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Matrix solid-phase dispersion (MSPD) in chromatographic analysis of essential oils in herbs

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ABSTRACT

Matrix solid-phase dispersion (MSPD) is a simple and cheap sample preparation procedure allowing for the reduction of organic solvent consumption, exclusion of sample component degradation, improvement of extraction efficiency and selectivity, elimination of additional sample clean-up and pre-concentration step before chromatographic analysis.

The paper shows the possibility of MSPD application for qualitative and quantitative analysis of essential oil components in the following herbs: thyme (*Thymus vulgaris* L.), mint (*Mentha piperita*), sage (*Salvia officinalis* L.), chamomile (*Chamomilla recutita* L.), marjoram (*Origanum majorana* L.), savory (*Satureja hortensis* L.), and oregano (*Origanum vulgare*). The results obtained using MSPD are compared to two other sample preparation methods: steam distillation (SD) and pressurized liquid extraction (PLE).

The results presented in the paper prove that the total amount and the composition of the essential oil component obtained by MSPD are equivalent to those gained by one of the most effective extraction technique, PLE.

1. Introduction

Sample preparation is a crucial step in the chemical analysis of plant material. Recently, research has been focused on those sample preparation methods which allow for the reduction of organic solvent consumption, the exclusion of sample component degradation, the elimination of additional sample clean-up and pre-concentration step before chromatographic analysis as well as the improvement of extraction efficiency, selectivity, and/or kinetics.

Matrix solid-phase dispersion (MSPD) is a simple and cheap sample preparation procedure involving simultaneous disruption and extraction of various solid and semi-solid materials [1–4]. It permits complete fractionation of the sample matrix components and has the ability to selectively isolate a single compound or several classes of compounds from the sample. MSPD involves direct mechanical blending of sample with a SPE sorbent (mainly octadecyl-modified silica). In this process, the sorbent acts both as an abrasive material disrupting sample architecture and as a 'bound' solvent that assists in accomplishing sample disruption. The sample is dispersed over the surface of the bonded-phase support material, producing a unique mixed-character phase for conducting target analyte isolation. In this phase non-polar components are dispersed in the non-polar organic phase bonded to the silica support; smaller, highly polar molecules are associated with silanols on the surface of the silica support as well as with matrix components able to polar interactions; large, less polar molecules are accumulated on the surface of mixed-character phase formed by bonded octadecyl phase and dispersed matrix. After homogenization, blended mixture is transferred into a SPE barrel and subjected to elution with an appropriate eluent. Finally, the obtained eluate undergoes the analytical procedure.

MSPD has been used for performing the extraction of a variety of matrices for a number of compounds, e.g.: caffeine in green tea leaves [5], rutin in *Sambucus nigra* L. [6], polybrominated diphenyl ethers and polychlorinated biphenyls in biota samples [7], phenolic compounds in fruit-green tea [8], pesticides in fruits [9], free fatty acids in chocolate [10], pesticides in single insects [11]. However, little is known about MSPD application as a sample preparation method for the analysis of essential oil components in herbs.

The presented paper discusses the possibility of MSPD application for qualitative and quantitative analysis of essential oil components in the following herbs: thyme (*Thymus vulgaris* L.), mint (*Mentha piperita*), sage (*Salvia officinalis* L.), chamomile (*Chamomilla recutita* L.), marjoram (*Origanum majorana* L.), savory (*Satureja hortensis* L.), and oregano (*Origanum vulgare*). The results obtained using MSPD are related to analogous ones gained applying two other sample preparation methods: steam distillation (SD), recognized as the standard essential oil preparation method, and

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pressurized liquid extraction (PLE), regarded as one of the most effective extraction techniques.

2. Experimental

2.1. Materials and chemicals

The following herbs were used in the experiments: thyme (*T. vulgaris* L.), mint (*M. piperita*), sage (*S. officinalis* L.), chamomile (*C. recutita* L.), marjoram (*O. majorana* L.), savory (*S. hortensis* L.), and oregano (*O. vulgare*). All of them were cultivated in eastern Poland (cultivation year 2008). About 2 kg portions of the herbs were air-dried, cut and stored at +4 °C. Immediately before extraction an appropriate plant material was ground and its exactly weighed portions were subjected to the applied sample preparation procedures. Hexadecane (Aldrich, Gilingham, UK) in n-octane (Merck, Germany) solution (104.1 mg hexadecane in 100 ml of n-octane), and pentadecane in n-octane (54.6 mg pentadecane in 50 ml of n-octane) were used as the internal standards. Hexane, ethyl acetate and 1,4-dioxane, all of them of analytical grade, were supplied by the Polish Chemical Plant POCH S.A. (Gliwice, Poland). The Sepra C_{18}-E sorbent (50 μm, 65 Å) used in the MSPD process was purchased from Phenomenex (Torrance, CA, USA).

2.2. Steam distillation

A herb sample (10 g) was submitted to steam distillation with 500 ml of water for 3 h using a Deryng-type apparatus. The distillation time was measured after the fall of the first distillate drop. The separated essential oil distillate was dried by freezing and, after filtration, stored at +4 °C until further experiments. An appropriate amount of the internal standard solution was added to each essential oil sample. The procedure was repeated 3 times, each time with a fresh portion of the herb.

2.3. Pressurized liquid extraction

PLE was performed with a Dionex ASE 200 instrument (Dionex, Sunnyvale, CA, USA). To reduce the volume of the extraction solvent, the exactly weighed portion of the plant material (0.5 g) was mixed with neutral glass [12] and placed into a 22 ml stainless steel extraction cell. In the case of sage herb, ethyl acetate was applied for the PLE extraction. For all other herbs hexane was used as the extraction solvent. PLE was carried out in the following conditions: extraction temperature: 100 °C; extraction pressure: 60 bar; static extraction time: 10 min. These conditions were established in a separate investigation (not reportable here) as optimal for the examined herbs [13]. An appropriate volume of the internal standard solution was added to each extract before analysis. The extraction procedure was repeated 3 times using fresh portions of plant material.

2.4. Matrix solid phase dispersion

The MSPD optimization procedure was carried out to determine such MSPD conditions which would be general for all seven herbs used in the presented experiments. MSPD process was optimized estimating the total amount of essentials oil components isolated from selected herbs. The MSPD conditions revealing the greatest total amount of essential oil components in herbs were assumed as optimal.

The evaluation of plant matrix to sorbent (SepraC_{18}-E) mass ratio was the first step of the optimization procedure. The following plant to sorbent mass ratio was examined 1:2, 1:4, 1:8, 1:12. Isopropanol (2 ml) was used in these experiments as MSPD dispersing liquid. 1:4 herb to sorbent mass ratio was found to be satisfactory for all examined herbs.

Table 1
Total amounts of essentials oils components (in mg/g) estimated in herbs using steam distillation, PLE and MSPD. Mean values ± sd, $n = 3$.

Type of herb	Sample preparation method		
	Steam distillation	PLE	MSPD
Chamomile	1.85 ± 0.08	2.19 ± 0.09	2.07 ± 0.15
Thyme	9.48 ± 0.44	9.99 ± 0.41	9.91 ± 0.65
Mint	8.72 ± 0.35	9.60 ± 0.30	9.87 ± 0.66
Sage	7.84 ± 0.36	8.54 ± 0.27	8.36 ± 0.55
Marjoram	3.35 ± 0.15	4.68 ± 0.17	5.06 ± 0.37
Savory	17.68 ± 0.63	17.32 ± 0.68	17.86 ± 0.95
Oregano	2.16 ± 0.08	3.04 ± 0.12	3.17 ± 0.26

The selection of dispersing solvent and its volume was the second step of MSPD optimization procedure. Water, methanol, ethanol, n-propanol, isopropanol, and 1,4-dioxane were used in this optimization step. These experiments were performed using 1:4 herb to sorbent mass ratio and 1, 2 or 3 ml of MSPD dispersing solvent. 1,4-Dioxane was regarded as the most appropriate. 1 ml of 1,4-dioxane allows for efficient isolation of essential oil components from herb matrix. Although, methanol and n-propanol have given the similar yields of essential oils components as dioxane but the latter has not evaporated as quickly as other organic solvents applied during MSPD dispersion process.

To remove the essential oil components from MSPD cartridge the hexane–ethyl acetate mixture (9:1, v/v) was used in all experiments [14]. The experiments revealed that 10 ml of this mixture was sufficient for effective elution of essential oil components.

The homogenization time in all experiments was constant (10 min).

In consequence of the described investigations the following procedure was assumed as optimal for all seven herbs used in the presented experiments. A 0.2 g sample of grounded herb, 0.8 g of the sorbent and 1 ml of 1,4-dioxane were mixed for 10 min in a glass mortar using a glass pestle to obtain a homogeneous mixture. After homogenization, the blend was quantitatively transferred with a spatula to a syringe barrel containing a filter disc at the bottom. The mixture was compressed using the syringe plunger. Plant components were then eluted to a 10 ml calibrated flask using the hexane–ethyl acetate mixture. An appropriate amount of hexadecane or pentadecane solution was added to the extract and subjected to GC analysis. The extraction procedure was repeated 3 times using fresh portions of plant material.

For the isolation of essential oil components by the above described methods the same plant material was used.

2.5. Chromatographic analysis

Qualification of essential oil components in the prepared samples from MSPD, PLE and steam distillation was performed using GC–MS QP2010 (Shimadzu, Kyoto, Japan). A ZB5-MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μl film thickness) (Phenomenex, USA) was used. Helium (grade 5.0) was used as a carrier gas. 1 μl of the sample was injected by AOC-20i type autosampler. The injector temperature was 310 °C. The following temperature program was applied: 1 min at 50 °C and then a linear temperature increase up to 310 °C at the rate 6 °C/min. The mass spectrometer was operated in EI mode at 70 eV; and the ion source temperature was 220 °C. The mass spectra were measured in the range 35–360 amu. Qualitative analysis was carried out comparing the obtained MS spectra with the NIST'05 library spectra. The presence of a given component was additionally confirmed by the published and our own temperature retention indexes.

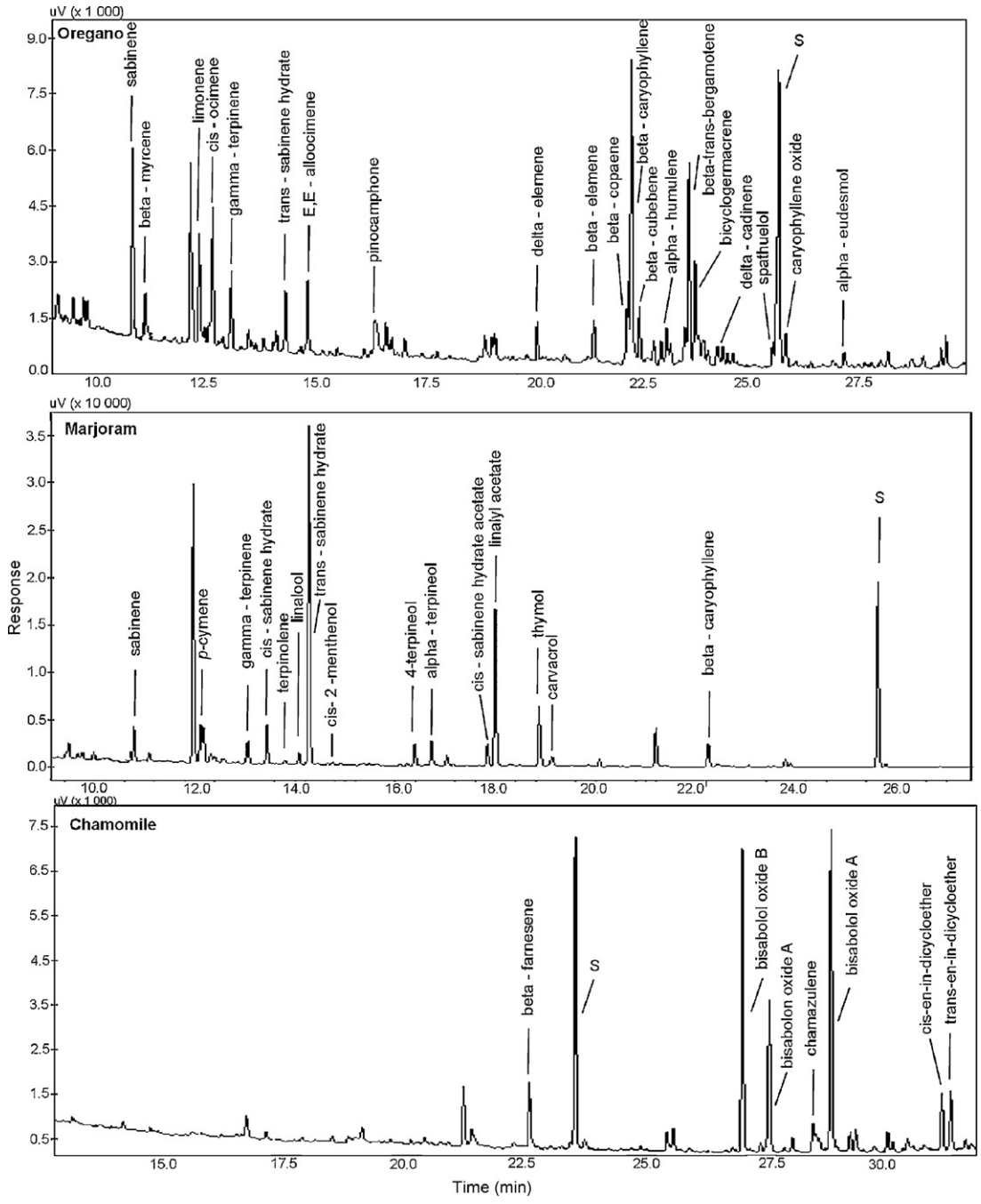

Fig. 1. Gas chromatograms of MSPD extracts of oregano, marjoram and chamomile.

Quantification of extracts was performed using a gas chromatograph with a flame ionization detector (GC-FID), model GC-2010. 1 μl of the sample was injected by AOC-20i type autosampler into a ZB5-MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μl film thickness). The temperature program during GC-FID separation was the same as for GC-MS. Peaks identification was carried out by comparing the GC retention indexes with those from GC-MS.

The amounts of essential oil components were expressed in micrograms relating the peak area of a given component to the peak area of hexadecane (or pentadecane in the case of chamomile), a known amount of which was added to the examined extracts before GC measurements.

3. Results and discussion

Table 1 contains the total amounts of essential oil components estimated in the examined herbs using SD, PLE and MSPD. The presented values were calculated in relation to the known amount of hexadecane (or pentadecane) added to the herb extracts as a quantity standard.

The consideration of the collected data leads to the conclusion that the greatest total amounts of essential oil components are obtained when PLE or MSPD are used for sample preparation in herb analysis. According to the literature [15], PLE is one of the most effective sample preparation methods. Its high extraction power results from the application of high pressure during the extraction process, allowing to use an extrahent at a tem-

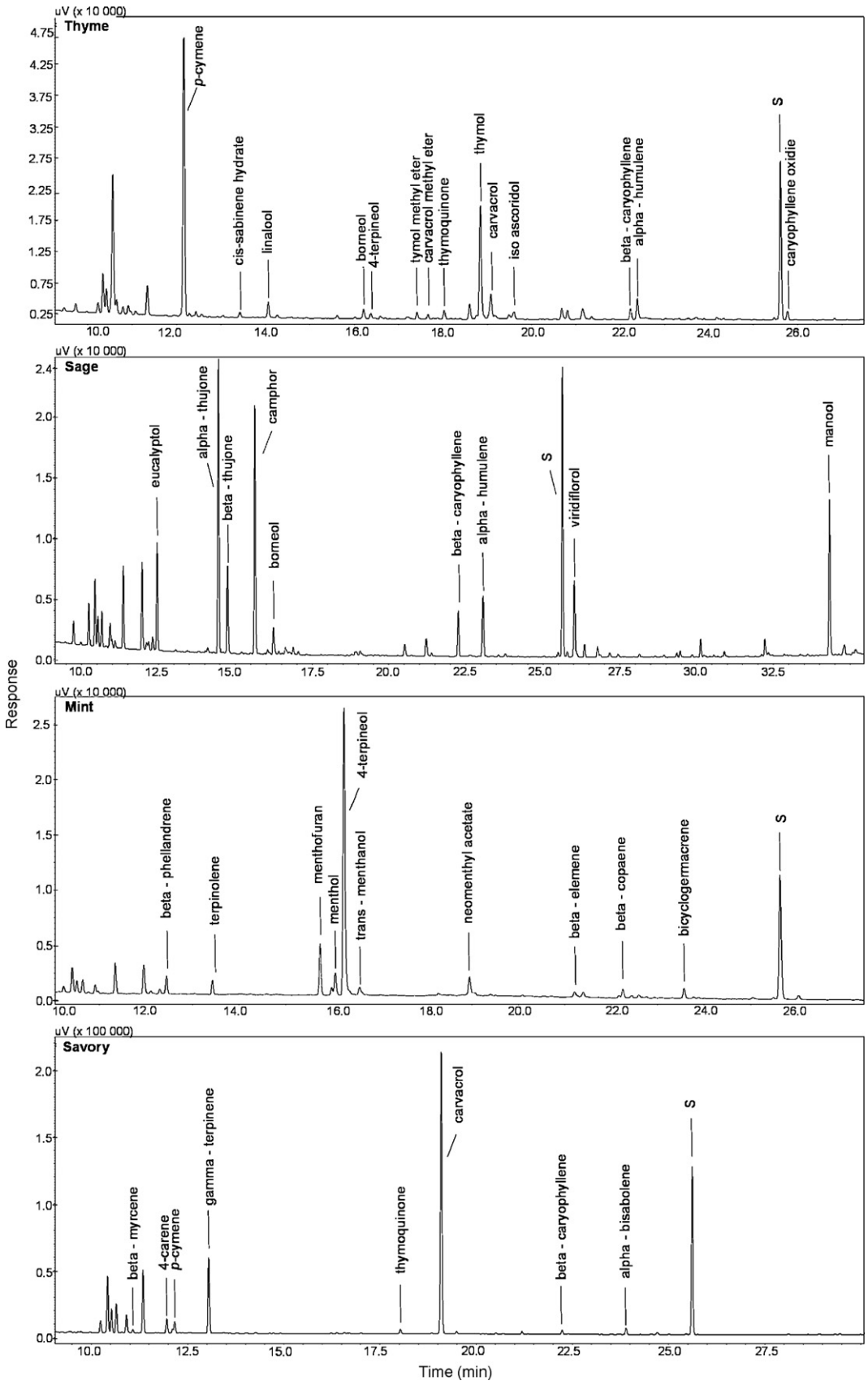

Fig. 2. Gas chromatograms of MSPD extracts of thyme, sage, mint and savory.

Table 2
Component amounts (expressed as peak area percentage) estimated in oregano, marjoram and chamomile herbs using steam distillation, PLE and MSPD methods.

No.	Compound	RI	Component amount [%]		
			Isolation method		
			Steam distillation	PLE	MSPD
Oregano					
1	Sabinene	981	11.3	11.3	8.4
2	β-Myrcene	992	2.0	1.8	1.7
3	p-Cymene	1031	1.4	1.5	1.1
4	Limonene	1034	4.8	4.2	5.2
5	cis-Ocimene	1053	7.3	6.1	5.9
6	γ-Terpinene	1066	4.9	4.9	3.5
7	trans-Sabinene hydrate	1112	0.8	3.2	2.6
8	E,E-Alloocimene	1132	4.4	3.5	2.8
9	Pinocamphone	1181	–	0.5	1.7
10	δ-Elemene	1345	2.4	2.2	1.7
11	β-Bourbonene	1390	–	–	7.1
12	β-Elemene	1401	1.6	2.1	2.0
13	β-Copaene	1437	3.5	3.5	3.7
14	β-Caryophyllene	1440	14.5	13.3	13.2
15	β-Cubebene	1449	2.6	2.6	2.5
16	α-Humulene	1475	1.9	1.8	1.8
17	β-trans-Bergamotene	1501	10.3	10.0	10.0
18	Bicyclogermacrene	1508	4.5	4.5	4.8
19	α-Farnesene	1516	2.6	2.1	1.9
20	δ-Cadinene	1533	3.0	0.8	1.2
21	Spathuelol	1598	1.7	6.5	4.4
22	Caryophyllene oxide	1609	2.0	1.7	1.8
23	α-Eudesmol	1678	2.9	–	0.6
Marjoram					
1	Sabinene	981	3.6	4.6	4.4
2	p-Cymene	1031	5.3	4.6	3.5
3	γ-Terpinene	1066	10.2	4.7	3.5
4	cis-Sabinene hydrate	1079	6.0	4.7	4.7
5	Terpinolene	1093	1.8	1.1	0.8
6	Linalool	1103	2.3	1.4	1.4
7	trans-Sabinene hydrate	1112	25.1	40.3	37.1
8	cis-2-Menthenol	1135	1.3	0.5	0.4
9	4-Terpineol	1188	20.0	3.8	3.5
10	α-Terpineol	1205	4.0	3.5	3.0
11	cis-Sabinene hydrate acetate	1250	1.2	2.4	2.5
12	Linalyl acetate	1256	–	8.6	16.8
13	Thymol	1292	8.2	7.8	6.0
14	Carvacrol	1302	0.8	1.3	1.6
15	β-Caryophyllene	1440	3.4	3.0	2.8
Chamomile					
1	β-Farnesene	1455	4.0	4.8	6.1
2	Bisabolol oxide B	1675	31.9	27.8	27.5
3	Bisabolon oxide A	1704	17.6	16.9	15.3
4	Chamazulene	1760	17.7	2.4	3.5
5	Bisabolol oxide A	1774	23.7	33.0	33.7
6	Cis-en-in-dicycloether	1905	1.0	6.2	6.0
7	Trans-en-in-dicycloether	1914	1.2	7.3	5.8

perature above its normal boiling point and, in consequence, to remove the analytes efficiently and quickly from various matrices. Hence, the PLE results presented in Table 1 can be treated as a confirmation of a high extracting ability of PLE in relation to essential oil components from herb. However, the MSPD results are more interesting as they indicate that the extraction efficacy of this very simple and cheap sample preparation procedure is equivalent to that for PLE which, contrary to MSPD, is a technically advanced and developed method. High yield of essential oil components in the MSPD process results both from the chemical character of the compounds and the capacity of the bonded C-18 layer, which plays the role of a reservoir for the isolated components.

As seen in Table 1, the standard essential oil separation method – steam distillation – is generally a less effective isolation process of essential oil components than MSPD and PLE. The isolation efficiency of steam distillation is equivalent to PLE and MSPD only in the case of essential oil components from savory.

The results in Table 1 allow us to formulate an opinion about isolation efficiency of the applied methods but do not allow for their more detailed comparison. The physicochemical foundations of all the applied sample preparation techniques are different and their deeper consideration requires the comparison of the essential oil compositions estimated by these methods. The essential oils are very complex mixtures which, beside main components, contain many compounds existing in very small amounts. The exemplary chromatograms of MSPD extracts from individual herbs are presented in Figs. 1 and 2.

In order to simplify the comparison of the methods it was decided to limit the number of essential oil components considered for individual herbs. The compositions of essential oil components from the examined herbs estimated by SD, PLE and MSPD, expressed as peak area percentage, are presented in Tables 2 and 3. The listed values were calculated taking into account all isolated essential oil components. For this reason the sum of peak area percentage in presented tables does not equal 100%. The data in

Table 3
Component amounts (expressed as peak area percentage) estimated in thyme, mint, sage and savory herbs using steam distillation, PLE and MSPD methods.

No.	Compound	RI	Component amount [%]		
			Isolation method		
			Steam distillation	PLE	MSPD
Thyme					
1	cis-Sabinene hydrate	1079	0.8	1.0	0.8
2	p-Cymene	1031	51.4	47.4	45.4
3	Linalool	1102	3.0	3.1	2.6
4	Borneol	1183	2.0	2.2	2.1
5	4-Terpineol	1188	0.9	0.8	1.0
6	Tymol methyl eter	1234	1.3	1.3	1.2
7	Carvacrol methyl eter	1244	0.9	0.9	0.8
8	Thymoquinone	1258	0.2	1.5	1.5
9	Tymol	1292	25.8	23.3	21.5
10	Carvacrol	1301	3.7	4.2	5.9
11	iso-Ascoridol	1318	–	–	0.8
12	β-Caryophyllene	1440	1.8	1.9	2.0
13	α-Humulene	1475	0.1	4.4	4.0
14	Caryophyllene oxide	1608	1.8	1.9	1.6
Mint					
1	β-Phellandrene	1039	3.4	3.0	3.0
2	Terpinolene	1093	1.9	2.8	2.7
3	Menthofuran	1166	10.6	9.7	9.7
4	Menthol	1179	4.7	4.4	4.5
5	4-Terpineol	1188	63.3	62.6	59.9
6	trans-Menthanol	1199	1.7	1.7	2.1
7	Neomenthyl acetate	1295	4.8	4.6	4.6
8	β-Elemene	1396	1.1	1.1	1.5
9	β-Copaene	1443	1.8	1.8	2.1
10	Bicyclogermacrene	1500	1.7	2.2	2.2
Sage					
1	Eucalyptol	1041	9.3	9.5	8.6
2	α-Thujone	1116	24.8	22.9	24.0
3	β-Thujone	1127	8.8	7.8	7.4
4	Camphor	1162	20.2	19.7	20.1
5	Borneol	1183	2.5	2.5	2.7
6	β-Caryophyllene	1440	3.6	3.5	4.0
7	α-Humulene	1476	5.5	5.2	5.3
8	Viridiflorol	1619	7.7	6.7	7.2
9	Manool	2086	9.5	13.9	13.0
Savory					
1	β-Myrcene	992	0.9	1.1	0.8
2	4-Carene	1025	1.7	1.7	1.7
3	p-Cymene	1031	3.9	3.7	2.9
4	γ-Terpinen	1066	22.0	23.4	19.4
5	Thymoquinone	1258	0.1	1.1	1.1
6	Carvacrol	1302	69.2	66.7	71.5
7	β-Caryophyllene	1440	0.9	0.9	1.0
8	α-Bisabolene	1517	1.3	1.4	1.6

Tables 2 and 3 indicate that in all the examined plants MSPD reveals almost the same qualitative composition of essential oil components as with the other methods used. The lack of iso-ascoridol in thyme oil components estimated by steam distillation and the lack of β-bourbonene in essential oil components from oregano estimated by PLE are the only quantitative composition differences.

The quantitative analysis of the data from Tables 2 and 3 shows a little greater difference in the composition of essential oil components established using individual methods of sample preparation.

The greatest variation in the quantitative composition of essential oil components estimated using MSPD, PLE and steam distillation is observed for oregano (see limonen, trans-sabinene hydrate, β-copaene, β-trans-bergamotene, bicyclogermacrene, and spathuelol), marjoram (see sabinene, γ-terpinene, cis-sabinene hydrate, 4-terpineol, cis-sabinene hydrate acetate, linalyl acetate, tymol, and carvacrol) and chamomile (chamazulene, bisabolol oxide A, cis-en-in-dicycloether, and trans-en-in-dicycloether). Significantly smaller quantitative variation is seen for mint (see terpinolene and bicyclogermacrene), sage (manool) and savory (p-cymene and thymoquinone). There is no essential difference in the quantitative composition of essential oil components from thyme estimated by the used methods.

More detailed consideration of the data from Tables 2 and 3 shows that the quantitative composition of essential oil components estimated by MSPD is close to that estimated by PLE. Only the behavior of p-cymene in savory departs from the observed pattern.

The discussed variation in the qualitative and quantitative composition of the essential oil components, revealed using MSPD, PLE and steam distillation, can result from different factors such as:

- different extraction abilities of the applied sample preparation methods in relation to individual essential oil components;
- different temperatures of the employed processes, which affects both the separation and the transformation processes in isolated compounds;
- different positions of individual essential oil components in plant matrix;

- presence of additional compounds (not only essential oil compounds) which can take part in or catalyze the transformation processes;
- different exposures of essential oil components with oxygen and water;
- different pHs of the mixtures formed during sample preparation.

All these factors can affect the essential oil composition. The influence of temperature seems to be the most important as the essential oil compositions estimated using steam distillation – the method involving long thermal exposure – differ most from the results gained using other methods. The fact that essential oil components can be formed and can take part in transformation processes presents special difficulty in explaining simply and unequivocally the observed composition differences in essential oil components estimated using the discussed sample preparation methods. This difficulty can be partly resolved by reference to some literature reports describing such transformations for a few chosen components, noticing simultaneously physicochemical conditions of the applied methods. Chamazulene, a chamomile oil component, is the product of matricin transformation which takes place in the presence of water in acidic environment [16]. Hence, the greatest amount of chamazulene estimated by steam distillation and the lowest by PLE and MSPD is not surprising. The lower amount of manool in sage essential oil composition, estimated using steam distillation, can also by explained by the presence of big water amount in the sample preparation system. Manool is formed by partial dehydration of non-essential oil component, scraleole [17]. It is probable that water in the steam distillation system suppresses this transformation.

The applied sample preparation methods differ in temperature, duration time and type of solvent used. Pressure is also a factor differentiating these methods. Its influence on the mentioned transformations is not recognized, however, it may be essential. The influence of pressure on the observed differences in essential oil composition can be taken into account not only in the PLE process but also in MSPD, due to local pressure increase resulting from pastel press on MSPD mixture. It is also possible that the applied MSPD sorbent (especially its residual silanol groups and silanol groups formed after sorbent crumble during MSPD) catalyzes some transformations, which may also be responsible for the observed differences in qualitative and quantitative composition of essential oil components.

4. Conclusions

MSPD has been demonstrated to be a suitable preparation technique for the isolation of essential oil components from herbs. The results presented in the paper prove that the total amount and the composition of essential oil components obtained by MSPD are equivalent to those gained by one of the most effective extraction techniques, PLE. However, unlike PLE, MSPD is performed with a very simple and cheep equipment. Moreover, the method requires a small sample size and offers considerable savings in terms of solvent consumption, cost of materials, sample manipulation and time involved. In consequence, MSPD can be regarded as a GRAS (generally recognized as safe) method.

The MSPD results in the present paper were obtained at a 1:4 mass ratio of herb to sorbent, which was found in preliminary experiments to be good enough for the estimation of essential oil components in all the examined herbs. Yet, as herbs differ in the amount of essential oil components, the optimal mass ratio should be established for each herb, making the application of MSPD method even more economical for the estimation of essential oil components than demonstrated.

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