CHEMIJA. 2012. Vol. 23. No. 1. P. 24–29

Comparative study of hydrodistillation headspace solvent microextraction and microwave-assisted distillation headspace solvent microextraction for analysis of volatile components in *Stachys inflata*

Mohammad Bagher Gholivand[1]*,

Mir Mahdi Abolghasemi[1, 2],

Marziye Piryaei[1],

Seyed Mohamad Maassoumi[3]

[1] *Department of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran*

[2] *Faculty of Chemistry, Maragheh University, Maragheh, Iran*

[3] *Department of Plant Protection, Agricultural College, Razi University, Kermanshah, Iran*

In this work, three different methods: hydrodistillation (HD), hydrodistillation headspace solvent microextraction (HD-HSME) and microwave-assisted distillation headspace single-drop microextraction (MA-HSME) have been applied for the extraction of volatile components of the *Stachys inflata Benth*. The effect of experimental parameters such as solvent selection, microdrop volume, microwave power and sample amount on MA-HSME and HD-HSME were investigated, and the methods precision was studied. The obtained extracts were analyzed by GC-MS (identification and determination of components), and the experimental results were compared. Thirty-five volatile compounds present in *Stachys inflata Benth* were identified by using the proposed methods.

The experimental results showed that MA-HSME is a rapid, reliable, simple and solvent-free technique for the determination of volatile compounds in *Stachys inflata Benth*.

Key words: microwave-assisted hydrodistillation headspace solvent microextraction, hydrodistillation headspace solvent microextraction, essential oils, *Stachys inflata Benth*

INTRODUCTION

The genus *Stachys* is one of the largest genera of the Labiata with a worldwide distribution. About three hundred *Stachys* species are reported of which 34 ones are found in the flora of Iran [1–2]. *Stachys inflata Benth* has been used in Iranian folk medicine for treatments of infections, rheumatic conditions, genital tumors, inflammatory tumors, sclerosis of the spleen and cancerous ulcers.

Several methods are available for the extraction of essential oils from medicinal plants such as hydrothermal extraction, water vapor extraction, solvent extraction, simultaneous purge and trap in solvent extraction, supercritical fluid extraction, stir bar sorptive extraction and solid-phase microextraction [3, 4].

In the last decade, there has been an increasing requirement in new extraction methods for decreasing extraction times, reducing organic solvent, preventing pollution, reducing sample preparation costs, miniaturization and automation.

To reach these goals, advances in microwave extraction have resulted in a number of techniques such as vacuum microwave hydrodistillation [5, 6], microwave-assisted solvent extraction [7, 8], microwave hydrodistillation [9, 10], and solvent-free microwave hydrodistillation [11, 12]. In recent years, solvent microextraction (SME) has been developed as an alternative to solid-phase microextraction SPME [13–15]. This method provides analyte extraction in a few microliters of an organic solvent. SME avoids some problems of the solid phase

* Corresponding author. E-mail: mbgholivand@yahoo.com

microextraction (SPME) method such as sample carry-over and fiber degradation. It is also fast, inexpensive and uses very simple equipments. For the first time, Fakhari and co-workers developed a new method involving concurrent headspace solvent microextraction coupled with continuous hydrodistillation of essential oil [16]. Zhang et al. reported coupling continuous microwave hydrodistillation with headspace solvent microextraction as an efficient method that was claimed to be superior to the traditional hydrodistillation [17].

In this paper, the microwave-assisted headspace solvent microextraction (MA-HSME) method as a new and green technique followed by gas chromatography-mass spectrometry was developed for the analysis of essential oil in the *Stachys inflata Benth*. A comparative study is also made between MA-HSME, hydrodistillation headspace solvent microextraction (HD-HSME) and the traditional hydrodistillation (HD) methods. A simplex method was used for rapid and efficient optimization of the conditions used for MA-HSME and HD-HSME.

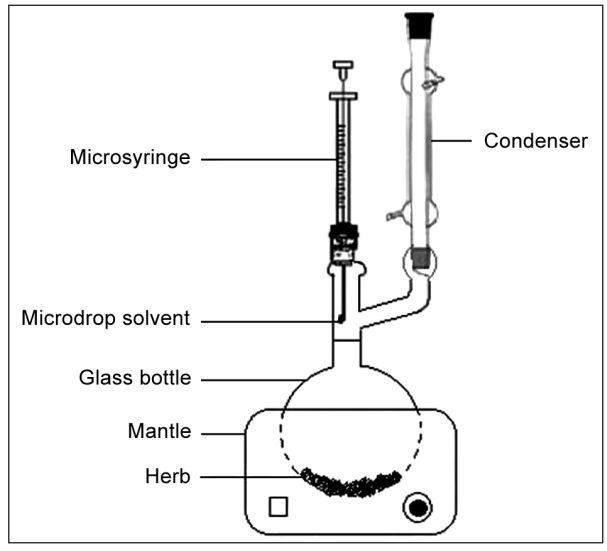

Fig. 1. HD-HSME apparatus

EXPERIMENTAL

Reagents and materials

n-Dodecane, *n*-hexadecane, *n*-heptadecane, *n*-heptane and nitrobenzene were purchased from Merck (Darmstadt, Germany) and used as received. All parts of the wild *Stachys inflata Benth* were harvested in Taghboostan region (Kermanshah, Iran) in June 2010.

Hydrodistillation (HD) apparatus and procedure

Air-dried aerial parts of *Stachys inflata* (100 g) were ground and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus as recommended. Briefly, the plant was immersed in water and heated to boiling, after that 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–MS. The yield of the yellowish oil from the aerial parts of *Stachys inflata* was 0.31% (w/w), based on the dry weight of the sample.

HD-HSME of essential oil

HD-HSME was performed by using the apparatus illustrated in Fig. 1. The ground sample (5.5 g) was placed in a 100 mL round bottomed flask containing 50 mL water, and the mixture was heated under reflux for 30 min. Five minutes after the end of reflux, the needle of the syringe was inserted into the headspace of the plant sample. The syringe plunger was depressed, and 3 µl of *n*-heptadecane containing *n*-hexadecane as an internal standard with a volume ratio of 1 : 200 v/v was suspended from the needle tip. After extraction, the plunger was withdrawn, and the micro drop was retracted back into the syringe. The needle was removed from the headspace, and its content was injected into the GC system. Finally, the analytical signal was calculated as the peak areas of the analytes relative to the internal standard.

MA-HSME of essential oil

The laboratory made MA-HSME apparatus is shown in Fig. 2. The microwave oven with a maximum delivered power of 900 W (model of GE614ST / GE614W, Samsung Company, Korea) was used as a heating device. In order to prevent microwave leaking, aluminum foil was tacked onto the inner wall and the outer wall of the microwave in the interface part [17–20].

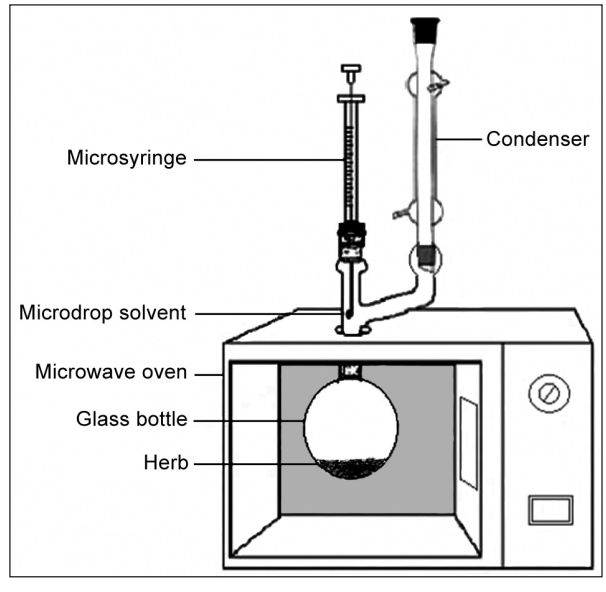

Fig. 2. The home-made apparatus of MA-HSME

MA-HSME extraction was performed according to the following procedure. Weighed amount (2 g) of powdered herb was transferred into a 50 ml round bottom flask containing 1 ml water. After assembling a condenser, a 10 µl micro syringe (Hamilton, USA) containing 3 µl of *n*-heptadecane as an extracting solvent and *n*-hexadecane as an internal standard (with a volume ratio of 1 : 200 v/v) was suspended on

the top of the flask, the herb was heated by microwave power (450 W). After extraction, the plunger was withdrawn, and the micro drop was retracted into the micro syringe, and finally the micro drop was injected directly into the GC–MS injection port.

Gas chromatography–mass spectrometry

An HP 6890N GC system with a split / splitless injector and a HP-5972 mass-selective detector was used. The extracted compounds were separated on an HP-5MS capillary column (30 m × 0.25 mm i. d., 0.25 μm film thickness). Analyses were performed in the electron ionization (EI) mode at 70 eV, and the mass range was *m*/*z* 40–550. Transfer line and ion source temperatures of 280 and 250 °C were used, respectively. Split injection was employed for both distillated and extracted samples with a ratio of 50 : 1, and the injector temperature was set to 250 °C.

The column oven temperature was programmed to rise from an initial temperature of 40 °C to 160 °C at 4 °C/min, then to 260 °C at 10 °C/min. Helium was used as the carrier gas with a flow rate of 1.1 ml/min. Compounds were identified using the Wiley 7 (Wiley, New York, NY, USA) Mass Spectral library and retention indices.

RESULTS AND DISCUSSION

In the present study, the volatile constituents in the *Stachys inflata* were transferred to the headspace by a heating source, and the analytes in the headspace were simultaneously extracted and concentrated into a suspended micro drop solvent. Isolation, extraction, and concentration of the volatile components were performed in one single step. The solvent selection, solvent volume, microwave power, irradiation time, extraction time and sample mass that can affect the extraction efficiency of MA-HSME were studied. Also for the HD-HSME method, the nature of the extracting solvent, solvent volume, extraction time, and sample mass were investigated.

The selection of a suitable extracting solvent is essential. This solvent should fulfil three requirements: it should not evaporate under the extraction condition (in order to be stable at the extraction period), it should have the ability to extract the analytes efficiently, and the solvent peak should not overlap with the analyte peaks in the chromatogram [16].

Five different solvents with different polarities and boiling points including nitrobenzene, *n*-heptane, *n*-dodecane, *n*-hexadecane and *n*-heptadecane were examined for MA-HSME and HD-HSME. Fig. 3 compares the sum of relative peak areas obtained for five main (Benzyl benzoate, γ-Curcumene, Cedrol, β-Pinene and α-Pinene) components. The highest signals, best extraction efficiency and a minimum overlap were obtained for *n*-heptadecane. For *n*-dodecane and nitrobenzene, the solvent peaks overlapped with some of the oil components. Heptane was almost disappeared because of its high volatility in the high temperature of the system. Hexadecane was added to heptadecane with a ratio of 1 : 200 (*v*/*v*) and utilized as an internal standard.

Optimization of MA-HSME and HD-HSME

A simplex method was used for optimization of effective parameters on extraction efficiency in MA-HSME and HD-HSME methods. Use of a simplex method can significantly reduce the number of experiments required for achievement of the maximum extraction efficiency. The relative areas of five main peaks in the GC-MS chromatogram (Benzyl benzoate, γ-Curcumene, Cedrol, β-Pinene and α-Pinene) were monitored during optimization. In the simplex method, (n + 1) initial experiments were designed (n is number of effective parameters on extraction efficiency in HSME method), the conditions corresponding to the worst response were reflected, and the reflection process was repeated until no further improvement in the response was observed. Some of the reflections were modified when appropriate [21–23].

The conditions used for the initial experiments and the subsequently designed experiments for MA-HSME and

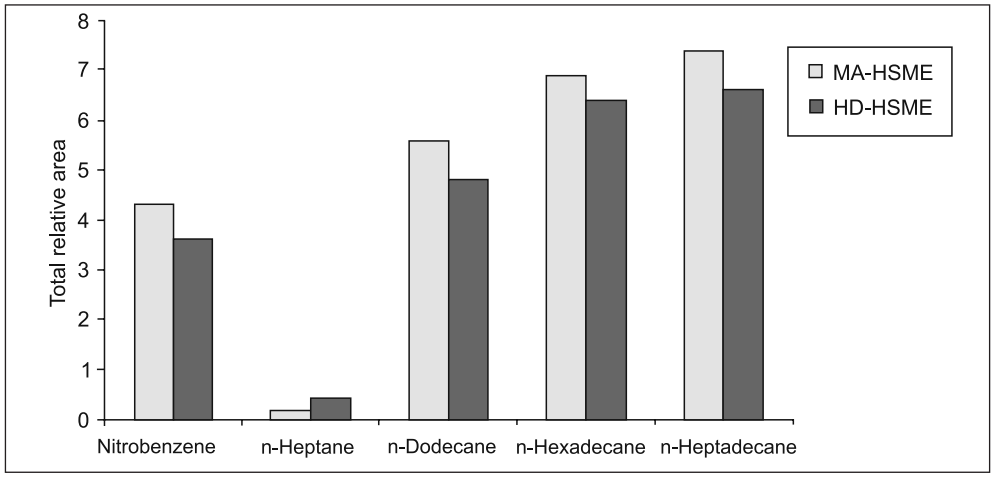

Fig. 3. Effect of solvents on the extraction efficiency at optimum conditions (total relative peak area for Benzyl benzoate, γ-Curcumene, Cedrol, β-Pinene and α-Pinene)

HD-HSME are summarized in Table 1 and Table 2, respectively. The experimental conditions were obtained by use of a modified reflection method. The modifications were usually performed in accordance with the practical limitation of some factors such as micro drop volume (larger drops are difficult to manipulate) [21].

The results clearly indicate the positive effects of micro drop volume, sample weight, microwave irradiation power and extraction time on the extraction efficiency of HD-HSME and MA-HSME methods.

Table 3 shows the optimum condition chosen for MA-HSME and HD-HSME for further studies. As seen from Table 3, the experimental results demonstrate that MA-HSME is a rapid method and needs a small weight of the sample for the determination of the volatile fraction in *Stachys inflata Benth*.

MA-HSME and HD-HSME of *Stachys inflata Benth*

The components of *Stachys inflata* oil and their percentages obtained by calculation of the peak area relative to the total peak area for conventional hydrodistillation, MA-HSME and HD-HSME methods, are presented in Table 4.

Almost the same number of components (thirty five) was found in HD, HD-HSME and MA-HSME.

MA-HSME and HD-HSME are based on the same extraction process: the volatile compounds are carried away by steaming, leading to a solvent phase. In this case, both extractions obtained must contain a majority of compounds with higher volatility, apart from the fact that MA-HSME is much faster than HD-HSME. Benzyl benzoate was found to be the main constituent of the essential oil components of *Stachys inflata* followed by Caryophyllen oxide, γ-Curcumene, Cedrol, β-Pinene and α-Pinene.

The hydrodistillation method required a long time (4 h) to isolate the volatile oil from *Stachys inflata Benth*. In the MA-HSME method, the isolation of volatile compounds in the herb was rapidly completed, and then the isolated volatile compounds were simultaneously extracted and concentrated by a suspended micro drop (total time 4 min). In the HD-HSME methods, the isolation, extraction and concent-

Table 1. **Experimental conditions used and results obtained for the MA-HSME experiments performed in the simplex optimization procedure**

Exp. No	Sample weight (g)	Droplet volume (µl)	Extraction time (min)	Microwave power (W)	Sum of relative peak area[a]
1	2	2	3	300	4.23
2	3	2	3	300	5.13
3	2	2.5	3	300	5.36
4	2	2	5	300	6.14
5	2	2	3	450	7.56
6 (Refl.[b])	2.5	3	4	450	9.36
7 (Refl.)	1.5	2.5	4.5	450	6.22
8 (Refl.)	2	3	3.5	600	5.83
9 (Refl.)	2	2	4	450	6.66
10 (Refl.)	2	2	4	180	4.13

[a] Total relative peak area for Benzyl benzoate, γ-Curcumene, Cedrol, β-Pinene and α-Pinene

[b] Reflection

Table 2. **Experimental conditions used and results obtained for the HD-HSME experiments performed in the simplex optimization procedure**

Exp. No	Sample weight (g)	Droplet volume (µl)	Extraction time (min)	Sum of relative peak area[a]
1	1	2	4	4.21
2	1	2.5	4	5.12
3	1	2	6	4.96
4	4	2.3	4	6.84
5 (Refl.[b])	5	2.5	5.5	7.32
6 (Refl.)	5.5	3	8	10.02
7 (Refl.)	9.2	3	6.5	7.78
8 (Refl.)	11.8	3	12	6.29
9 (Refl.)	5	2.5	5	7.11
10 (Refl.)	2	2	4	4.62

[a] Total relative peak area for Benzyl benzoate, γ-Curcumene, Cedrol, β-Pinene and α-Pinene

[b] Reflection

Table 3. **Optimum extraction condition of MA-HSME and HD-HSME**

Method	Sample weight (g)	Droplet volume (µl)	Extraction time (min)	Microwave power (W)
MA-HSME	2	3	4	450
HD-HSME	5.5	3	8	–

Table 4. **Constituents of the oil of *Stachys inflata Benth***

	Compounds	RI[a]	HD[b]	MA-HSME[c]	%RSD[d]	HD-HSME[e]	%RSD[f]
1	α-Thujene	929	0.33	0.25	8.3	0.23	9.6
2	α-Pinene	932	4.76	7.21	1.3	6.23	1.6
3	Sabinene	972	0.22	0.10	9.6	0.12	10.3
4	β-Pinene	975	5.91	4.38	4.8	4.28	3.2
5	Myrcene	992	0.49	0.42	9.9	0.39	10.3
6	α-Phellandrene	1006	0.22	0.11	10.6	0.18	11.3
7	δ-3-Carene	1008	1.01	1.03	7.8	1.18	9.3
8	p-Cymene	1019	0.21	0.11	9.9	0.12	11.1
9	o-Cymene	1022	0.38	0.37	8.3	0.33	10.3
10	Limonene	1027	2.02	1.06	1.6	1.26	3.9
11	*cis*-Ocimene	1039	0.96	0.93	7.3	0.99	6.6
12	(E)-β-Ocimene	1048	0.22	0.10	5.1	0.14	4.6
13	γ-Terpinene	1060	0.25	0.16	8.6	0.18	7.1
14	α-Terpinolene	1085	0.12	0.10	8.8	0.15	8.9
15	Linalool	1099	0.57	0.47	7.3	0.44	5.4
16	α-Terpineol	1189	0.30	0.29	6.9	0.26	4.8
17	Neral	1239	0.38	0.44	10.3	0.52	8.3
18	E-Carveol	1269	0.95	0.85	5.3	0.78	3.9
19	Thymol	1300	1.73	0.97	0.7	1.16	1.1
20	Carvacrol	1303	0.22	0.18	8.8	0.12	9.6
21	α-Copaene	1371	0.73	0.57	11.3	0.49	12.0
22	β-Bourbonene	1381	1.31	1.35	1.3	1.28	1.9
23	α-Cedrene	1408	1.81	0.58	8.3	0.53	7.2
24	Caryophyllene	1413	1.09	0.98	1.6	0.83	1.5
25	Aromadendren	1443	2.03	0.77	0.8	0.56	0.9
26	Germacrene D	1475	2.62	1.18	1.4	1.12	0.9
27	γ-Curcumene	1480	10.61	12.01	0.9	10.3	0.8
28	Bicyclo germacrene	1488	2.83	0.59	5.3	0.45	6.8
29	Bisabolene	1505	1.52	0.13	7.8	0.36	5.6
30	δ-Cadinene	1515	2.55	1.05	1.9	0.87	0.8
31	*trans*-Nerolidol	1563	1.63	0.46	4.5	1.36	1.9
32	Caryophyllen oxide	1574	18.4	27.6	0.9	25.6	0.6
33	Cedrol	1587	7.55	0.64	1.2	1.25	1.8
34	Epi-α-Cadinol	1646	2.18	1.43	0.3	1.69	0.9
35	Benzyl benzoate	1749	18.1	26.7	1.3	25.6	2.1

[a] Retention indices using a DB-5 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 MA-HSME method

[d] RSD values for MA-HSME method (n = 3)

[e] Relative area (peak area relative to total peak area except for the solvent peak) for HD-HSME method

[f] RSD values for HD-HSME method (n = 3)

ration were performed in a single step (total time 45 min). Moreover, MA-HSME required only a small sample amount (2.0 g) and very little extraction solvent (3.0 μL). So MA-HSME is an easy, rapid, low-cost and solvent-free method for the determination of the volatile oil in the *Stachys inflata*.

The precisions of the methods performed under the optimized conditions were determined by analyzing the samples in triplicate. The percentage of relative standard deviation (%RSD) values was calculated by the peak areas obtained by replicate analyses. It was observed that RSD values for the compounds were less than 12% (Table 4).

CONCLUSIONS

In this study, MA-HSME and HD-HSME techniques were successfully performed for the determination of volatile compounds in *Stachys inflata Benth*. Thirty-five compounds were identified in *Stachys inflata Benth* using the proposed methods. Compared with HD and HD-HSME methods, MA-HSME is an easy, rapid and efficient method for the analysis of essential oils in *Stachys inflata Benth*. This technique may also be used to follow variations in the concentrations of volatile components of a plant with time, or for different species.

The simplex method was shown to be a rapid and efficient means of optimization of the microextraction conditions by performing just a few experiments.

Received 6 October 2011
Accepted 20 October 2011

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Mohammad Bagher Gholivand, Mir Mahdi Abolghasemi, Marziye Piryaei, Seyed Mohamad Maassoumi

HIDRODISTILIAVIMO IR MIKROBANGOMIS SKATINAMO DISTILIAVIMO PALYGINAMASIS TYRIMAS *STACHYS INFLATA* LAKIŲJŲ KOMPONENTŲ ANALIZEI

S a n t r a u k a

Trys skirtingi metodai panaudoti *Stachys inflata Benth* lakiųjų komponentų ekstrakcijai. Ištirta kelių parametrų įtaka ir įvertintas metodų tikslumas. Ekstraktai, gauti panaudojus tirtuosius metodus, buvo tiriami dujų chromatografijos būdu. Ekstraktuose nustatyti 35 lakieji komponentai.