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Determination of the Volatile Components of *Stachys Lavandulifolia* with Periodic Mesoporous Organosilica as the Fiber Coating for Headspace Solid Phase Microextraction

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A microwave-assisted headspace solid-phase microextraction (MA-HS-SPME) approach using a periodic mesoporous organosilica based on alkylimidazolium ionic liquid (PMO-IL) was proposed and used as a greatly porous fiber covering substance for effectively investigating the *Stachys lavandulifolia's* essential oil composition. The specimen was exposed to microwave radiation and its volatile constituents were gathered *via* the fiber from the specimen headspace and straightly inserted into a GC-MS addition port for investigation. A simplex technique was utilized for optimizing three various factors influencing the extraction effectiveness. Under the enhanced circumstances (with the sample weight of 2 g, extraction time of 2.0 min and microwave power of 300 W), the PMO-IL nanoporous fiber could proficiently adsorb volatile components of *Stachys lavandulifolia*. In optimum conditions, the repeatability for one fiber (n = 3), expressed as relative standard deviation (R.S.D.%), was between 3.5% and 12.1% for the test compounds. The suggested technique, relative to hydrodistillation can equally be used to monitor all the sample components easily, though it will require less sample quantity and duration. A few experiments based on the simplex method proved it would be fast and efficient method to optimize the micro-extraction conditions.

Keywords: Alkylimidazolium ionic liquid, Headspace microextraction, Periodic mesoporous organosilica, *Stachys lavandulifolia*

INTRODUCTION

The mesoporous substances with uniform holes and high specific areas have attracted great interest of researchers due to their potential uses such as separation, catalysts, adsorbents for biomolecules, organic molecules, guest-host chemical supporters and controlled drug release [1]. This instance deals with combining an organic functionality with the inorganic matrix stability. To insert a vigorous functionality over the mesopore surface, various organosilane precursors are utilized as a silica basis for synthezing the naturally altered hybrid mesoporous substances [2,3]. The periodic mesoporous organosilica (PMOs) is amongst these organic-inorganic hybrid substances appealed incrementing attentions as a result of their latent function in various frontier fields. Potential usage of PMOs relies censoriously on macroscopic morphologies and on the available organic groups loading into the framework. This leads to very fascinating features such as great adsorption attraction for organic combinations, high surface area, constricted distribution of pore size, and changeable diameter of pore. Room-temperature ionic liquids (ILs) are a class of organic salts containing a collection of various anions and organic cations as liquids at room temperature. They include different benefits over conventional organic solvents, like high stability, low vapor pressure, adaptable miscibility and polarity, large viscosity, decent extractability for various inorganic and organic compounds [4,5]. Furthermore, because of their low volatility, toxicity, and flammability,

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ILs have been suggested as the green solvents for extraction , a substitute for organic solvents. Some uses were also confirmed for different analytical objectives such as the stationary point for liquid phase microextraction [6], gas chromatography [7-9], or liquid chromatography [10], matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) [11-13], the mobile phase modifier in HPLC [14], and hybrid liquid-solid phase microextraction [15,16].

Within numerous approaches utilized to extract the plant materials essential oil, microextraction methods have obtained widespread application in recent years. Both solid-phase microextraction and solvent microextraction techniques were used for this aim. Headspace-solid phase microextraction, headspace solid-phase microextraction is a fairly innovative concentration and sampling method for extracting the essential oils of the plants. Headspace-solid phase microextraction after gas chromatography-mass spectrometry (GC-MS) was demonstrated as a sensitive, simple, and solvent-free technique for analyzing the components of the essential oil. Nevertheless, nearly 30-40 min is required in conventional HS-SPME for extracting the compounds of essential oil in medical plants [17].

Stachys lavandulifolia is used in Iranian traditional medicine as the herbal tea in inflammatory diseases, gastrointestinal disorders, cough, anxiolytic, antispasmodic, sedative, ulcers, diuretic, diarrhea and fevers. The *S. lavandulifolia's* aerial part was utilized by tribal people of west province of Iran, as a sedative, carminative, and cardiotonic, and for treating indigestion, rheumatism, and headache. The aqueous extract of the S. *lavandulifolia* aerial parts indicates potential wound healing and anti-inflammatory activities in rat [18,19].

In this examination, PMO-IL nanocomposite is used by HS-SPME as a covering for *solid-phase microextraction* fiber for analyzing the volatile compounds in *Stachys lavandulifolia* enabling the identification and separation volatile compounds by supporting MA-HS-SPME with GC-MS. These experimnetal consequences agree with Clevenger method. The factors influencing on the extraction efficiency of volatile compounds including sample temperature, extraction time and sample weight were investigated and optimized. Finally, the proposed method was successfully applied to the analysis of volatile compounds in the medicinal plant.

MATERIALS AND METHODS

Materials

This work deals with the application of investigative level substances n-alkanes especially C6-C24, sulphuric acid, and sodium hydroxide acquired from Merck a company situated in Darmstadt in Germany with no more purifying over the application. All experiments were performed using distilled water. The wild *S. lavandulifolia* aerial parts were gathered in July 2019 in western Iran. The plant substances were dehydrated in the air and kept in sealed bags in a cool place. Identifying and validating of the plants were performed by the botanic research laboratory of Razi University and a specimen was prepared at the herbarium.

Synthesizing the Priodic Mesoporous Organosilica with the Ionic Liquid Basis (PMO-IL)

Synthesizing the PMO-IL was in a way similar to the method presented by Karimi *et al.* [18-20]. An argon atmosphere is quite vital in a distinctive PMO-IL synthesis. This procedure includes setting sodium imidazolide (3.002 g, 30 mmol) and 3-chloropropyltrimethoxysilane (6.082 g, 30 mmol) in a dried flask with 100 ml of super dry THF and agitating for 24 h at 65 °C in an argon atmosphere. The solvent was eliminated under decreased pressure, followed by cooling the reaction combination to room temperature, and the resulting oil was transmitted to another bottle with 30 mmol of 3-chloropropyl-trimethoxysilane in absolute toluene (100 ml) and refluxed in darkness for 48 h. The reaction mixture was initially rinsed carefully with toluene (5 × 50 ml), followed by cooling the solution to room temperature, and super dry CH_2Cl_2 was then inserted to NaCl precipitation. The solution of supernatant dichloromethane was transmitted to another well-dried bottle. The PMO-IL preparation procedure includes two mixtures that were agitated at 40°C. The initial combination contains KCl (8.8 g) and Pluronic P123 (1.67 g) and the second mixture comprises of 2 M hydrochloric acid with distilled water. In addition, a fusion of ionic liquid with 2.74 g of tetramethoxysilane in absolute methanol and a

weight of 0.86 g was introduced to the previous mixture and stirred for 24 h. The mixture was heated statically at 100 °C for 72 h in Teflon-lined autoclave. The mixture was filtered to achieve the solid which is then carefully rinsed with deionized water and dried. Using 3 ml of concentrated HCl and 100 ml of ethanol via a Soxhlet apparatus, the surfactant was extracted. This is reiterated 4 times for 12 h with 1 g. The final product is PMO-IL.

Preparation of the SPME Fiber

A piece of stainless steel wire with a 200-m diameter was twice cleaned with methanol in an ultrasonic bath for 20 min and dried at 70 °C. One centimeter of the wire was limed with epoxy glue and the PMO-IL nanocomposite was immobilized onto the wire. Then, PMO-IL was immobilized on the sample and later heated for 48 h in a 50 °C oven and then slightly cleaned to remove non-bonded particles to permit to assemble on the SPME apparatus. Finally, the SPME fiber is cleaned and acclimatized in a helium atmosphere at 260 °C for 2 h.

Hydrodistillation (HD) Device and Procedure

Utilizing a Clevenger-type device, 100 g of air-dried of *S. lavandulifolia* was hydrodistilled for 2.5 h. The plant is temporarily heated in water to ease evaporating the oil; which is then gathered in a condenser. To dry the oil, anhydrous sodium sulfate is utilized, prior storage at 4 °C and analyzing through the GC-MS. Utilizing the samples dry weight obtained from *S. lavandulifolia* aerial parts, 0.81% (w/w) of the yellowish oil was created.

Gas Chromatography-mass Spectrometry

A Hewlett-Packard HP 6890 GC equipped with a splitless injector or split. To identify the essential oils an HP 5973 mass-selective indicator device was utilized. The restrictions were 0.25 mm by 30 m HP-5 MS column. The diameter of the layer was 0.25 µm. The column was mainly kept at 50 °C for 3 min. At a rate of 15 °C per min, it was also enlarged to 180 °C. Then, it was raised to 260 °C at the rate of 20 °C per min and kept at this temperature for 5 min. The gas streamed with 1.1 ml min^{-1} rate.

The injections were carried out for 2 min at 250 °C. GC-MS interface at 280 °C, quadruple temperatures at 150 °C, and ion source temperature at 230 °C were the other main control temperatures. The ions features in each compound were vital to set the MS mode utilizing Scan mode (chosen ion observing) within the range of 50-550 m/z. To recognize these compounds, Wiley 7N(Wiley, New York, NY, USA) Mass Spectral Library was arranged. By introducing the determined solutions and recording the total number of ions over the chromatogram, ion discrimination was enabled. The measureable ion was calculated as the most abundant ion, however, the others were utilized as a standard for confirming the particular analytes.

MA-HS-SPME of Essential Oil

To heat in the MA-HS-SPME process, a 900W microwave oven (GE614ST/GE614W) was positioned. The powdered herb with the weight of 2 g was inserted into the bottle; then heating was performed *via* microwave power (300 W). Followed by extracting, the fiber was removed from the flask and introduced into the GC-MS injection port for analyzing.

RESULTS AND DISCUSSION

Within this study, the fiber porosity utilized in extracting the compounds was determined once heating with no solvents. Extracting, secluding and concentrating were performed simultaneously on volatile substances. Transmission electron microscopy (TEM) and thermal gravimetric analysis (TGA) measurements were performed in our previous work [16,17]. The average pore diameter is also calculated from the adsorption branch of the isotherm to be 7.0 nm by using the BJH method [16]. The SEM images and FT-IR are in our previous work [16].

Optimization of MA-HS-SPME

A simplex method was used for optimization of effective parameters on the extraction efficiency in the MA-HS-SPME method. Use of a simplex method can significantly reduce the number of experiments required for achievement of the maximum extraction efficiency. The relative areas of four main peaks in the GC-MS chromatogram were monitored during the optimization. In the simplex method, (n + 1) initial experiments were designed (n is the number of effective parameters on

Table 1. Experimental Conditions Used and Results Obtained for the SPME Experiments Performed in the Simplex Optimization Procedure

Exp. no	Sample weight (g)	Extraction time (min)	Temperature (°C)	Droplet volume (µl)
1	1	25	70	3
2	2	25	70	3
3	1	30	70	3
4	1	25	75	3
5	1	25	70	2
6 (Refl.)[a]	1.5	28	73	3
7 (Refl.)	1.75	29	74	3
8 (Refl.)	2.1	31	76	3
9 (Refl.)	2.6	34	72	3
10 (Refl.)	2	34	78	3
11 (Refl.)	2.7	35	77	3
12 (Refl.)	3	35	78	3
13 (Refl.)	3	35	80	3
14 (Refl.)	3	35	85	3

[a]Reflection.

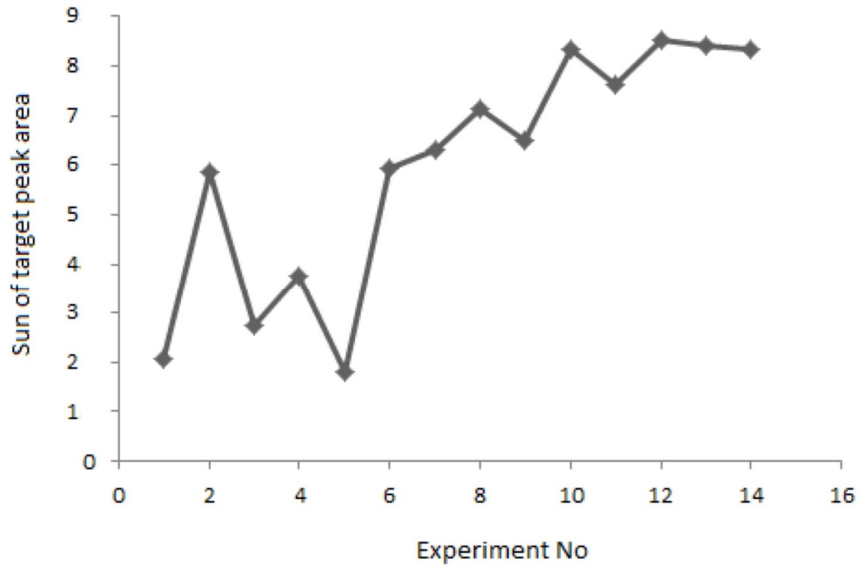

Fig. 1. The response (sum area of four main peaks samples) for the designed experiments mentioned in Table 1 (SPME).

the extraction efficiency in the 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 modifed when needed [21-23].

Table 1 contains a summary of the conditions under which primary and subsequent experiments were conducted (Fig. 1 illustrates the optimum response for SPME). The tests indicate the positive influences on extracting efficiency of MA-HS-SPME based on the weight of the specimen, the microwave radiation power, and the extraction time. The tests indicated that by the weight of the specimen, the extracted compounds are incremented. The extracted quantities are increased by incrementing the weight of the specimen. These were the elements of the chosen volatile mixtures. Figure 2 illustrates the optimum response for MA-HS-SPME. The maximum specimen weight of 2 g was chosen in terms of the experimental clarifications of the observations. Moreover, incrementing the fiber introduction time in the considered headspace, increasing the analyte quantity extracted was anticipated *via* the MA-HS-SPME method.

The test also revealed that at 300 W the optimum volatile compounds relative climax area was obtained, however, the best consequences were found followed by extracting over 2 and a half min (Table 2). Hence, it is obvious that extracting MA-HS-SPME requires roughly 2 g of the specimen, for about 2 min. Evaporating the volatile oils from the plants was made possible by a 300 W power in the reheating system.

The Effect of Adding Water

It was found that the moisture or adding water to the specimens may have an important effect on extracting in the SPME test. Since in this work, the specimens were then a dried S. *lavandulifolia* plant, the humidity influence was investigated by adding various quantities of water to the specimens in the enhanced circumstances. Adding water involved negatively in the response. Hence, it can be found that the positively extracted analytes decreased by the water vapor in the gaseous specimens. It shows that the fiber surface can be deactivated by the water molecules through blocking the active sites.

HD and MA-HS-SPME of *S. lavandulifolia*

Table 3 indicates the components and percentages of *S. lavandulifolia* oil obtained by calculating the optimum area related to the overall peak area for the MA-HS-SPME and conservative hydrodistillation technique. In headspace-solid phase microextraction and hydrodistillating, a comparable amount of combinations was created. Utilizing a regression line technique for data comparing, revealed a linear association within the nanoporous fibers results, and the commercial fiber, with the HD data. The hydrodistillation technique needed an extended time (3 h) to separate the volatile oil from *S. lavandulifolia*. The HD method takes a lot of time, while extracting with MA-HS-SPME only takes 2 min. In the MA-HS-SPME technique, isolating the volatile mixtures in the plant was accomplished quickly, and the separated volatile compounds were then concurrently took out and became rigorous *via* fiber. Figure 3 shows the chromatogram.

To evaluate the reproducibility (fiber-to-fiber) and repeatability (for one fiber), we made 4 fibers under the same circumstances and, 3 repeated tests were performed by each fiber. The RSD% for each fiber and fiber-to-fiber for menthol as a model compound was determined. The RSD% (12.1%) indicates the good technique replicability for menthol. Therefore, the coating process should be enhanced to confirm the reproducible and uniform thickness. Alternatively, the SPME fiber is mechanically steady and no need to use various fibers for analyzing.

The lifetime of the homemade SPME device was estimated after more than 50 analyses, using a 0.1 m g^{-1} of Myrcene added to dry plants. The extraction of thymol was used to measure the prepared nanocomposite fiber stability after repeating the sampling/desorption cycles. The results reveal that the extraction capability of menthol after 50 cycles remained almost unchanged. These results, vigorously indicate that the mentioned fiber has a moderately high strength, Thus the lifetime of the prepared device should be longer in comparison with commercial SPME fibers.

CONCLUSIONS

It was noted that there was a rapid completion in the separation of volatile compounds in the herb. Subsequently,

Table 2. Experimental Conditions Used and Results Obtained for the MH-SPME Experiments Performed in the Simplex Optimization Procedure

Exp. no	Sample weight (g)	Extraction time (min)	Temperature (°C)	Droplet volume (µl)
1	1	2	300	3
2	2	2	300	3
3	1	3	300	3
4	1	2	450	3
5	1	2	300	2
6 (Refl.)[a]	1.5	2.5	450	3
7 (Refl.)	1.75	3	450	3
9 (Refl.)	2	2	450	3

[a]Reflection.

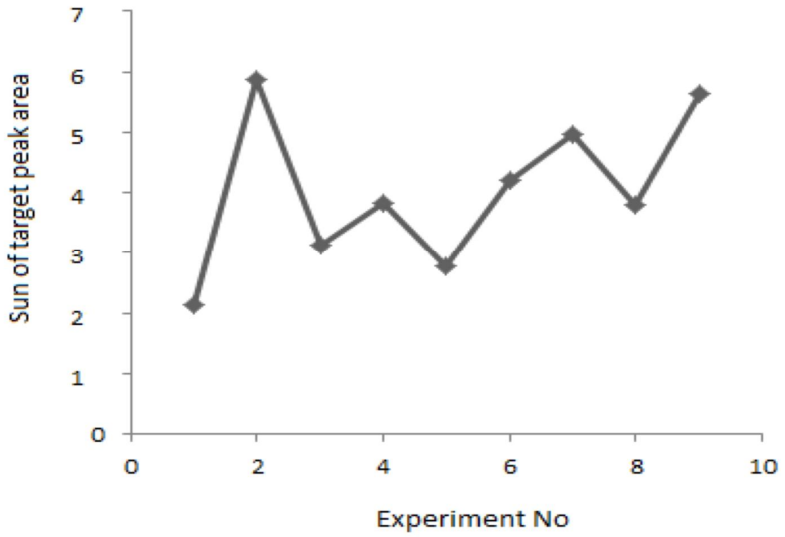

Fig. 2. The response (sum area of four main peaks samples) for the designed experiments mentioned in Table 2 (MA-HS-SPME).

with the use of fiber, the compounds were separated and concentrated simultaneously within a time frame of 3 h for hydrodistilation and 2 min in MA-HS-SPME method. As such, the MA-HS-SPME method proves to be the most suitable technique in oil determination and extraction from *S. lavandulifolia* owing to its advantageous aspects of cost effectiveness, simplicity and solvent independence. Based on the experiments performed here, the PMOIL particles are

Table 3. Constituents of the Oil of *Stachys lavandulifolia*

No.	Compounds	RI[a]	(HD) Area (%)[b]	SPME Area (%)[c]	(MA-HS-SPME)	Repeatability R.S.D. (%)
1	α-Thujene	931	2.11	1.73	1.02	4.2
2	α-Pinene	936	6.42	4.68	3.27	7.1
3	Camphene	952	0.85	0.41	0.24	5.1
4	Sabinene	974	0.21	0.11	0.21	3.8
5	β-Pinene	979	8.37	5.23	3.68	8.0
6	Myrcene	997	6.21	5.58	4.26	7.5
7	α-Phellandrene	1007	2.07	1.06	0.86	4.8
8	δ-3-Caren	1010	0.84	0.32	0.11	6.7
9	α-Terpinene	1020	0.27	0.08	0.10	8.4
10	β-Phellandrene	1029	6.34	3.16	2.37	11.6
11	1,8-Cineole	1031	0.12	0.05	0	-
12	Z-β-Ocimene	1038	1.25	0.36	0.19	3.5
13	E-β-Ocimene	1045	2.11	0.13	0.06	9.2
14	γ-Terpinene	1061	1.08	0.27	0.08	9.5
15	Z-Sabinenehydrate	1070	0.81	0.25	0.15	7.3
16	Terpinolene	1082	2.16	0.86	0.72	5.5
17	Linalool	1105	0.69	0.53	0.44	3.2
18	Allo-ocimene	1119	0.14	0.08	0	-
19	Camphor	1140	1.27	0.63	0.45	6.7
20	Borneol	1161	0.13	0.10	0.05	7.4
21	Terpinen-4-ol	1178	3.09	1.06	0.83	6.5
22	α-Terpineol	1193	1.58	0.65	0.56	5.9
23	Nerol	1224	0.67	0.24	0.14	4.7
24	Neral	1236	0.15	0.04	0	-
25	Geranio	1253	0.22	0.06	0	-
26	Linalool acetat	1259	0.17	0.04	0	-
27	Bornyl acetate	1285	0.10	0.12	0	-
28	γ-Teqpinen-7-al	1288	0.73	0.08	0	-
29	δ-Elemene	1339	1.58	0.66	0.28	6.2
30	Terpin-4-ol acetate	1342	1.06	0.18	0.12	5.3
31	α-Cubebene	1350	0.07	0.27	0.17	2.8
32	Eugenol	1359	0.11	0.15	0.07	4.6
33	α-Copaene	1380	3.46	1.76	1.16	9.5
34	β-Bourbonene	1382	0.26	0.14	0.09	3.8
35	β-Elemene	1389	1.75	1.37	0.08	8.6

Table 3. Continued

36	E-Caryophyllene	1420	2.65	0.92	0.61	2.8
37	Z-β-Farnesene	1440	0.94	0.53	0.38	7.8
38	α-Humulene	1451	1.14	0.17	0.04	6.3
39	E-β-Farnesene	1457	0.64	0.61	0.52	7.4
40	Germacrene D	1478	4.39	3.23	3.07	10.7
41	Bicyclogermacrene	1495	8.12	6.62	4.39	12.1
42	β-Bisabolene	1505	1.27	0.87	0.07	7.8
43	γ-cadinene	1510	3.51	2.54	1.46	10.5
44	δ-Cadinene	1528	3.32	1.79	1.12	11.7
45	α-Cadinene	1536	0.24	0.15	0.03	5.2
46	Spathulenol	1574	2.71	1.39	1.15	7.4
47	β-Bisabolol	1673	2.35	2.13	1.62	9.7
48	α-Bisabolol	1680	1.48	0.82	0.35	8.2

[a]Retention indices (relative retention times normalize to closely eluting *n*-alkanes). [b]Relative area (peak area relative to total peak area) for hydrodistillation method. [c]Relative area (peak area relative to total peak area) for SPME method.

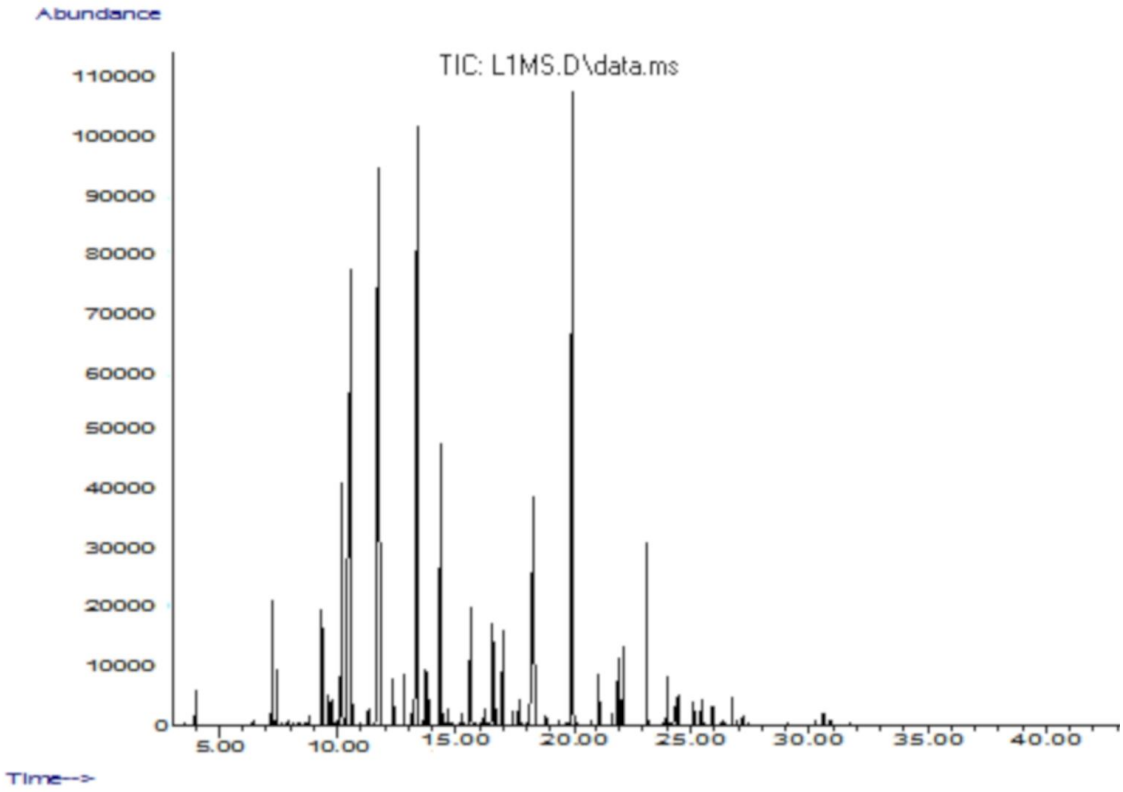

Fig. 3. The chromatograms of essential oil *S. lavandulifolia*.

appropriate in analysis of MA-HS-SPME in the essential oil. The suggested technique compared to HD can equally be used to monitor all the sample components easily, but it will require less sample quantity and duration. A few experiments based on the simplex method proved it would be fast and efficient method to optimize the micro-extraction conditions.

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