Headspace gas chromatographic analysis of Bulgarian *Lavandula Angustifolia* mill Herbs. I. optimization of the analysis conditions

Rumyana Milina, Zilya Mustafa, Stanko Stanev, Dilyana Zvezdova, Stoyanka Stoeva

Abstract: *An analytical method for evaluation of Lavandula angustifolia Mill herbs is proposed. The method consists of quick extraction and simultaneously determination of volatile components using headspace gas chromatography (HS-GC). Some HS parameters were experimentally considered to maximizing the signal and sensitivity and minimizing the relative standard deviation of the results: weight of sample in the headspace vial, the time of the headspace oven (sample equilibration time) and the temperature of the oven. The evaluations were made using 7 Bulgarian kinds of herb. Using our earlier developed GC-MS method of qualitative and quantitative analysis of lavender essential oils, some tests were carried out to determine the contents of volatile components in the headspace gas of the herbs. Preliminary results for the main components are given.*

Key Words: *headspace, Lavandula angustifolia Mill,gas chromatography, herb.*

INTRODUCTION

Lavender is one of the most useful medicinal plants. It provides important essential oils, having sedative and soothing effect on the nervous system, antiseptic and repellent properties, in addition to its primary use in fragrance industry.

Bulgarian lavender essential oils are volatile liquids with characteristic scent and taste of lavender flowers and are obtained by steam distillation of the fresh flowering spikes of different Bulgarian kinds of *Lavandula angustifolia* Mill. Yields and oil quality depend on conditions of the climate and cultivation.

Detailed analyses of essential oils are very important for their medicinal quality, but also in the plant breeding for the selection of superior cultivars. Earlier we developed GC-MS method of qualitative and quantitative analysis of lavender essential oils [thesis].

In general an analytical procedure for essential oil includes extraction (steam- or hydro-distillation, organic solvents extraction and other techniques) and analysis by gas chromatography (GC) or mass spectrometry (GC-MS) [1,3,6,8,11]. The extraction methods are time consuming and could result in losses of some volatiles.

Headspace gas chromatography (HS-GC) is a tool for analysis of light volatiles in samples that can be efficiently partitioned into the headspace gas volume from the liquid or solid sample. Complex sample matrices, which may be difficult to analyze directly or would require sample extraction or preparation can be placed in the vial with little or no preparation. This saves both time and money. The popularity of this technique has grown over recent years and has now gained worldwide acceptance for analyses of biological liquids, pharmaceutical products, environmental. Variations of HS-GC like solid phase microextraction and purge-and-trap have been used for rapid extraction of volatile compounds from aromatic plants [6-10].

So, to evaluate the quality of herbs, an analytical method of quick extraction and simultaneously determination of volatile components would be useful. HS-GC is suitable for this purpose. To our knowledge there has been no HS-GC method reported for investigation of Bulgarian kinds of *Lavandula angustifolia* Mill herb.

The aim of this work is to develop rapid and effective HS-GC method for evaluation of the quality of the herb and to compare obtained results with those from essential oils analyses by GC-flame ionization or mass spectrometry. So, a preliminary quick HS-GC analysis of the herb could give the producer an information about expected quality of the essential oil obtained.

EXPERIMENTAL

1. Plant materials

Lavender flowers of seven kinds of herb *Lavandula angustifolia* Mill, named Raya, Hebar, Hemus, Yubileina, Drujba, Karlovo, Sevtopolis, were collected in July 2012 in the valley near Kazanlak area of Bulgaria.

Each herb was subjected to extraction of essential oil by steam distillation and essential oil was analyzed by GC. At the same time each herb (fresh flowers) undergo headspace extraction and GC analysis to obtained the relative chromatograms.

2. Methods

GC-FID analyses

GC analyses were carried out using gas chromatograph Agilent Technologies 7890A equipped with flame ionization detector (FID). The fussed silica column used was HP-5, 30 m x 0.320 mm I.D. film thickness 0.25 μm. The oven temperature program was from 50°C, 5 min., to 180°C at 5°C/min and held isothermally at 180°C for 10 min. Injector temperature was 200°C, FID temperature was 300°C. Essential oils samples were injected by Agilent 7693A Automated Liquid Sampler, using helium as carrier gas – flow rate 1.5 ml/min, split ratio 1:80, injection volume 0.2 μl pure oil. For FID: Hydrogen rate 40 ml/min, air rate 400 ml/min, make up gas nitrogen 20 ml/min. The concentrations of the components were calculated using relative areas of the peaks without correction factors. Agilent ChemStation was used for data acquisition and data evaluation.

HS-GC analyses

Static headspace GC analyses were carried out using Agilent 7694 Headspace Sampler at the following operating parameters:

Heated zones (°): oven – 70, loop – 110, transfer line – 120.

Equilibration time (minutes): vial eq. time – 60, pressurization time – 0.2, loop fill time – 0.2, loop eq. time – 0.05, inject time – 0.5, GC cycle – 40.

Pressure values (psi): vial pressure – 15, carrier gas pressure – 9.

Sample loop – 1 ml.

A weigh amount of each herb (usually 7 g) was put in a sample vial (20 ml size) and sealed with PTFE rubber septum.

RESULTS AND DISCUSSION

The components, identified by GC-MS are reported in Table 1. The components identification was carried out by comparison of retention data with those of standard compounds or literature data [4,7,9,12,13] and by computer matching with commercial mass spectral libraries. In the same table the concentrations of these components in the essential oils by GC-FID of each herb kind are given.

Some HS parameters were experimentally considered to maximizing the signal and sensitivity and minimizing the relative standard deviation of the results. Data were analyzed statistically using Student test and $p < 0.5$. Optimization of conditions was carried out using 2 kinds of herbs: “Hemus” and “Hebar”, and a lot of experiments combining 4 weights of sample in the headspace vial, 3 sample equilibration times and 3 temperatures of the oven and triplicate analyses. Relative standard deviation calculated on the base of peak area of some components from FID signal, showed reliable results of the HS-GC method. After statistical analyses of data the following conditions were chosen.

Sample weight

Headspace sample vials are typically in 10 ml and 20 ml sizes. We used 20 ml vials in order to have more possibilities to vary the sample weight.

The analytical chemist would increase the concentration of the sample or inject more sample onto a column to get a better signal. With headspace, more sample volume does not always provide the expected increase in peaks areas. We changed the sample weight from 1 to 10 g. The other parameters were maintained the same – sample equilibration

time 60 minutes, oven temperature 70°C. The observed peak areas were those of the two main compounds – Linalool and Linalyl acetate.

Table 1. Composition of essential oils of *Lavandula angustifolia* Mill (Bulgarian kinds).

Compound/Kind	Raya	Hebar	Hemus	Yubileina	Drujba	Karlovo	Sevtopolis	RSD (%)
Origanene	tr*	0.05	tr	0.23	0.14	tr	tr	
α - Pinene	0.12	0.18	0.13	0.44	0.22	0.71	0.25	6.1
Camphene	0.21	0.37	0.28	0.22	0.31	0.50	0.38	7.6
β - Pinene	0.23	0.20	0.29	0.15	0.34	0.53	0.36	5.4
3-Octanone	2.10	1.52	1.12	1.35	2.40	0.50	2.98	4.3
Myrcene	0.67	0.76	0.90	0.77	1.07	0.53	0.85	5.6
1-Octen-3-ol	0.21	0.16	0.10	0.19	0.56	0.17	0.45	5.3
6-Methyl-5-hepten-2-one	0.11	0.46	0.16	0.14	0.49	0.06	0.20	6.1
Hexyl acetate	0.44	0.89	0.44	0.64	1.10	0.73	1.21	7.5
α - Phelandrene	tr	0.08	tr	0.04	0.07	tr	0.05	
p – Cymene	0.10	0.19	0.13	0.27	0.26	0.08	0.15	7.7
1,8 - Cineole	0.83	1.29	2.39	0.44	4.23	0.31	2.17	5.0
Limonene	0.70	0.83	1.96	0.25	1.93	1.11	1.12	6.8
cis – β - Ocimene	2.22	6.17	1.70	7.09	1.87	3.88	2.53	4.3
trans – β - Ocimene	1.63	4.18	1.03	4.07	1.73	0.55	3.26	4.4
γ-Terpinene	tr	tr	tr	0.24	0.10	tr	tr	
cis – Linalool oxide	tr	tr	tr	0.15	0.08	tr	tr	
trans – Linalool oxide	0.05	0.10	0.09	0.18	0.05	0.24	0.05	6.9
α - Terpinolene	0.11	0.16	0.15	0.21	0.12	0.22	0.11	5.8
Linalool	40.58	30.55	36.26	25.24	40.02	47.29	28.92	1.4
Chrisanthenone	0.09	0.08	0.08	0.10	0.07	0.09	0.08	7.2
Octene-3-yl-acetate	1.04	0.94	0.91	1.17	0.79	1.15	2.27	6.5
5 - Caranol	0.19	0.12	0.09	0.12	0.15	tr	0.33	7.3
Camphor	0.18	0.38	0.22	0.31	0.19	0.09	0.33	5.3
Borneol	0.50	1.81	1.24	0.86	0.96	1.08	2.06	4.6
Lavandulol	0.09	0.07	0.11	tr	0.11	0.11	0.12	6.3
1-Terpinen-4-ol	0.07	0.08	0.08	7.35	1.79	0.20	0.10	7.1
p-Cymene-1-ol-8	0.41	0.60	0.76	0.22	0.87	0.21	0.79	5.3
α- Terpineol	1.70	1.44	1.76	1.24	1.63	1.55	1.60	5.1
Hexyl butyrate	0.34	0.31	0.36	0.24	0.24	0.25	0.33	6.2
Geraniol	0.15	0.17	0.28	0.07	0.27	0.09	0.22	6.1
Linalyl acetate	32.36	36.41	37.63	34.82	19.91	29.32	27.55	1.8
Bornyl acetate	0.33	0.25	0.21	0.25	0.05	tr	0.25	5.4
Lavandulyl acetate	3.43	2.63	2.90	3.78	2.66	2.45	4.43	4.0
Neryl acetate	0.37	0.34	0.42	0.27	0.26	0.34	0.37	6.3
Geranyl acetate	0.66	0.65	0.78	0.51	0.40	0.59	0.56	5.8
β- Caryophylene	3.30	1.73	1.77	2.71	3.28	3.32	5.19	4.2
α-Santalene	0.10	0.09	0.07	0.11	0.15	0.17	0.12	7.1
β-Farnesene	1.92	1.96	1.59	1.00	4.41	1.05	4.65	5.6
Germacrene D	0.58	0.24	0.08	0.41	0.69	0.23	1.05	8.5
γ-Cadinene	0.07	0.07	0.04	0.15	tr	tr	0.10	8.1
Caryophylene oxide	0.41	0.24	0.17	0.36	0.23	0.30	0.45	5.3
α-Cadinol	tr	tr	0.26	tr	0.23	0.12	0.18	4.2

*tr (trace) < 0.05 %

The results obtained running triplicates are given in Table 2. With 7 g the sample vial is filled about half way. The results show, that sample weight higher than 7 g do not give the expected increase in signal. Higher weights (above 10 g) would decreased the headspace volume and the needle would came in contact with the material, which is not desirable.

Table 2. Influence of sample weight in headspace vial.

Kind	Hemus				Hebar			
Weight (g)	1	4	7	10	1	4	7	10
Linalool*	138 ±5.0	385 ±6.1	1175 ±5.0	1205 ±4	156 ±5.0	438 ±5.2	1306 ±5.1	1398 ±5.2
Linalyl acetate*	203 ±5.3	350 ±6.0	1079 ±6.2	1203 ±5.0	221 ±5.6	495 ±5.5	1578 ±5.0	1602 ±5.8

*Compound/ Area ± RSD (%)

Sample equilibration time

In Table 3 the peak areas from triplicates analyses are shown using equilibration time 30, 60 and 100 minutes. They were chosen on the base of duration of steam distillation of herbs and some references related to HS of oils [2,7,10,12]. The other parameters were maintained the same – sample weight 7 g, oven temperature 70°C. As can be seen, equilibration time longer than 60 min do not give a significant increase in peaks areas. Considering all chromatograms we noted more scatter in the data in 100 min, which could be indicative of secondary reactions. These data show 60 min to be sufficient with less risk of secondary reactions occurring.

Table 3. Influence of sample equilibration time.

Kind	Hemus			Hebar		
Time (min)	30	60	100	30	60	100
Linalool*	1110 ±4.5	1175 ±5.0	1099 ±5.6	1300 ±4.6	1306 ±5.2	1340 ±5.3
Linalyl acetate*	1060 ±4.6	1079 ±5.2	1085 ±5.4	1620 ±4.7	1578 ±5.0	1650 ±5.2

*Compound/ Area ± RSD (%)

Temperature of the oven

The temperature of the oven was maintained at 40°C, 70°C and 100°C (Table 4). The investigations were carried out using the herb “Hemus”. The temperature 70°C was chosen because of the statements that the steam distillation of the herbs begins at 70°C [2,4]. The chromatograms obtained in 70°C showed higher signals than those in 40°C. The chromatograms obtained in 100°C were very reach of peaks and the reproducibility was low. On the other hand, the compositions of the headspace gas in 100°C and those of the respective essential oils are very similar. For further work, we intend to continue the investigations upon temperatures higher 70°C.

Table 4. Influence of temperature of the oven.

Kind	Hemus		
Temperature (ºC)	40	70	100
Linalool*	238 ±5.6	1175 ±5.0	3892 ±11
Linalyl acetate*	303 ±5.4	1079 ±5.3	3934 ±12

*Compound/ Area ± RSD (%)

As we used the standard headspace extraction of herbs, the influence of the partition coefficient caused by solid samples has to consider. Unfortunately, with solid materials it is very difficult to eliminate the influence of the sample matrix and it is not possible to apply commonly used method in HS analyses. Because of the complexity of the headspace gas, it was not possible to reproduce exactly the mixture of all volatile compounds for each herb, as the external standard method requires. So, the results of GC-FID analyses of essential oils can not direct compared with those of analyses of HS gas. But the calculation of HS gas composition using relative peak areas could give the available components and their distribution in the herb.

In Table 5 the results from HS-GC analyses of fresh flowers of the investigated Bulgarian kinds are shown. It is observed significant difference between the composition of the oils and respective herbs in the content of lighter than β-pinene compounds. While the first components in the chromatograms of oils are origanene and β-pinene, there are about 10 lighter components in the chromatograms of herbs. Probably, at steam distillation of the herbs, these components fly out and do not remain in the oils (Fig.1 and Fig.2).

The chromatographic profiles of the used essential oils meet the requirements of ISO 3515:2000.

Table 5. HS-GC of fresh flowers.

Compound/Kind	Raya	Hebar	Hemus	Yubileina	Drujba	Karlovo	Sevtopolis
∑ lights before β - Pinene	13.25	14.35	17.87	15.64	18.06	20.52	15.79
β - Pinene							
3-Octanone	7.52	3.98	4.96	3.60	5.20	1.46	6.17
Myrcene	1.04	1.01	1.61	1.49	1.90	1.50	2.05
1-Octen-3-ol							
6-Methyl-5-hepten-2-one							
Hexyl acetate	1.39	2.42	1.50	1.63	2.34	1.61	2.50
α - Phelandrene							
p – Cymene							
1,8 - Cineole	1.12	0.79	3.68	0.68	1.45	0.65	4.06
Limonene	1.75	0.63	3.74	0.19	1.65	2.01	1.18
cis – β - Ocimene	6.45	16.85	5.06	18.40	5.26	13.71	7.56
trans – β - Ocimene	5.92	13.03	3.34	12.92	5.17	1.94	9.75
γ-Terpinene							
cis – Linalool oxide							
trans – Linalool oxide							
α - Terpinolene							
Linalool	35.29	21.86	27.77	22.01	12.32	32.96	25.43
Chrisanthenone							
Octene-3-yl-acetate	1.08	0.95	1.23	1.13	1.56	1.49	0.63
5 - Caranol							
Camphor							
Borneol	0.42	0.32	0.31	0.54			0.78
Lavandulol							
1-Terpinen-4-ol				4.60	1.34		1.30
p-Cymene-1-ol-8							
α- Terpineol		0.24	0.37	0.36		0.42	0.63
Hexyl butyrate							
Geraniol							
Linalyl acetate	21.50	22.25	23.00	16.85	22.93	19.40	18.10
Bornyl acetate							
Lavandulyl acetate	1.16	0.77	0.83	1.68	1.21	1.19	2.50
Neryl acetate							
Geranyl acetate							
β- Caryophylene	1.59	0.85	0.83	1.30	1.46	1.54	2.60
α-Santalene							
β-Farnesene	0.77	0.75	0.71	0.49	2.19	0.59	2.29
Germacrene D							
γ-Cadinene							
Caryophylene oxide							
α-Cadinol							

When comparing data and chromatograms of the herbs and respective oils one can see that although they are not the same, the profiles are very closed. Increasing or reducing the content of any component in the oil or herb is proportional and it is an useful mean for evaluation of the quality of herbs. Chemometric analysis will be reported in our next papers.

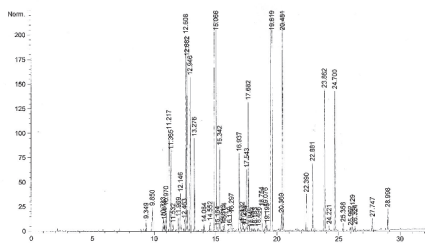

Fig. 1. GC – FID Chromatogram of essential oil of “Hemus”.

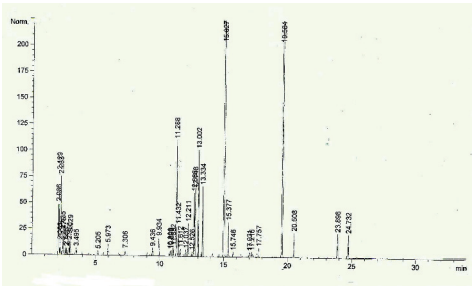

Fig. 2. HS – GC Chromatogram of fresh flowers of “Hemus”.

CONCLUSION

HS-GC method is developed for extraction and simultaneously GC monitoring of volatile components composition of aromatic herbs. Investigations of the influence of sample weight, sample equilibration time and temperature of the oven are carried out and the optimal conditions giving reproducible peak areas are given. The method is used for analyses of different Bulgarian kinds of *Lavandula angustifolia* Mill. For comparison, data of essential oil compositions are given, using GC-FID analyses and GC-MS identification. By calculation of the relative peak areas of HS chromatograms of herbs, an information about the profiles of the respective oils can be received. Further studies are in progress in our laboratory concerning new seed populations and other plant materials.

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About the authors:

Assoc. Prof. Dr Rumyana Milina, Central Research Laboratory, Faculty of Natural Science, Assen Zlatarov University – Burgas 8010, Bulgaria, Phone: +35956858332, e-mail: rumjana_milina@abv.bg.

Chemist Zilya Mustafa, Central Research Laboratory, Faculty of Natural Science, Assen Zlatarov University – Burgas 8010, Bulgaria, Phone: +35956858332, e-mail btu_cnil@abv.bg.

Dr Stanko Stanev, Institute for roses and aromatic plants, Kazanlak 6100, Bulgaria, e-mail: sdstanev@abv.bg.

Assistant Prof.Dr Dilyana Zvezdova, Department of Organic Chemistry, Assen Zlatarov University - Burgas, Phone: +35956858254, e-mail: dzvezdova@yahoo.com.

Assoc. Prof. Dr Stoyanka. Stoeva, Department of Organic Chemistry, Assen Zlatarov University - Burgas, Bulgaria, Phone: +35956858371, e-mail: sstoeva@btu.bg.

The paper is reviewed.