

ADVANTAGES OF “HEADSPACE” TECHNIQUE FOR GC/MS ANALYSIS OF ESSENTIAL OILS

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Abstract

The essential oils of Fir needle and Juniper obtained in Romania by industrial scale steam distillation of fresh material collected from wild flora were analyzed by gas chromatography-mass spectrometry (GC-MS), using two different techniques of injection: “split-splitless” and “headspace” (HS/GC-MS). The determined chemical composition of each essential oil was compared with literature data and previous results obtained by the authors. Qualitative and quantitative differences were obtained between the two injection used techniques.

Rezumat

Uleiurile volatile de brad și ienupăr, obținute prin procedeul industrial de distilare cu vapori de apă a materialului vegetal proaspăt recoltat din flora spontană, au fost analizate prin gaz cromatografie cuplată cu spectrometrie de masă (GC-MS), utilizând două tehnici diferite de injectare a probei: “split-splitless” și “headspace” (HS/GC-MS). Compoziția chimică determinată pentru fiecare ulei volatil a fost comparată cu datele din literatură și cu rezultatele obținute anterior de către autori. S-au observat atât diferențe calitative cât și cantitative între cele două tehnici de injectare folosite.

Keywords: essential oils, fir needle, juniper, GC-MS, HS/GC-MS

Introduction

Fir and Juniper essential oils are very important pharmaceutical products obtained from species growing wild in the temperate climate.

The generic name of fir needle essential oil refers to the oils obtained by extraction from needles and twigs of different species of *Abies* (*Pinaceae* family). The best known specie is the silver fir, *Abies alba* Mill. [7].

The Juniper essential oil is extracted from ripe berries (cones) of *Juniperus communis* L. (*Cupresaceae* family) collected in the second year of vegetation [4, 7].

Materials and Methods

Essential oils

The essential oils used in this study were samples of fir needle and juniper essential oils obtained in Romania by industrial scale steam distillation of fresh material collected from the wild flora.

Experimental method

The analysis of the essential oils was performed using a gas chromatography – mass spectrometry (GC-MS) method [1, 9]. The analysis method was developed on a Shimadzu GC-MS QP-2010 model gas chromatograph – mass spectrometer equipped with an AOC-5000 autosampler (CombiPAL). Alltech, USA, AT-5 capillary column of 30m x 0.25mm i.d. and 0.25µm film thickness was used. The parameters for the method were: injector temperature 250°C; pressure 37.1 kPa; column flow 1.5 mL/min; linear velocity 44.7 cm/s; split ratio 1:300, sample volume 2.5 µL (fir needle oil) and 1 µL (juniper oil). Carrier gas helium; detector: MS, ion source temperature 250°C; interface temperature 250°C; MS mode: EI; mass range: 40-650u (fir needle oil), 40-450u (juniper oil); scan speed: 769u/s. The temperature program for column oven is presented in Table I for the essential oils analyzed. In the case of “*headspace*” technique (HS/GC-MS) the samples were incubated at 85°C for 15 minutes prior the injection.

The identification of the components separated by GC-MS was made by comparing the obtained mass spectra for each component with the values stored in mass spectra libraries, NIST27 and NIST147. The percentage composition of the oils was calculated in peak areas using the normalization method.

Table I
Column oven temperature program

Entry	Step 1			Step 2			Step 3		
	T	t	r	T	t	r	T	t	r
Fir needle oil	35	2	-	250	-	5	250	5	-
Juniper oil	60	3	-	210	-	3	210	3	-

T - temperature (°C); t – time (min); r – rate (°C/min)

Results and Discussion

Fir needle oil

The results of the GC-MS analysis, using split-splitless injection revealed 19 components in the essential oil, and 15 of them were identified,

representing 96.32% of total oil composition. Using HS/GC-MS, 38 components were separated using the same working conditions, from which 22 components were identified, representing 96.34% of the total composition of the oil (Figure 1, Table II). Almost all identified components were reported previously in the literature data available for fir needle oils [4, 7, 8].

Table II
Composition of Fir needle essential oil
(the components identified in the highest yields are in bold)

RT	Component	A1 (%)	A2 (%)	MW	S%	BP
8.95	Unknown C₉H₁₂O	3.133	3.133	136	89	79
10.20	Tricyclene	1.652	2.634	136	97	93
10.60	α-pinene	30.172	26.957	136	97	93
11.09	Camphene	15.176	13.539	136	97	93
12.05	β-pinene	31.130	30.939	136	96	93
12.56	β -myrcene	0.869	0.800	136	97	41
13.00	α -phellandrene	0.055	0.158	136	92	93
13.22	3-carene	Not found	0.082	136	92	93
13.69	(+)-4-carene	Not found	0.021	136	86	93
13.67	o/m-cymene	0.083	0.257	134	93	119
13.83	Limonene	10.066	12.356	136	91	93
13.92	1,8-cineole	0.063	Not found	154	89	81
14.85	γ -terpinen	Not found	0.039	136	88	93
15.83	Terpinolen	0.114	0.362	136	96	93
15.98	α -campholenal	Not found	0.087	152	88	108
16.18	Linalyl acetate	Not found	0.047	196	87	93
17.68	Camphor	Not found	0.047	152	91	95
18.35	Borneol	Traces	0.209	154	91	95
19.11	α -terpineol	0.130	0.256	154	89	93
22.04	Bornyl acetate	4.123	7.110	196	95	95
25.94	Caryophyllene	Not found	0.176	204	95	41
26.12	Himachala-2,4-diene	Not found	0.110	204	89	133
26.86	α -humulene	Not found	0.155	204	94	93
Component groups						
	Monoterpenes hydrocarbons	89.234	87.887			
	Oxygenated monoterpenes	4.123	7.756			
	Esters	4.123	7.157			

RT - Retention time (min); A1 – Area GC-MS (%); A2 – Area HS/GC-MS (%); MW – molecular weight; S – Similarity with mass spectra libraries, NIST27 and NIST147 (%); BP – Base Peak.

The molecular weight, base peak and similarity were obtained following HS/GC-MS analysis.

The main group of identified components highlighted in Table II is that of monoterpenes hydrocarbons with 89.234% of the total composition for GC-MS and, respectively 87.887% for HS/GC-MS.

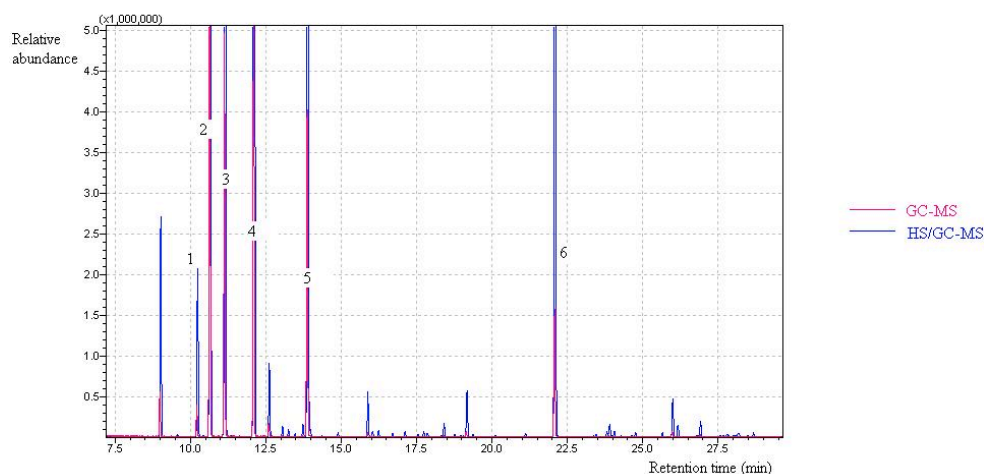


Figure 1

GC-MS versus HS/GC-MS profiles of fir needle essential oil (1 - Tricyclene, 2 - α -pinene, 3 - Camphene, 4 - β -pinene, 5 - Limonene, 6 - Bornyl acetate).

Considering the literature data [7], the high content of monoterpenes hydrocarbons and low content of esters is specific for fir needle oils obtained from *Abies alba* Mill. species. Also, the key components which can be found only in the oils extracted from *Abies* species, are santene and tricyclene. These two components are not found in the composition of oils from other conifers [7]. Santene was not identified in our sample. Tricyclene was identified and represents 1.652% (GC-MS), respectively 2.634% (HS/GC-MS), which confirms with the literature data which gives a content of tricyclene between 1.3-2.1% [7].

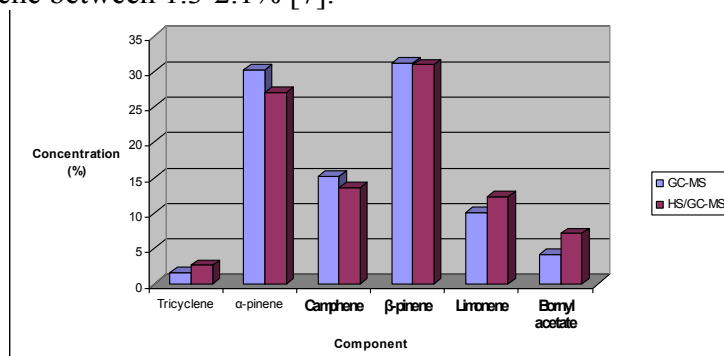


Figure 2

Concentration of the main components in Romanian Fir needle essential oil.

In the case of fir needle oil the percent of detection of monoterpene hydrocarbons decreased when headspace injection was used, with exception of tricyclene and limonene. In the same time, the detection of bornyl acetate was improved (Figure 2). The observed decrease of some major components when HS/GC-MS technique was used may be explained by identification of minor components from the classes of oxygenated monoterpenes and esters. These minor components are not detected by the GC-MS due to the shielding of major components located in the first part of the chromatograms (Table II).

Juniper oil

HS/GC-MS permitted the identification and quantification of 22 components from the 30 components separated from juniper oil. Thus, 95.88% of the total composition of this oil was characterized. Nine compounds from a total of 16 were identified when using normal injection and the same conditions for GC-MS, representing 99.018% from total amount. The main components were α -pinene, *p*-cymene, sabinene, β -pinene, β -myrcene and limonene (Table III). All these compounds were reported before as components of juniper oils [2, 3, 5, 6, 7].

The investigated juniper essential oil was of α -pinene chemotype. The first major component identified was α -pinene (84.768% from the total amount by GC-MS and 60.978% from the total amount when using HS/GC-MS). The second main component identified was *p*-cymene (5.187% when using GC-MS and 16.175% when using HS/GC-MS). The third component detected was sabinene (2.042% when using GC-MS and respectively 5.384% when using HS/GC-MS). When using GC-MS, β -pinene was the fourth component with the same amount as sabinene (2.042%), unlike limonene as fourth component when using HS/GC-MS (2.608%). The fifth and the sixth components were β -myrcene and limonene (GC-MS), respectively β -pinene and β -myrcene (HS/GC-MS) (Table III). The reversal in the content of the components illustrates the versatility of headspace technique for the determination of the composition of essential oils, particularly of minor components.

The main group of components in juniper oil was represented by monoterpene hydrocarbons (91.706% for GC-MS, and respectively 74.449% for HS/GC-MS). The second group, in fact *p*-cymene component, was represented by aromatic terpenes (5.187% for GC-MS, and respectively 16.175% for HS/GC-MS). The amounts of the other two groups (oxygenated monoterpenes and sesquiterpenes) were markedly smaller than those of monoterpene hydrocarbons (Table III).

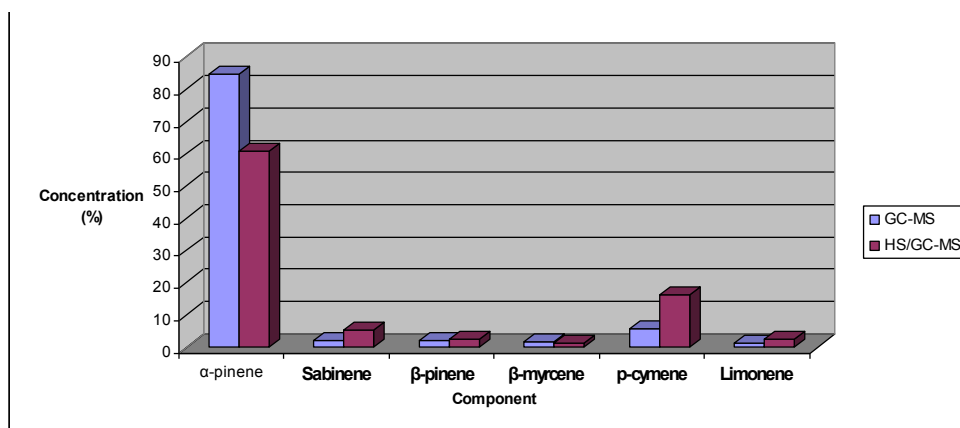
Table III
Composition of Juniper essential oil
(the components identified in the highest yields are in bold)

RT	Component	A1 (%)	A2 (%)	MW	S%	BP
7.25	β -thujene	Not found	0.122	136	89	93
7.40	α -phellandrene	0.924	2.778	136	96	93
7.65	α-pinene	84.768	60.978	136	97	93
8.18	Camphene	0.242	0.496	136	96	93
8.38	Unknown C10H14	0.240	0.312	134	84	91
9.11	Sabinene	2.042	5.384	136	93	93
9.25	β-pinene	2.042	2.522	136	96	93
9.79	β-myrcene	1.627	1.344	136	96	93
10.82	1,4-cineole	0.152	0.197	154	90	43
11.19	<i>p</i>-cymene	5.817	16.175	134	97	119
11.39	Limonene	1.404	2.608	136	93	68
14.10	Unknown C10H12	Traces	0.285	132	88	117
14.93	Trans-verbenol	Traces	0.680	152	89	91
15.85	α -camphonelal	Traces	0.298	152	86	108
16.45	Trans-pinocarveol	Traces	0.204	152	85	91
16.75	Carveol	Traces	0.487	152	84	91
18.29	Unknown C10H18O	Traces	2.620	154	87	93
18.62	Unknown C12H16O2	Traces	0.439	192	84	117
18.92	α -terpineol	Traces	0.255	154	86	93
19.82	Verbenone	Traces	0.199	150	89	107
23.47	Bornyl acetate	Not found	0.213	196	84	95
26.43	α -cubebene	Not found	0.175	204	85	105
27.65	Copaene	Not found	0.139	204	81	105
28.35	β -elemene	Traces	0.216	204	95	93
32.05	Germacrene D	Not found	0.183	204	89	161
33.08	α -muurolene	Not found	0.227	204	81	105
33.66	Unknown C15H24	Not found	0.230	204	87	161
Component groups						
	Monoterpene hydrocarbons	93.049	76.232			
	Oxygenated monoterpenes	0.152	2.533			
	Sesquiterpene hydrocarbons	0	0.94			
	Aromatic terpenes	5.187	16.175			
	Total	98.388	95.88			

RT - Retention time (min); A1 – Area GC-MS (%); A2 – Area HS/GC-MS (%); MW – molecular weight; S – Similarity with mass spectra libraries, NIST27 and NIST147 (%); BP – Base Peak.

The molecular weight, base peak and similarity were obtained following HS/GC-MS analysis.

The headspace injection favoured the identification of aromatic terpenes, as *p*-cymene (Figure 3).

**Figure 3**

Concentration of the main components in Romanian Juniper essential oil

Compared with the literature data [2, 3, 5, 6], the content of monoterpene hydrocarbons found in Romanian juniper oil was in the same range with other juniper oils from European origin (Estonia 58%, Lithuania 61.4-80.8%, Greece 59.42% and Serbia 72.4-82.8%). The content of the main component, α -pinene, of the Romanian juniper oil (60.978-84.768%) was higher than the one found in the previously named essential oils (Estonia 47.9% [6], Lithuania 39.7-64.9 % [2], Greece 40.29% [3] and respectively Serbia 36.6-40.5% [5]). The content of *p*-cymene found in Romanian juniper oil (5.817-16.175%) was significantly higher than the ones found in the oils previously analyzed. In fact, only for the Serbian juniper oils, this content was 2.2-2.3%, for one of the oils from Lithuania was 2%, and much smaller for the other oils [2, 3, 5, 6]. The amount and the number of sesquiterpenes of Romanian juniper oil were smaller than those found in literature data [2-7].

Conclusions

The composition of the tested samples of fir needle and juniper oils originating from Romania is in agreement with data reported in the literature, with some significant particularities. However, santene from fir needle oil and few sesquiterpenes from juniper oil were not identified in this work.

Both types of sample injection (liquid and headspace) showed high applicability for the evaluation of essential oils composition. Headspace injection offers a reliable and fast method for the evaluation of different samples of essential oils. In both cases, a significant improvement of the

number of components identified was observed when using HS/GC-MS. The further development of the method may be of interest.

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