

Application of SPE for selective fractionation of essential oils constituents from plant materials

Joanna Szewczyk*, Michał P. Dybowski and Andrzej L. Dawidowicz

*Maria Curie-Skłodowska University, Faculty of Chemistry,
Department of Chromatographic Methods, Maria Curie-Skłodowska Sq.3,
20-031 Lublin, Poland*

*email: asiaszewczyk2@wp.pl

Solid-phase extraction (SPE) is simple and inexpensive sample preparation procedure which can be applied for the isolation/fractionation of essential oil compounds from wide variety of samples, such as foodstuffs, biological and environmental. Due to the complex nature of the examined matrices and frequently low concentration level of target components, analytical procedures require the use of initial sample preparation stage. The paper shows the possibility of essential oil components fractionation from different plant materials using SPE method.

The results presented in this paper shows that the proposed SPE procedure allows for easy and total fractionation of essential oil constituents (especially low-molecular oxygen compounds) from the sample matrix.

Keywords: SPE, essential oils from herbs, fractionation

1. INTRODUCTION

Essential oils (EO) isolated from aromatic herbs and plants are multi-component mixtures of monoterpenes and sesquiterpenes, and their oxygenated derivatives such as aldehydes, alcohols and esters [1-4]. As EO are commonly used by food, pharmaceutical, cosmetic and fragrance industries, hence, it is important to evaluate the aroma-active

essential oil components, generally existing in different concentration range from high level of concentration to traces. Nevertheless, presence of traces has also significant impact as they are responsible for the specific natural flavor and odor. Therefore, it is important to applied the sample preparation stage used in order to recover EO from plant sample, enabling maintain the natural percentage of its primary compounds [5-7].

Many approaches of EO components fractionation have been reported in literature. Traditional methods applied in order to isolate of EO constituents from plant material are hydrodistillation, steam-distillation and liquid-liquid extraction [8-10]. Steam distillation is pharmacopoeial method recommended to separate EO from plant matrixes [11]. However because of time consumption and low extraction efficiency in analysis of organic components from numerous plant samples, in recent years it is frequently replaced by more effective technological approaches [12–13], such as pressurized liquid extraction (PLE) [14], supercritical fluid extraction (SFE) [15] and matrix-solid phase dispersion (MSPD) [16].

Among assisted extraction techniques, solid phase extraction (SPE) [17] is actually the most commonly studied application. Due to allows for selective and rapid preparation step, is applied to a wide variety of samples such as foodstuffs, biological and environmental [18–21]. SPE complies with the requirements of green chemistry by reduction or total elimination of solvent consumption during in analytical procedures. Its versatility allows the application of SPE for many purposes, i.e. purification, isolation, pre-concentration and class fractionation [18, 22]. This method is a good approach for the extraction/concentration of components from aromatic plants, as many solid phases are available enabling increase of selectivity of the process. In addition, enrichment of the aromatic components from extract can be accomplished by using a small amount of organic solvent during the elution step from the solid phase [23–24].

This paper reports the possibility of essential oil components fractionation using SPE method. The article shows results for following plant materials: peppermint, juniper, thyme, lemon and grapefruit.

2. EXPERIMENTAL

2.1. *Materials and chemicals*

The following plant materials were used in the experiments: peppermint (*Mentha piperita L.*), juniper (*Juniperus L.*), thyme (*Thymus vulgaris L.*), lemon (*Citrus limon L.*) and grapefruit (*Citrus paradisi*). All of them were cultivated in eastern Poland. The herbs were air-dried, cut and stored at +8°C. Immediately before steam distillation, an appropriate amount of plant material was ground and its exactly weighed portions were subjected to the applied sample preparation procedures. Hexane, methanol, acetonitrile, 1,4-dioxane, all of them of analytical grade, were supplied by the Chemical Plant POCH S.A. (Gliwice, Poland). The Septra C18-E sorbent (50 mm, 65 Å), used in the SPE process, was purchased from Phenomenex (Torrance, CA, USA).

2.2. *Steam distillation*

Essential oils from the used plants were obtained by steam distillation. The steam distillation process was performed for 3 h applying the Deryng apparatus (the Deryng apparatus is a Clevenger-type apparatus; it is described in detail in the Polish Pharmacopea V) which contained a plant sample (50 g) and 600 cm³ of water. The distillation time was measured after the fall of the first drop of the distillate. The separated essential oil distillate was dried by freezing and, after filtration, stored at +4°C until further experiments.

2.3. *SPE*

SeptraC18-E SPE cartridge (0.5 g) was washed with hexane (5 mL) and then vacuum-dried (5 min). Afterwards, examined essential oil was loaded into SPE cartridge. The elution of components was carried out using methanol/water mixtures of a differential composition (2 cm³ portion)- fraction I and 5 cm³ of hexane- fraction II. Reference solutions of essential oils were prepared combining 50 mm³ of essential oil with 5 cm³ of hexane solution. The recoveries for essential oil components eluted in the fractions I and II were obtained by comparison of the peak area of compound eluted in fraction II to its peak area in applied reference solution. The reference materials were analyzed applying to the proposed procedure in order to determine the accuracy of the method. Recoveries, calculated as percent of the true value for each

essential oil compound, provide determination of the accuracy of the proposed analytical SPE procedure.

2.4. Chromatographic analysis

Qualitative analyses of components in the prepared samples of essential oils were carried out using GC/MS QP2010 (Schimadzu, Kyoto, Japan) equipped with 30 m × 0.25 mm id. 0.25 μm film thickness ZB5-MS fused silica capillary column (Phenomenex). The column was operated with helium as carrier gas (1 cm³/min). The temperature of injection was 310 °C and the volume of injected sample was 1 μL. During injection the split mode was applied (purge time- 0.7 min) The following temperature program was administered: 1 min at 50 °C and it was then linearly raised at the rate of 6 °C/min to 310 °C. EI mode at 70 eV was used in the mass spectrometer and 220 °C was the temperature of ion source. The mass range was from 35 to 360 amu. Qualitative analysis was performed by comparing the retention indexes and MS spectra for the obtained peaks with the analogous data from mass spectrometry library (NIST'05).

Quantitative analyses were carried out using gas chromatograph with a flame ionization detector (GC/FID, Schimadzu 2010). Hydrogen was used as carrier gas (1 mL min⁻¹). Experimental conditions were the same as for GC/MS. Peaks identification was performed basing on the experimentally determined indexes of retention.

3. RESULTS AND DISCUSSION

Table 1 shows the percent amounts of mint essential oil components retained in the SPE column after its washing out with methanol-water mixtures of different composition.

The values were obtained by relating the peak area of essential oils components eluted from the SPE column (fraction II) to the peak area in an appropriate reference solution. It should be remembered that essential oil reference solution contained 50 mL of the oil in 5 mL hexanic solution and that the SPE column was loaded with 50 mL of the oil and then, in the second elution step, was washed out with n-hexane to reach 5 mL of hexanoic eluate.

The application of C-18 sorbent and 70% methanol causes total or almost total elution of certain components from the sorbent (cis-4-thujanol, p-menthon, isomentol, menthol, piperitone) with

simultaneous retention of other essential oil components. The average recovery of components eluted from the SPE column by 70% methanol equals almost 100%, whereas an average content of compounds retained in SPE column after its elution with 70% methanol exceeds 85% (without taking into account the above ingredients, for which the recovery was below 5%) – see the last line of Table 1, which is a satisfactory result of preliminary separation for analytical and preparative purposes.

Table 1. The percentage of mint essentials oil components retained by SPE cartridge after its washing out using methanol/water mixture (i.e. composition of fraction II); n = 5, RSD < 3.5%.

No.	Compound	RI*	Methanol concentration (%)						
			30	40	50	65	70	75	80
1.	3-Thujone	962	100	100	100	100	100	94.13	90.35
2.	Pinene	977	100	100	100	100	100	97.48	91.74
3.	β -Phellandrene	1046	100	100	100	100	100	97.21	92.18
4.	cis-Sabinene hydrate	1068	100	100	100	100	100	97.29	91.82
5.	3-Octanol	1088	100	100	100	100	100	98.64	93.98
6.	3,5-Dimethyloctane	1094	100	100	100	100	100	91.64	80.98
7.	α -Terpinene	1141	100	100	100	100	100	97.45	91.57
8.	o-Cymene	1161	100	100	100	100	100	94.48	91.01
9.	D-Limonene	1173	100	100	100	100	100	97.65	91.74
10.	Eucaliptol	1184	100	96.14	91.42	88.49	20.82	9.53	4.53
11.	γ -Terpinene	1251	100	100	100	100	100	97.78	92.3
12.	cis-4-Thujanol	1291	100	92.65	75.14	0.81	T	T	T
13.	Terpinolene	1338	100	100	100	100	100	92.79	86.29
14.	Linalool	1384	100	95.32	75.19	12.18	10.48	9.50	9.09
15.	2-(methylbutyl)-iso-valerat	1414	100	100	100	100	93.83	87.93	39.48
16.	p-Menthanon	1634	100	100	100	100	30.81	12.58	1.75
17.	Menthofurane	1662	100	100	100	100	100	95.58	91.82
18.	p-Mentone	1671	100	91.97	76.50	23.71	6.03	2.5	2.35
19.	Isomenthol	1689	100	98.00	83.68	30.01	7.27	4.8	0.93
20.	Menthol	1730	100	95.20	81.94	4.24	0.99	0.84	T
21.	γ -Terpineol	1782	100	75.32	66.92	35.98	33.59	31.63	27.68
22.	Pulegone	1911	100	96.98	82.74	21.37	21.03	14.74	6.72
23.	Piperitone	1955	100	91.67	77.57	0.79	0.77	0.62	0.52
24.	Menthyl acetate	1995	100	100	100	100	92.83	91.16	75.38
25.	o-Menth-8-en	2136	100	100	100	100	100	100	100

cont. Table 1.

No.	Compound	RI*	Methanol concentration (%)						
			30	40	50	65	70	75	80
26.	α -Burbonene	2249	100	100	100	100	100	96.51	90.05
27.	Caryophyllene	2323	100	100	100	100	100	100	100
28.	Muurolene	2391	100	100	100	100	100	100	100
29.	α -Cubene	2438	100	100	100	100	100	100	100
30.	Eriksene	2464	100	100	100	100	100	100	100
31.	δ -Cadinene	2498	100	100	100	100	100	99.5	97.89
32.	Wiridofloren	2561	100	100	100	100	93.2	80.34	14.81
Average recovery (%)			100	97.91	94.10	78.67	74.57	70.81	65.23

* – Kovats Retention Index; T – less than 0.05%.

The comparison of the chromatogram A (chromatogram of the mint essential oil reference solution) and the chromatogram B (chromatogram of the second fraction of mint essential oil, which contains all components retained by the SPE column beforehand washed out with 70% methanol) shows the difference between both fractions (Figure 1).

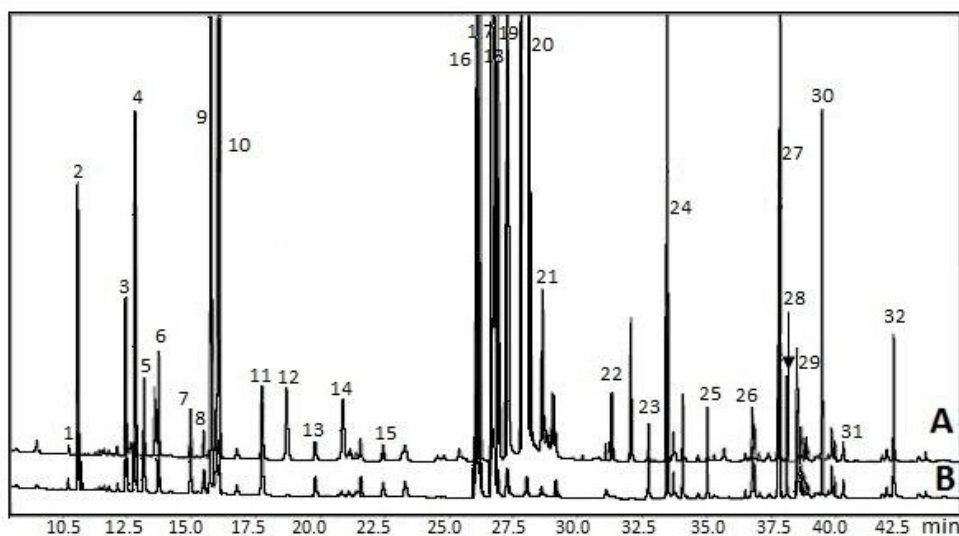


Fig. 1. Exemplary GC/FID chromatogram of mint essential oil (A) and chromatogram of SPE fraction II from mint essential oil (B).

As results from the comparison, peaks relating to: *cis*-4-thujanol, *p*-menthon, isomentol, menthol, piperitone in the Table 1 were decreased on the chromatogram B, indicating removal these compounds during fractionation process.

Data from Table 1 and Figure 1 show that the application of column packed with octadecyl silica sorbent can be used for the isolation of mint essential oil components. To check validity of this statement, it was decided to carry out the same experiments using by SPE C-18 procedure for various plant materials.

In the case of juniper essential oil (Table 2) washing out the SPE column with 75% methanol causes complete removal of some components: borneol, terpinen-4-ol, α -terpineol (low-molecular oxygen compounds). The average recovery of the remaining components of juniper essential oil exceeds 94%.

Table 2. The percentage of juniper essential oil components retained by SPE cartridge after its washing out using methanol/water mixture (i.e. composition of fraction II); $n = 5$, RSD < 3.5%.

No.	Compound	RI*	Methanol concentration (%)						
			10	15	25	50	65	75	100
1.	α -Pinene	935	100	100	100	100	100	100	1.93
2.	Camphene	952	100	100	100	100	100	100	1.69
3.	Sabinene	972	100	100	100	100	100	100	1.82
4.	β -Pinene	980	100	100	100	100	100	100	2.02
5.	β -Myrcene	990	100	100	100	100	100	100	1.65
6.	Pseudolimonene	1003	100	100	100	100	100	100	2.35
7.	<i>m</i> -Cymene	1021	100	100	100	100	100	100	2.90
8.	Limonene	1031	100	99.90	95.11	91.64	90.41	88.65	1.59
9.	Eucaliptol	1035	100	100	100	100	100	100	2.37
10.	γ -Terpinene	1060	100	100	100	100	100	29.96	0.96
11.	Terpinolen	1089	100	100	100	100	100	100	2.80
12.	Camphor	1152	100	100	100	100	100	100	2.73
13.	Borneol	1176	100	100	100	93.66	T*	T	T
14.	Terpinen-4-ol	1178	100	100	100	94.61	6.78	T	T
15.	α -Terpineol	1189	100	99.35	93.27	91.49	1.80	T	T
16.	Bornyl acetate	1287	100	100	100	100	100	95.69	0.59
17.	α -Cubebene	1345	100	100	100	100	100	89.61	3.11
18.	α -Copaene	1377	100	100	100	100	100	100	8.86
19.	β -Elemene	1393	100	100	100	100	100	94.97	3.02

cont. Table 2.

No.	Compound	RI*	Methanol concentration (%)						
			10	15	25	50	65	75	100
20.	(E)-Caryophyllene	1424	100	100	100	100	100	100	2.68
21.	α -Caryophyllene	1453	100	100	100	100	100	100	2.62
22.	Cadina-1(10),4-dien	1519	100	100	100	100	100	100	10.72
Average recovery (%)			100	99.97	99.47	98.70	90.43	94.68	2.97

* – Kovats Retention Index; T – less than 0.05%.

The total elution of six components from thyme essential oil: linalool, camphor, borneol, α -terpineol, terpinen-4-ol, thymol was achieved using 75% methanol. Average recovery of remaining EO components after SPE procedure of thyme herb essential oil was more exceed 86% (see Table 3).

Table 3. The percentage of thyme essential oil components retained by SPE cartridge after its washing out using methanol/water mixture (i.e. composition of fraction II); n = 5, RSD < 3.5%.

No.	Compound	RI*	Methanol concentration (%)						
			15	25	50	65	75	100	
1.	Tricyclene	927	100	100	100	100	94.73	5.04	
2.	α -Pinene	935	100	100	100	100	95.98	5.09	
3.	Camphene	952	100	100	100	100	94.79	4.71	
4.	β -Pinene	980	100	100	100	100	90.44	4.56	
5.	β -Myrcene	990	100	100	100	100	92.60	4.14	
6.	Pseudolimonen	1003	100	100	100	100	100.0	99.06	
7.	α -Phellandren	1006	100	100	100	100	93.77	78.77	
8.	α -Terpinene	1012	100	100	100	100	93.59	41.74	
9.	p-Cymene	1023	100	100	100	100	89.05	T	
10.	Eucaliptol	1035	100	100	100	100	96.91	0.84	
11.	γ -Terpinene	1060	100	100	100	100	94.15	T	
12.	Terpinolene	1088	98.91	96.31	95.46	90.88	81.23	16.05	
13.	Linalool	1090	98.89	97.77	97.37	26.50	T*	T	
14.	Camphor	1139	100	96.98	95.33	64.42	T	T	
15.	Borneol	1176	100	98.56	97.20	19.72	T	T	
16.	Terpinen-4-ol	1178	97.95	98.28	95.52	53.58	T	T	

cont. Table 3.

No.	Compound	RI*	Methanol concentration (%)					
			15	25	50	65	75	100
17.	α -Terpineol	1189	100	97.47	97.17	27.41	T	T
18.	Linalol acetate	1256	100	98.55	95.66	65.35	31.49	T
19.	Thymol	1291	98.76	95.01	85.34	2.37	T	T
20.	α -Cubebene	1345	100	100	100	97.89	64.32	T
21.	(E)-Caryophyllene	1424	100	100	100	100	94.76	6.21
22.	α -Caryophyllene	1453	100	100	100	100	94.67	8.57
23.	Caryophyllene oxide	1589	100	100	100	98.86	65.78	T
Average recovery (%)			99.76	99.08	98.22	80.30	86.37	22.90

* – Kovats Retention Index; T – less than 0.05%.

Application of 75% methanol allows to total elution of one lemon essential oil component- linalol and almost total elution: α -terpineol, neral and geranial. Average recovery of other components exceeds 89% (see Table 4).

Table 4. The percentage of lemon essential oil components retained by SPE cartridge after its washing out using methanol/water mixture (i.e. composition of fraction II); n = 5, RSD < 3.5.

No.	Compound	RI*	Methanol concentration (%)						
			10	15	25	50	65	75	100
1.	Tricyclene	927	100	100	100	100	100	100	6.06
2.	α -Pinene	935	100	100	100	100	100	100	4.38
3.	Camphene	952	100	100	100	100	100	100	3.67
4.	Sabinene	972	100	100	100	100	100	100	2.71
5.	β -Pinene	980	100	100	100	100	100	100	1.45
6.	β -Myrcene	990	100	100	100	100	100	100	1.59
7.	α -Terpinene	1012	100	100	100	100	100	100	4.55
8.	p-Cymene	1023	100	100	100	100	100	100	T
9.	Limonene	1031	100	100	100	100	100	100	11.21
10.	Ocimene	1052	100	100	100	100	100	100	T
11.	γ -Terpinen	1060	100	100	100	100	100	100	0.38
12.	Terpinolene	1088	100	100	100	100	100	94.49	T
13.	Linalool	1090	100	100	100	88.13	25.65	T*	T
14.	Limonene oxide	1134	100	100	100	96.94	96.54	48.04	T

cont. Table 4.

No.	Compound	RI*	Methanol concentration (%)						
			10	15	25	50	65	75	100
15.	trans-Limonene oxide	1147	100	100	100	100	100	48.48	T
16.	α -Terpineol	1189	100	100	100	81.39	23.87	2.85	T
17.	cis-Carveol	1230	100	100	100	100	20.08	11.21	T
18.	Neral	1247	100	100	100	75.31	44.72	3.19	T
19.	Linalool acetate	1256	100	100	100	100	88.83	82.41	T
20.	Geranial	1276	100	100	100	100	78.15	4.03	T
21.	Geraniol acetate	1382	100	100	100	100	100	100	1.10
22.	(E)-Caryophyllene	1424	100	100	100	100	100	100	5.54
23.	α -Caryophyllene	1453	100	100	100	100	100	100	7.32
25.	Caryophyllene oxide	1589	100	100	100	100	100	100	3.14
Average recovery (%)			100	100	100	97.57	86.58	78.03	4.08

* – Kovats Retention Index; T – less than 0.05%.

In the case of grapefruit essential oil washing out the SPE column with 75% methanol causes total elution of α -terpineol, ostarol and almost total elution of 1-octanol and linalool. Average recovery of other components is more than 87% (see Table 5).

Table 5. The percentage of grapefruit essential oil components retained by SPE cartridge after its washing out using methanol/water mixture (i.e. composition of fraction II); n = 5, RSD < 3.5%.

No.	Compound	RI*	Methanol concentration (%)					
			10	25	50	65	75	100
1.	α -Pinene	935	100	100	100	100	100	2.48
2.	Sabinene	972	100	100	100	100	100	2.14
3.	β -Myrcene	990	100	100	100	100	100	1.85
4.	Octanal	1001	100	100	98.58	61.29	55.09	2.79
5.	Limonene	1031	100	100	100	100	100	2.52
6.	trans-Ocimene	1038	100	100	100	100	100	T
7.	1-Octanol	1078	100	98.50	94.04	3.70	1.58	T
8.	Linalool	1090	100	100	96.74	8.36	3.90	6.37
9.	Nonanal	1102	100	100	98.32	87.61	66.08	2.64
10.	Limonene oxide	1134	100	100	88.65	91.29	60.50	7.50
11.	(R)-(+)-Citronellal	1159	100	100	96.82	69.74	49.49	T

cont. Table 5.

No.	Compound	Methanol concentration (%)						
		RI*	10	25	50	65	75	100
12.	α -Terpineol	1189	100	100	100	5.47	T*	T
13.	Decanal	1209	100	100	100	100	84.28	T
15.	α -Cubebene	1345	100	100	100	100	100	3.78
16.	α -Copaene	1378	100	100	100	100	100	9.51
17.	(E)-Caryophyllene	1424	100	100	100	100	100	T
18.	α -Caryophyllene	1453	100	100	100	100	100	T
19.	Germacrene D	1487	100	100	100	100	100	T
20.	Cadina-1(10),4-dien	1519	100	100	100	100	100	4.89
21.	Elemol	1546	100	100	100	100	91.68	6.89
22.	Nootkaton	1814	100	100	100	100	75.05	T
23.	Ostaol	2144	100	100	98.69	81.20	T	T
Average recovery (%)			100	99.93	98.72	82.21	79.38	4.45

* – Kovats Retention Index; T – less than 0.05%.

The comparison of the chromatographic data for juniper, thyme, lemon, and grapefruit EO leads to the analogous conclusion as in case of SPE application to fractionation of mint essential oil components, i.e. oxygen compounds of low molecular mass occurring in all the used oils can be eluted from the SPE column using 75% methanol.

The average recovery of these compounds in methanolic fraction is almost 100%, whereas the average recovery of the remaining essential oil compounds (fraction II) is above 94% for juniper, above 86% for thyme, above 89% for lemon and above 87% for grapefruit – see Table 2-5.

Results proved that using of methanolic solution in the initial step of SPE causes the selective fractionation of essential oil components. Hence, it was decided to check what situation will be observed, when we replace character of solvent by e.g. dioxane or acetonitrile. The percentages of essentials oil components from mint retained in the SPE column after its washing out using ACN/water and 1,4-dioxane/water mixtures of different composition are presented in Table 6.

As results from the data, the concentration increase of the organic modifier in the SPE eluents causes a decrease of the amount of almost all mint essential oil components deposited on the SPE column. After the total removal of some components in the first fraction, the average recovery of the rest in second fraction is lower than 60%. Only one component – α -terpinene – still remain in the SPE column after washing it

out with 70% 1,4-Dioxane and only one component – muurolene – after washing out the column with 70% ACN.

Table 6. The percentages of mint essentials oil components retained by SPE cartridge after its washing out using ACN/water and 1,4-dioxane/water mixtures (i.e. composition of fraction II).

No.	Compound	Concentration (%) of					
		ACN			1,4-Dioxane		
		50	60	70	50	60	70
1.	3-Thujone	93.52	84.09	77.43	83.94	76.51	74.06
2.	Pinene	95.59	89.44	78.66	86.07	80.68	80.19
3.	β -Phellandrene	94.52	89.15	78.62	87.25	77.5	72.25
4.	cis-Sabinene hydrate	96.84	91.69	89.32	91.37	89.88	75.21
5.	3-Octanol	95.31	93.92	90.43	87.61	85.45	79.98
6.	3,5-dimethyloctane	89.31	84.92	72.43	84.61	75.45	79.40
7.	α -Terpinene	94.49	87.05	77.21	100	100	100
8.	o-Cymene	97.7	93.65	75.83	96.19	83.98	65.35
9.	D-Limonene	95.61	89.25	78.45	86.79	79.37	77.37
10.	Eucaliptol	67.59	34.52	11.85	87.47	25.24	7.29
11.	γ -Terpinene	93.87	88.04	78.34	86.88	79.67	75.28
12.	cis-4-Thujanol	8.54	3.04	T	47.05	2.13	0.79
13.	Terpinolene	92.1	84.4	74.48	97.91	97.35	95.01
14.	Linalool	17.29	8.46	8.54	78.93	11.73	8.50
15.	2-(methylbutyl)-iso-valerat	85.84	79.38	32.23	91.63	71.82	40.95
16.	p-Menthanon	58.99	14.34	5.71	88.34	44.67	5.40
17.	Menthofuran	88.42	83.07	57.36	87.4	71.79	46.26
18.	p-Menthon	37.63	9.59	5.38	88.4	16.62	4.23
19.	Isomenthol	46.14	15.30	3.40	81.68	21.6	2.09
20.	Menthol	28.66	12.56	1.10	84.28	4.42	1.35
21.	γ -Terpineol	7.58	T*	T	35.13	T*	T
22.	Pulegone	30.26	14.61	3.07	87.05	4.25	3.56
23.	Piperitone	25.91	7.64	T	40.07	1.18	1.13
24.	Menthyl acetate	85.09	79.41	53.74	88.33	70.8	43.88
25.	o-Menth-8-en	100	96.12	88.15	92.19	86.59	85.18
26.	α -Burbonene	100	86.63	79.48	88.96	83.67	81.51
27.	Caryophyllene	100	100	94.32	90.37	86.17	83.90

cont. Table 6.

No.	Compound	Concentration (%) of					
		ACN			1,4-Dioxane		
		50	60	70	50	60	70
28.	Murolene	100	100	99.32	97.9	93.41	89.43
29.	α -Cubenene	98.67	95.32	84.1	90.17	85.72	83.04
30.	Eriksene	98.96	92.94	83.33	82.53	78.03	76.55
31.	δ -Cadinene	100	97.71	87.07	100	91.06	81.34
32.	Wiridofloren	93.76	80.69	42.89	88.02	62.81	8.62
Average recovery (%)		74.94	77.37	59.04	84.52	62.56	52.55

* – Kovats Retention Index; T – less than 0.05%.

Results from Table 6 indicate that applied 70% acetonitrile allows for the total elution of following EO components from SPE sorbent: cis-4-thujanol, γ -terpineol, piperitone and almost total elution of pulgeone, menthol, isomenthol, p-menthol, and p-menthanon. Average recovery of remaining EO components was 70.57%.

In case of 70% dioxane application, the total elution of EO constituents was only in the case of γ -terpineol. For components (numbered 12, 18-20, 22 and 23) the elution was more than 95%. Recovery of other components was only 64.64 % (without taking into account the above constituents, for which the recovery was below 5%).

4. CONCLUSIONS

The presented SPE method allows to carry out fractionation/isolation of essential oil components from plant materials. It is especially effective for isolation of low-molecular oxygen compounds, for which is easy and almost complete from the remaining compounds. Total removal of some components from essential oil can be achieved by application of methanol/water mixture as the eluent. The SPE method for fractionation of essential oils may be adapted to isolation of valuable components (e.g. menthol from mint essential oil) for foodstuffs, cosmetics or pharmaceutical applications.

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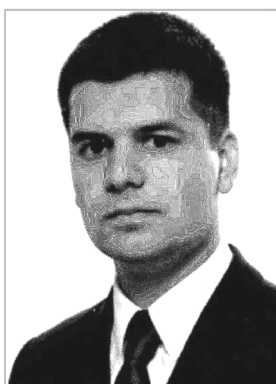
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CURRICULA VITAE



Joanna Szewczyk. Born in Poland in 1989. Graduated from the University of Maria Curie-Skłodowska (UMCS) in Lublin (2013). Since 2013 Ph.D. student in the Department of Chromatographic Methods at University of Maria Curie-Skłodowska (UMCS). Successfully completed the International Master Programme „*Measurement Science in Chemistry*” (2014). Since 2013 Member of student section of „Polish Chemical Society”.



Michał Paweł Dybowski. Born in Poland in 1985. Graduated from the University of Maria Curie-Skłodowska (UMCS) in Lublin (2009). Received his Ph.D. (2014) in analytical chemistry from the University of Maria Curie-Skłodowska in Lublin. Since 2009 an assistant at Department of Chromatographic Methods UMCS.



Andrzej L. Dawidowicz. Born in Lublin (Poland) in 1950. Graduated from Maria Curie-Skłodowska University in Lublin (1973). Received his Ph.D. and D.Sc. degree (1978 and 1986, respectively) in physical and analytical chemistry from University of Maria Curie-Skłodowska in Lublin. Since 1991 titular professor. Since 2000 Head of Chromatographic Methods Department. Since 1973 member of Polish Chemical Society. Member of numerous University teaching and research committees. Since 1996 Head of Doctoral Studies at the Faculty of Chemistry. His main field of interest is clinical, pharmaceutical, food, forensic and environmental analysis, development of sample preparation methods for chromatographic analysis, and studies of porous materials. He is an author over 200 papers.