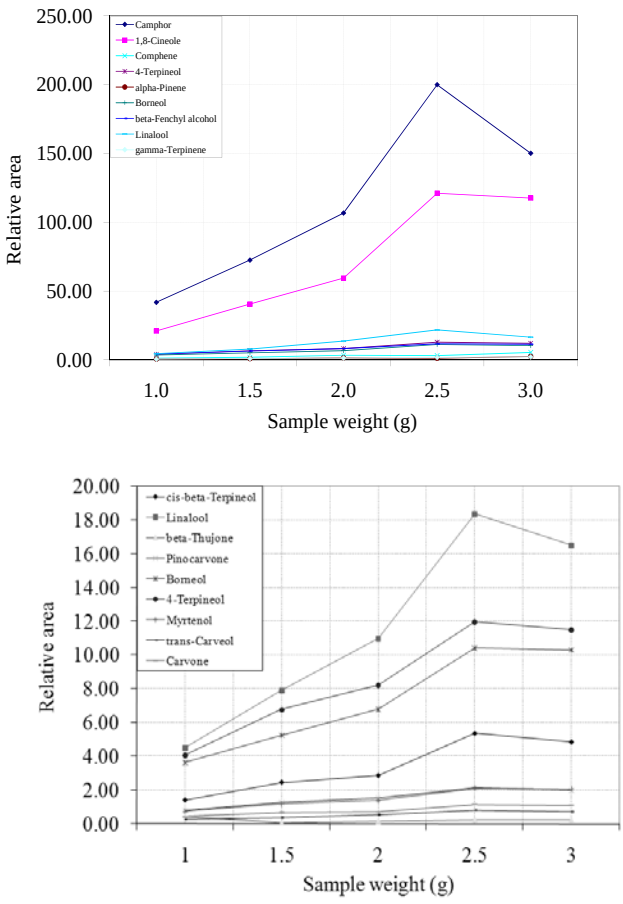

Figure 4. Effect of sample weight on extraction efficiency. Extraction conditions: microdrop volume, 1.0 µl; extraction time, 4 min.

Optimization of the microdrop volume. It is important to find a suitable solvent volume for both the extraction and the chromatographic analysis. Solvent volumes from 0.5 to 2.0 µl were tested and the results are shown in Figure 5. It can be seen that the peak area of the analyte has increased with increasing solvent volume from 0.5 to 1.0 µl and then has decreased. The decrease after 1.0 µl can be attributed to the insufficient equilibration time. On the other hand, large injection volumes bring about bigger band broadening in capillary GC (Marriotta *et al.* 2001). Taking these into consideration, a drop volume of 1.0 µl was chosen in the present contribution.

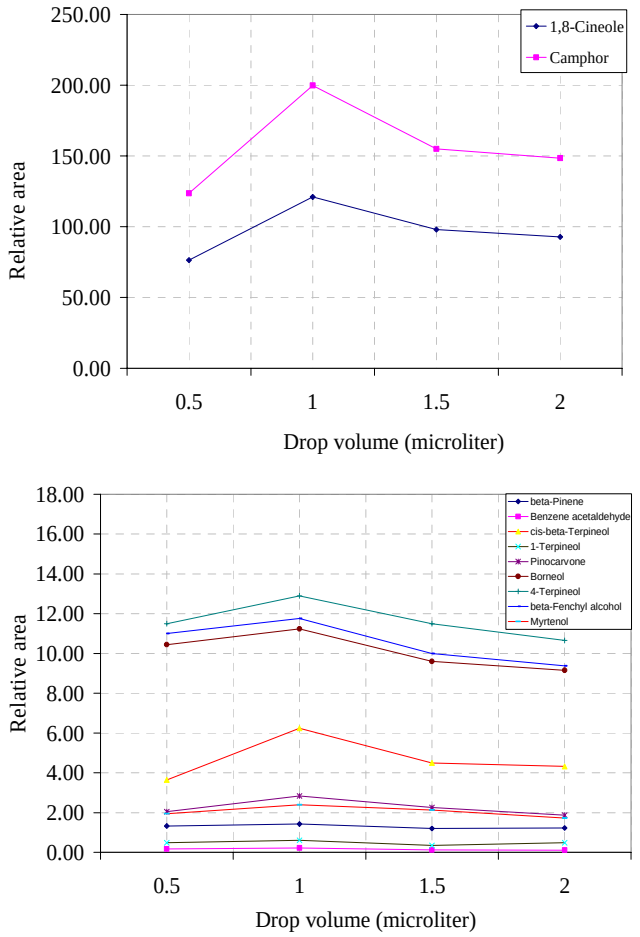

Figure 5. Effect of drop volume on the extraction efficiency. Extraction conditions: sample weight, 2.5 g; extraction time, 4 min.

Optimization of HD-DHLPME parameters. There are some parameters such as the extracting solvent, sampling volume, solvent volume, extraction cycle and the withdrawal rate, which directly affect the results of the extraction and control the optimum performance. In the present study, we have optimized these parameters. All quantifications made were based on the relative peak area of the analytes to the internal standard (*n*-heptadecane).

Selection of extracting solvent. In addition to the criteria for selecting a solvent mentioned in the section 3.1.1, the extraction has a relation to the partition coefficient and the film formation which are controlled by the characteristics of the solvent (e.g. the solvent viscosity, surface tension, etc.). Taking these into account, in order to choose the best solvent and to obtain the optimum extraction yield, several solvents with a range of polarity and vapor pressure such as *n*-heptadecane, *n*-hexadecane, *n*-hexane, *n*-pentane and dichloro-methane were examined.

Figure 6 shows that, among the tested solvents, *n*-hexane offered the highest extraction efficiency and produces a nonoverlapping peak at the start of chromatogram, thus was finally adopted as the extraction solvent. The other solvents showed lower extraction efficiency and/or broad overlapping peaks.

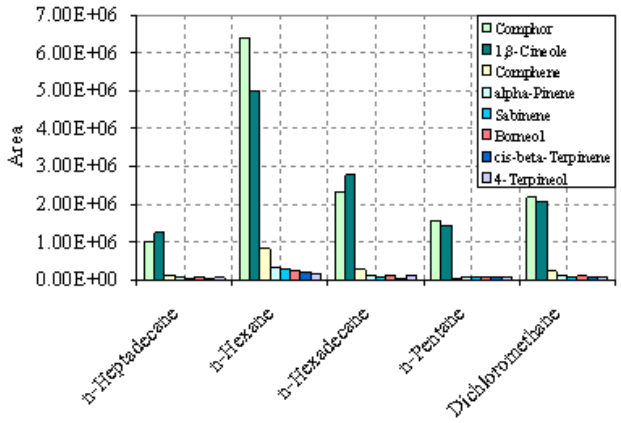

Figure 6. Effect of various solvents on the extraction efficiency. Extraction conditions: sample weight, 2.5g; extraction cycles, 25; sampling volume, 4 µl, solvent volume, 1 µl; withdrawal rate, 1µl/s.

Optimization of sampling volume The sampling volume is referred to the volume of gas phase sample (GPS) that is withdrawn into the microsyringe (Liang *et al.* 2006). In this work, five different sampling volumes; 2.0, 3.0, 4.0, 5.0 and 6.0 µl were tested (Fig. 7). V_{gps} cannot be increased indefinitely, since it is limited by the volume of the microsyringe, and also difficulty of manually manipulating the plunger repeatedly. As can be seen, the efficiency increases almost linearly with V_{gps} in the range of 2-4 µl and then decreases. It can be concluded that by increasing the sampling volume, a larger OSF will form in the barrel, and extraction efficiency increases in this range Sampling volume larger than 4 µl causes the concentration of compounds in the organic solvent plug (OSP) decreases. This decrease may be attributed to the vaporization of organic solvent in the microsyringe due to large V_{gps} of hot gaseous sample which withdrawn in it, since the high temperature in the vial would enhance the loss of solvent. The larger the sampling volume, the slope of decrease becomes faster. Considering the above explanations, a sample volume of 4.0 µl, was selected for subsequent experiments.

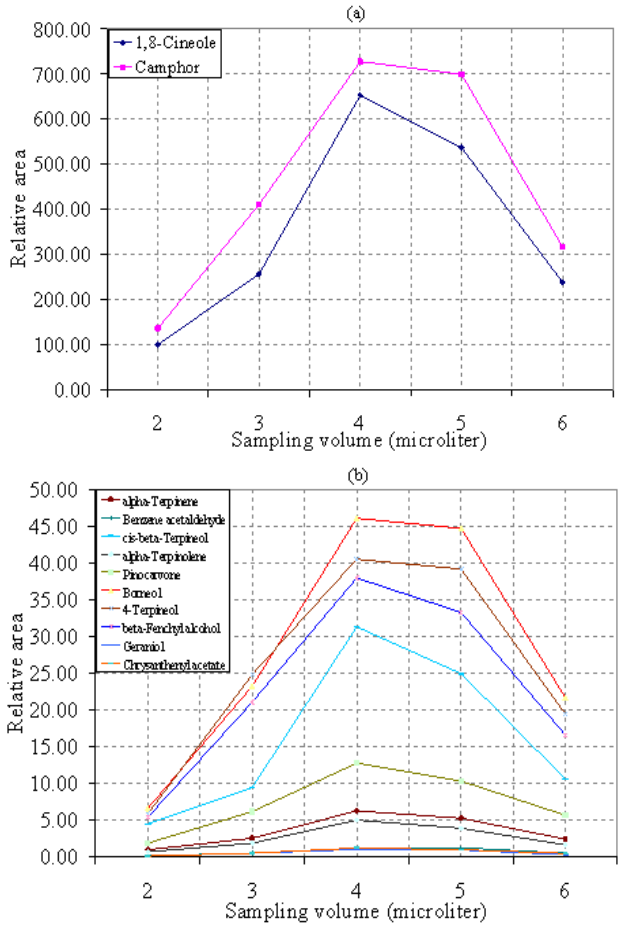

Figure 7. Effect of sampling volume on the extraction efficiency. Extraction conditions: solvent volume, 1.0 µl; withdrawal rate, 1.0 µl/s; extraction cycles, 25

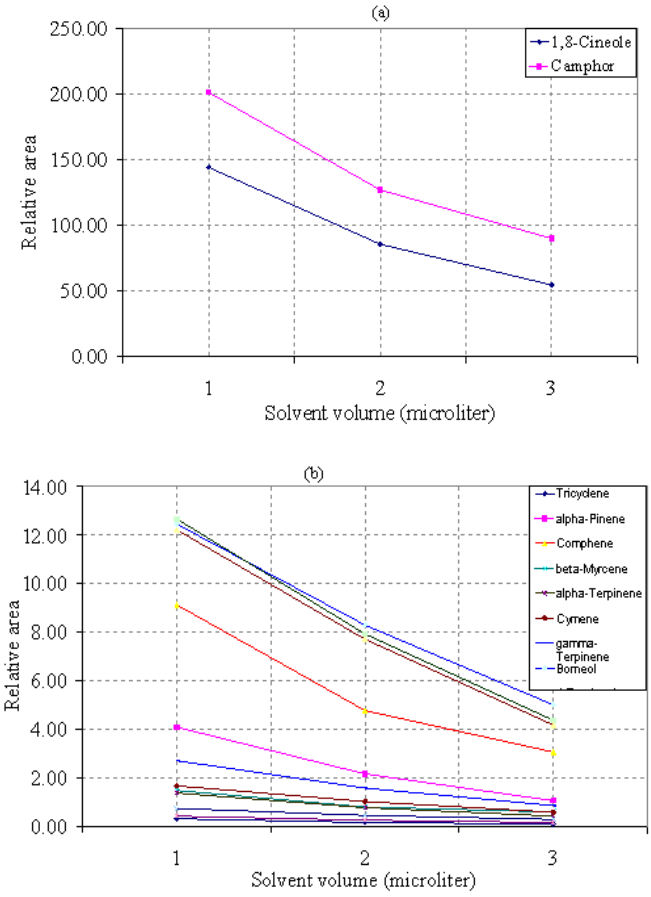

Figure 8. Effect of solvent volume on the extraction efficiency. Extraction conditions:sampling volume, 4.0 µl; syringe withdrawal rate, 1.0 µl/s; extraction cycles, 25.

Optimization of solvent volume. In the present experiment, solvent volumes of 1-3 µl were tested. As shown in Figure 8, the amount of analytes extracted is inversely proportional to the solvent volume. Although the smaller solvent volumes gave higher extraction efficiencies, but it was impossible to use a solvent volume smaller than 1µl, from the practical viewpoint, since this is the injection volume. Therefore, in the present work, an optimum solvent volume of 1µl was chosen which is good for GC injection, because large volume injections result in peak broadening in capillary GC.

Optimization of syringe (plunger) withdrawal rate. The extraction efficiency greatly depends on how fast the plunger in the syringe barrel moves (Wang *et al.* 1998). When the plunger is withdrawn, a very thin organic solvent film is formed on the inner surface of microsyringe barrel and simultaneously, sample headspace is drawn in. The analytes in the gaseous phase equilibrate between the organic film and the gaseous sample. In the present experiment, the withdrawal rates of 0.5, 1, 2 and 4 µl/s were investigated. As shown in Figure 9, although the OSF formed at 0.5 µl/s was supposed to be thinnest, and the time available for the compounds to reach partitioning equilibrium was the longest, the extraction efficiency was relatively low. This may be due to the partly loose of OSF by solvent evaporation because the plunger movement is slow (8 s duration at 0.5 µl/s). On the other hand, at low plunger moving speed (less than 1.0 µl/s), a weak enrichment of all analytes was obtained. The maximum peak area of all analytes was obtained at

the speed of 1.0 µl/s. Also, with faster plunger movement, such as 2 and 4 µl/s, the extraction efficiency was low. The possible reasons are: (a) the fast movement made the OSF thicker, but the exposure time to reach the equilibrium condition is short, and (b) a heterogeneous OSF might have been formed by a fast plunger movement, which would affect the extraction efficiency (Saraji 2005). Therefore, the withdrawal rate was fixed at 1.0 µl/s for the experiment.

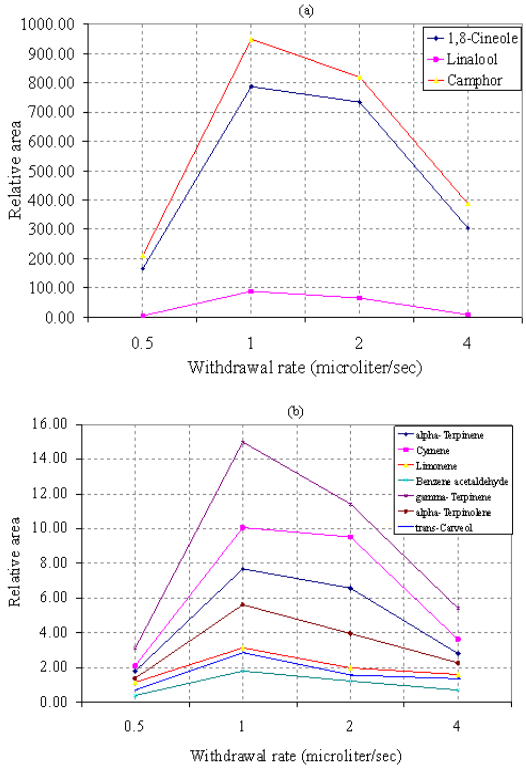

Figure 9. Effect of syringe withdrawal rate on the extraction efficiency. Extraction conditions: sampling volume, 4.0 µl; solvent volume, 1.0 µl; extraction cycles, 25.

Optimization of number of sampling. The key operation step in dynamic LPME is, repeating the movement of the plunger. The experiments carried out in 10, 15, 20, 25, 30 cycles. Higher extraction cycles would lead to poorer precision because the procedure was manually controlled. As shown in Figure 10, the amount of extracted analyte for all components has increased with increasing the number of extraction cycles (*n*) in the range of 10-25. After 25 cycles, almost 50 % of the extracted components (low molecular weight) has decreased (Fig. 10) and the amounts of the others have increased (higher molecular weight) (Fig. 10c). This can be due to increasing the temperature of the solvent in the barrel and evaporating low molecular weight compounds. In higher cycles, evaporation of the solvent in the needle and the barrel takes place more rapidly, because it remains longer at the exposure of the hot gaseous sample. Thus the extraction efficiency decreases. Here, we chose 25 extraction cycles which took 4.5 min.

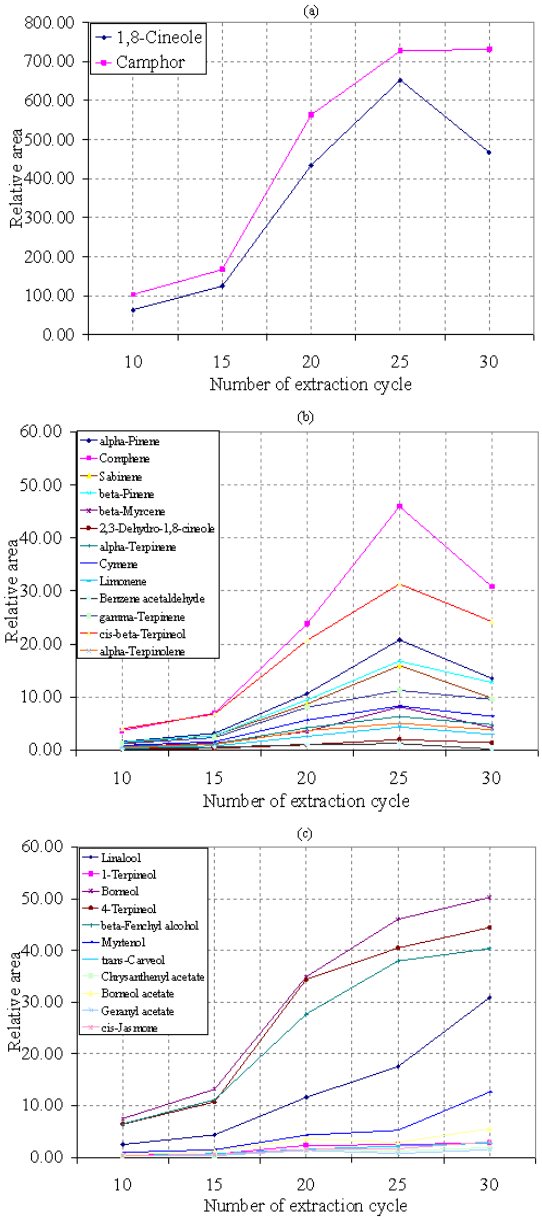

Figure 10. a, b and c- Effect of number of extraction cycle on the extraction efficiency. Extraction conditions: sampling volume, 4.0 µl; withdrawal rate, 1.0 µl/s; solvent volume, 1.0 µl.

Comparison of hydrodistillation with static and dynamic HD-LPME. Fifty six components were obtained for the essential oil of *Artemisia Haussknechtii Boiss.*, by HD-SHLPME-GCMS method in this work (Table 1). Retention indices using HP-5MS column together with the relative areas obtained for HD and HD-SHLPME are shown in this table. Comparing the results that have been

obtained by these two methods showed high efficiency of the latter. Obvousely, by HD-SHLPME not only all of the components of essential oil has been extracted but also the amounts of 29 compounds obtained are larger than those by HD method. The process has been done in a short period of time (4.0 min.) and consumed small amounts of plant material (2.5 gr.). But the HD method took a long period of time (3.5 hr.: 52.5 times longer) consuming a large amount of plant material (50 gr.: 20 times larger). Therefore, HD-SHLPME is much more efficient than HD which is the most common method of extracting essential oils. For comparison purposes, the chromatograms obtained for the extracted components of the essential oils using hydro-distillation and HD-HLPME are shown in Figure 11.

The RSD for the main components of the plant (camphor and 1,8-cineole) is between 17-19%, which is relatively satisfactory, but for the some minor components is not as good as for the main components. There could be five possible reasons for this discrepancy. First, the partition equilibrium has not been reached in the experiments; therefore, a precise timing is essential for a good precision. Second, the organic drop evaporates slightly with increasing the exposure time. Third, the plunger was stopped based on visual inspection when the organic drop was withdrawn back into the syringe after sampling was completed. This conceivably contributed to the error. Fourth, since the sample boils and the microdrop is exposed to a hot flowing steam, it is not as stable as ordinary LPME, and the microdrop evaporates more rapidly. Fifth, the turbulent flow in the bubble headspace, increases extraction efficiency, however, results in higher RSDs. Also the extraction efficiency for hydro-distillation and HD-DHLPME has been compared graphically in Figure 12. Obviously, the efficiency of the HD-DHLPME method is good and comparable with the results obtained by hydro-distillation. Figure 13 shows the results obtained by HD-DHLPME and static LPME (HD-SHLPME) under the same conditions. The results of HD-DHLPME method is superior over those obtained by HD-SHLPME.

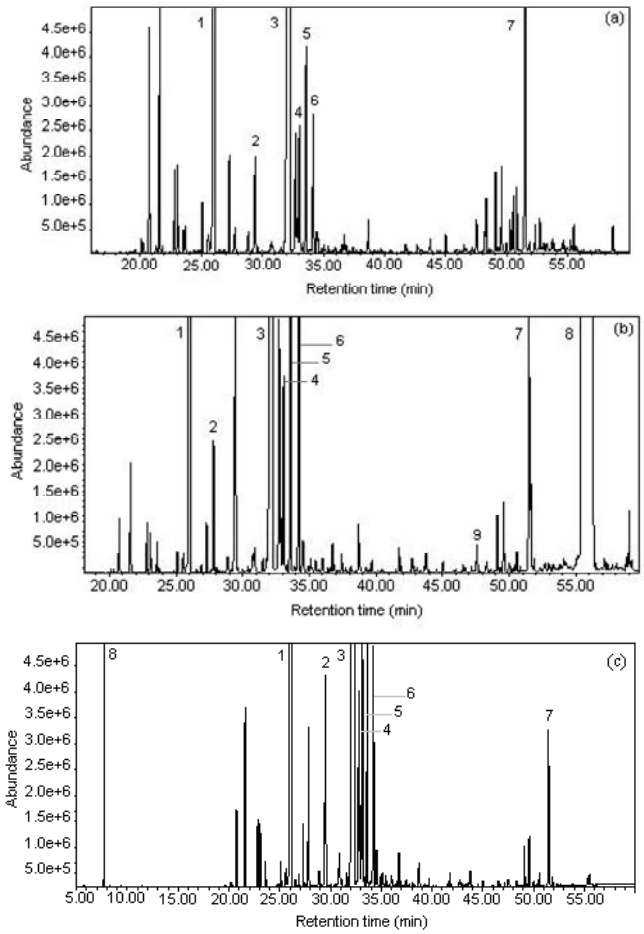

Figure 11. Total Ion Chromatograms of essential oil components of *Artemisia Haussknechtii Boiss.*, extracted by a) hydro-distillation, b) HD-SHLPME, c) HD-DHLPME 1) 1,8-Cineole, 2) Linalool, 3) Camphor, 4) Borneol, 5) 4-Terpineol, 6) *beta*-Fenchyl alcohol, 7) *cis*-Davanone, 8) Solvent, and 9) Internal standard.

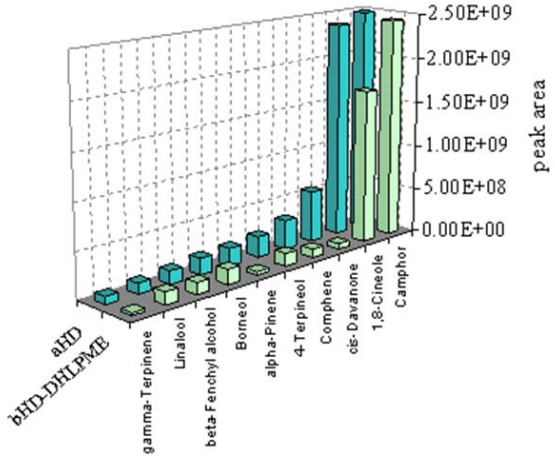

Figure 12. Comparison of extraction efficiency resulted by methods HD and HD-DHLPME.

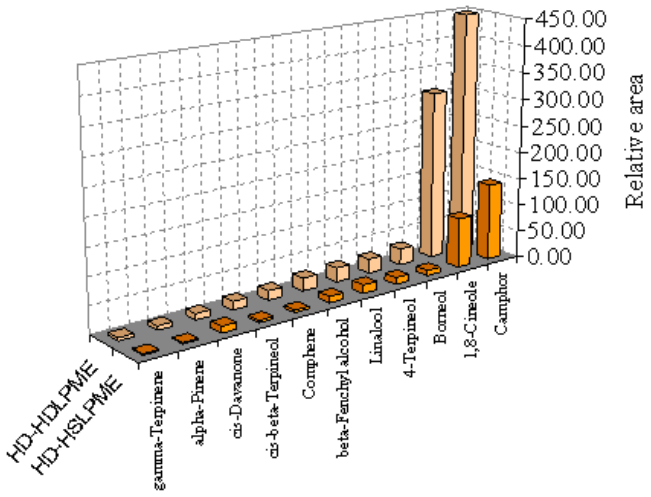

Figure 13. Comparison of relative peak areas for HD-SHLPME and HD-DHLPME methods.

Conclusions

The method of HD-SHLPME-GC-MS, which has been used in this work for analysis of the components of *Artemisia Haussknechtii Boiss.*, is a combination of hydro-distillation and static headspace liquid phase microextraction via headspace sampling followed by GC-MS analysis. The aim of this study was to offer a fast, low cost, facile, low consuming plant material and efficient method for analysis of the essential oils. In comparison with the hydro-distillation (HD) which is time consuming and needs large amount of botanical material, the proposed method offers advantages such as: (1) high extraction speed; (2) does not need a large amount of plant material; (3) inexpensive apparatus; (4) simplicity, (5) low consumption of a nontoxic solvent, and (6) small injection volume. Comparing the results of the two methods, it is obvious that most of the components of essential oil has been determined using HD-SHLPME. Therefore, it can be considered as a useful method for fast analysis of essential oils.

Increasing the extraction efficiency for the method presented in this work because of turbulent flow of gas phase sample in the headspace, allows using small volumes of the microdrop, which is enriched of analytes in a short time. Therefore, problems due to large volume injection in GC system will be removed, the extracted compounds would be increased and the trace components can be identified.

The method of HD-DHLPME-GCMS which is a combination of hydro-distillation and dynamic headspace liquid phase microextraction followed by GC-MS analysis was described here for determination of the essential oils. In fact, this is the first time that dynamic LPME has been employed under the condition of boiling water using an extracting organic solvent with a relatively low boiling point. The extraction in a very small volume of the microsyringe (about 4 µl) protects the solvent from evaporation and makes the choice of the extraction solvent for the present method very flexible.. In comparison with hydro-distillation (HD), method offers advantages such as: (1) high extraction speed; (2) consuming very small amount of plant material; (3) inexpensive and simple apparatus; (4) low consumption of a nontoxic solvent, (5) the possibility to use the solvents with high vapor pressures, (6) fast equilibrium between gaseous analytes and organic solvent film; (7) high enrichment factor and detection of trace compounds; (8) clear chromatogram with sharp and resolved peaks, without interference from the solvent peak and (9) feasibility of analysis with 1µl injection, so eliminating undesirable effects due to large volume injections to the GC system. In **c**omparison with the static method (HD-SHLPME), efficiency of HD-DHLPME is considerably higher (Fig.13). On the other hand, for the essential oils, a larger number of components have been determined by this method. Finally, it is shown that HD-DHLPME method is more efficient and useful from the viewpoint of time and size of the material consumed. moving the plunger of microsyringe automatically, improves the reproducibility of HD-DHLPME method.

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