

Matrix Solid-Phase Dispersion with Sand in Chromatographic Analysis of Essential Oils in Herbs

Andrzej L. Dawidowicz,* Dorota Wianowska and Ewelina Rado

ABSTRACT:

Introduction – Matrix solid-phase dispersion (MSPD) is a very simple, cheap and relatively quick sample preparation procedure which involves simultaneous disruption and extraction of various solid and semi-solid samples due to the direct mechanical blending of the sample with a SPE sorbent, mainly C₁₈. Little is known about MSPD application as a sample preparation method for the analysis of essential oil components in herbs.

Objective – To evaluate if C₁₈ sorbent, commonly used in MSPD process, can be substituted with sand in the procedure of essential oil analysis.

Methodology – Essential oil extracts were obtained from mint, sage, chamomile, marjoram, savory and oregano using MSPD with C₁₈ sorbent or sand, pressurised liquid extraction and steam distillation. Their qualitative and quantitative compositions were established by GC-MS and GC-FID.

Results – The results prove that C₁₈ sorbent can be substituted with sand in the procedure of essential oil analysis in herbs. The recoveries of essential oil components estimated using MSPD/sand are almost equal to those using pressurised liquid extraction.

Conclusion – The results presented in the paper reveal that MSPD with sand is suitable for the isolation of essential oil components from herbs. Its extraction efficiency is equivalent to pressurised liquid extraction, recognised as one of the most efficient extraction methods. The cost of MSPD procedure for essential oil analysis can be significantly diminished by substituting C₁₈ with sand. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: MSPD; sand application; essential oil analysis; sample preparation in herb analysis

Introduction

Because of the character and complexity of plant material, the analytical procedure of examining plant constituents involves the application of a sample preparation procedure to fully isolate the analysed substances from the plant matrix. Recently, some interest has been focused on those sample preparation methods which enable 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, and the improvement of extraction efficiency and selectivity, and/or kinetics. Matrix solid-phase dispersion (MSPD) can be numbered among such methods. This very simple, cheap and relatively quick sample preparation procedure involves simultaneous disruption and extraction of various solid and semi-solid samples due to the direct mechanical blending of sample with an SPE sorbent (mainly octadecyl modified silica) (Baker *et al.*, 1989; Baker, 2000, 2007; Kristenson *et al.*, 2006). There are many examples of analytical procedures in which, instead of C₁₈ sorbent, sand is applied in the MSPD process (Chiacchierini *et al.*, 2006; Ziakova *et al.*, 2003). Recently published results (Dawidowicz and Rado, 2010) showed that MSPD efficiency in essential oil components isolation process from herbs is better than steam distillation (SD), which is the routine method recommended by pharmacopoeias for the isolation of essential oil in controlling the quality of plant material as its source (*European Pharmacopoeia*, 2008), and equivalent to the efficiency of pressurised liquid extraction (PLE), regarded as one

of the most effective techniques of extracting essential oil components (Dawidowicz *et al.*, 2008).

As essential oil components are ranked as easily recoverable analytes, it was assumed in the present study that C₁₈ sorbent, commonly employed in MSPD process, can be substituted with sand and a proper MSPD dispersing solvent, diminishing significantly the cost of analytical procedure by means of MSPD. This was demonstrated by comparing the results obtained by MSPD with sand with those obtained by PLE with hexane, by SD and by MSPD with C₁₈ sorbent.

Experimental

Materials and chemicals

The following herbs were used in the experiments: mint (*Mentha piperita*), sage (*Salvia officinalis* L.), chamomile (*Chamomilla recutita* L.), marjoram (*Origanum majorana* L.), savory (*Satureja hortensis* L.) and oregano (*Origanum 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

* Correspondence to: A. L. Dawidowicz, Faculty of Chemistry, Chromatographic Method Department, Maria Curie Skłodowska University, Pl. Marii Curie Skłodowskiej 3, 20-031 Lublin, Poland. E-mail: dawid@poczta.umcs.lublin.pl

material was ground and its exactly weighed portions were subjected to the applied sample preparation procedures. Hexadecane (Aldrich, Gillingham, 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 internal standards. Hexane, ethyl acetate, 1,4-dioxane, methanol, *n*-propanol, propanol-2, butanol and *n*-octane, all of them of analytical grade, were supplied by the Polish Chemical Plant POCH S.A. (Gliwice, Poland). The Septra C₁₈-E sorbent (50 µm, 65 Å) purchased from Phenomenex (Torrance, CA, USA) and sand (obtained as a gift from a glassworks) were both used in the MSPD process. The sand, brazilian quartz, was fractionated, leached with 1 M hydrochloric acid, washed out with distilled water to neutrality and dried. Fractions of 80–100 µm were applied for the MSPD process.

Steam distillation

A herb sample (10 g) was exposed 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. After distillation the essential oil was separated from distillate, dried by freezing and stored at +4°C until its analysis. An appropriate amount of the internal standard solution (hexadecane or pentadecane) was added to each essential oil sample. The procedure was repeated three times, each time with a fresh portion of the herb.

Pressurised 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 powder (80–160 µm) (ASE 200, 1995) and placed into a 22 mL stainless steel extraction cell. In the case of sage, ethyl acetate was applied for 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 as optimal for the examined herbs (Dawidowicz *et al.*, 2008). An appropriate volume of the internal standard solution was added to each extract before analysis. The extraction procedure was repeated three times using fresh portions of plant material.

Matrix solid phase dispersion

Matrix solid phase dispersion with C₁₈ sorbent. MSPD with C₁₈ sorbent was performed according to the procedure described elsewhere (Dawidowicz and Rado, 2010). According this procedure, a 0.2 g sample of ground herb was mixed with 0.8 g of SeptraC₁₈-E sorbent. After homogenisation the mixture was transferred into a syringe barrel and then eluted to a 10 mL calibrated flask using the hexane–ethyl acetate mixture (9:1, v/v).

Matrix solid phase dispersion with sand. The optimisation procedure of MSPD with sand was carried out to determine MSPD conditions suitable for all six herbs used in the presented experiments. The MSPD process was optimised estimating the total amount of essential oil components isolated from the selected herbs. The MSPD conditions yielding the greatest total amount of essential oil components in herbs were assumed as optimal.

The influence of the following factors was examined in the experiments:

- sample/sand mass ratio (1:4, 1:8, 1:12 and 1:24);
- type (methanol, *n*-propanol, propanol-2, 1,4-dioxane, butanol and *n*-octane) and volume (1, 2 and 3 mL) of MSPD dispersing liquid;
- volume of hexane–ethyl acetate mixture (9:1, v/v) used for the elution of essential oil components from the homogenised mixture (5, 10 and 15 mL).

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

In consequence of the described investigations the following MSPD/sand procedure was found optimal for all six herbs used in the presented experiments. A 0.2 g sample of ground herb, 4.8 g of sand and 3 mL of 1,4-dioxane were used in the MSPD procedure. The components were blended in a glass mortar for 10 min using a glass pestle to obtain a homogeneous mixture. After homogenisation, 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 MSPD procedure with sorbent and sand was repeated three times using fresh portions of plant material.

Repeatability of MSPD/sand method. The repeatability was evaluated by only one analyst within one day. Six independent samples were examined for this purpose. The experiments were performed using only one herb, oregano.

Recovery of MSPD/sand method. To estimate the recovery of the MSPD/sand method, oregano samples were spiked with pentadecane (C15) and hexadecane (C16) solutions to obtain three different concentration levels of these standard compounds in the plant material. The obtained referenced materials contained 1292, 2584 and 3876 µg per 1 g of herb in the case of C15 and 1279, 2558 and 3837 µg/g in the case of C16. The above-mentioned amounts correspond to the amounts of the main essential oil components existing in the examined plants. The spiked samples were subjected to the MSPD procedure and obtained extracts were GC analysed. The recoveries were calculated relating the peak areas of C15 and C16 to the peak area of these compounds for standard solutions.

Chromatographic analysis

Qualification of essential oil components in the samples prepared by MSPD, PLE and SD 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 the carrier gas. A 1 µL aliquot of the sample was injected using an AOC-20i type autosampler. The injector temperature was 310°C. The following temperature programme was applied: 1 min at 50°C followed by 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; 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 by our own temperature retention indexes.

Quantification of extracts was performed using a gas chromatograph with a flame ionisation detector, GC-FID model GC-2010 (Shimadzu, Kyoto, Japan). A 1 µL aliquot of the sample was injected using an AOC-20i type autosampler into a ZB5-MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µL film thickness; Phenomenex, USA). The temperature programme during GC-FID separation was the same as for GC/MS. Peaks were identified 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), a known amount of which was added to the examined extracts before GC measurements.

Results and Discussion

Table 1 contains the total amounts of essential oil components estimated in the examined herbs using SD, PLE, MSPD/C₁₈ sorbent and MSPD/sand. The values collected in Table 1 were

Table 1. Total amounts of essential oils components estimated in herbs using SD, PLE and MSPD with C₁₈ sorbent and sand. Mean values \pm SD, $n = 3$

Type of herb	Sample preparation method			
	Steam distillation	PLE	MSPD/C ₁₈	MSPD/sand
Chamomile	1.85 \pm 0.08	2.19 \pm 0.09	2.07 \pm 0.15	2.07 \pm 0.11
Mint	8.72 \pm 0.35	9.60 \pm 0.30	9.87 \pm 0.66	9.13 \pm 0.65
Sage	7.84 \pm 0.36	8.54 \pm 0.27	8.36 \pm 0.55	8.44 \pm 0.58
Marjoram	3.35 \pm 0.15	4.68 \pm 0.17	5.06 \pm 0.37	4.79 \pm 0.34
Savory	17.68 \pm 0.63	17.32 \pm 0.68	17.86 \pm 0.85	16.16 \pm 0.82
Oregano	2.16 \pm 0.08	3.04 \pm 0.12	3.17 \pm 0.26	3.10 \pm 0.16

calculated relating the peak area of essential oil components to the peak area of the known amount of hexadecane (or pentadecane) added to the herb extracts as a quantity standard. According to the literature (Dawidowicz *et al.*, 2008; Carabias-Martinez *et al.*, 2005), 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 for the use of an extractant at a temperature above its normal boiling point and, in consequence, for the quick and efficient removal of the analytes from various matrices. This makes the extraction ability of the applied methods and relating them to the extraction ability of PLE worth closer investigation. The results from the collected data show that the yields of essential oil components obtained by MSPD are almost the same (within experimental error) as those obtained by PLE. Only for savory was the difference between MSPD (MSPD/sand) and PLE data visible, although it was still less than 7%. In the case of other herbs the difference does not exceed 5%. Hence, the presented results indicate that the extraction efficacy of MSPD, a very simple and cheap sample preparation procedure, is equivalent to that of PLE which, contrary to MSPD, is a technically advanced and complex method. Moreover, the results in Table 1 prove that essential oil components belong to easily recoverable analytes and that C₁₈ sorbent, commonly employed in the MSPD process, can be substituted with sand in analysing essential oil in herbs, which significantly lowers the cost of the analytical procedure.

As seen in Table 1, the standard steam distillation method is generally a less effective process of isolating essential oil components than MSPD and PLE. Only in the case of essential oil components from savory is the isolation efficiency of SD equivalent to PLE and slightly exceeds that of MSPD/sand. Therefore, the results in Table 1 allow us to formulate an opinion about the isolation efficiency of the applied methods, but they 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 a comparison of the essential oil compositions estimated by each of them. The chromatograms of SD, PLE, MSPD/C₁₈ and MSPD/sand extracts from three exemplary herbs are presented in Figs 1–3. As results from the figures show, there were no significant differences in qualitative and quantitative compositions of the extracts from the exemplary herbs. The same conclusion can be drawn considering the chromatograms of the extracts from other examined herbs, not presented in the paper. The essential oils are very complex mixtures which, apart from their main components, contain very small amounts of many other compounds. In order to simplify the comparison of the methods it was decided to limit the number of essential oil com-

ponents considered to the main components for individual herbs; see Fig. 4. Figure 4 indicates that noticeable differences are seen only in the SD extracts. The observed differences concern solely four compounds considered in Fig. 4: caryophyllene and β -bergamotene in oregano, manool in sage and bisabolol oxide A in chamomile. The lower amounts of these compounds in SD extracts can be connected with their relatively high boiling temperature (Corey *et al.*, 1964; Larsen and Monti, 1977) or with their transformation during the relatively long time of the applied SD process (3 h; Popa and Salei, 1974; Waleczek *et al.*, 2003). The quantitative composition of the main essential oil components estimated for individual herbs by means of PLE, MSPD/C₁₈ and MSPD/sand is almost the same. Hence, the detailed consideration of the main components quantities in the examined herbs confirms the already formulated conclusion that C₁₈ sorbent can be substituted with sand in the MSPD process applied for essential oil analysis in herbs. Moreover, the amounts of the main components established in the examined herbs using MSPD/sand are the same as their amounts estimated using PLE.

The data presented above were obtained using the MSPD mixture which was established as optimal in the case of all the examined herbs using 0.2 g plant portions. The relationships between MSPD components were as follows: 0.2 g of plant, 4.8 g of sand and 3 ml of dioxane as MSPD dispersing liquid. However, the preliminary experiments leading to the optimisation of MSPD/sand for essential oil analysis also indicated a significant influence of MSPD dispersing liquid type on the extraction efficiency of the MSPD process.

Octadecyl modified silica used in the classic MSPD process for essential oil analysis in herbs (Baker, 2000) acts not only as an abrasive material disrupting the plant sample architecture but also as a 'bound' solvent that accumulates extracted compounds. When C₁₈ sorbent is replaced with sand, the role of solvent accumulating essential oil components from the herb should be played by a properly selected MSPD dispersing liquid employed for mechanical blending of the sample with sand. The dispersing liquid should:

- be a good solvent for all essential oil components;
- not disturb the analytical procedure; and
- be used in the amount giving the MSPD mixture proper consistency (not too dry and/or not too liquid).

The influence of the MSPD dispersing liquid type (methanol, *n*-propanol, propanol-2, butanol, 1,4-dioxane and octane) on the total yields of essential oil components from herbs is shown in Table 2 (exemplified for oregano). The presented data were obtained using the MSPD mixture established as optimal for all

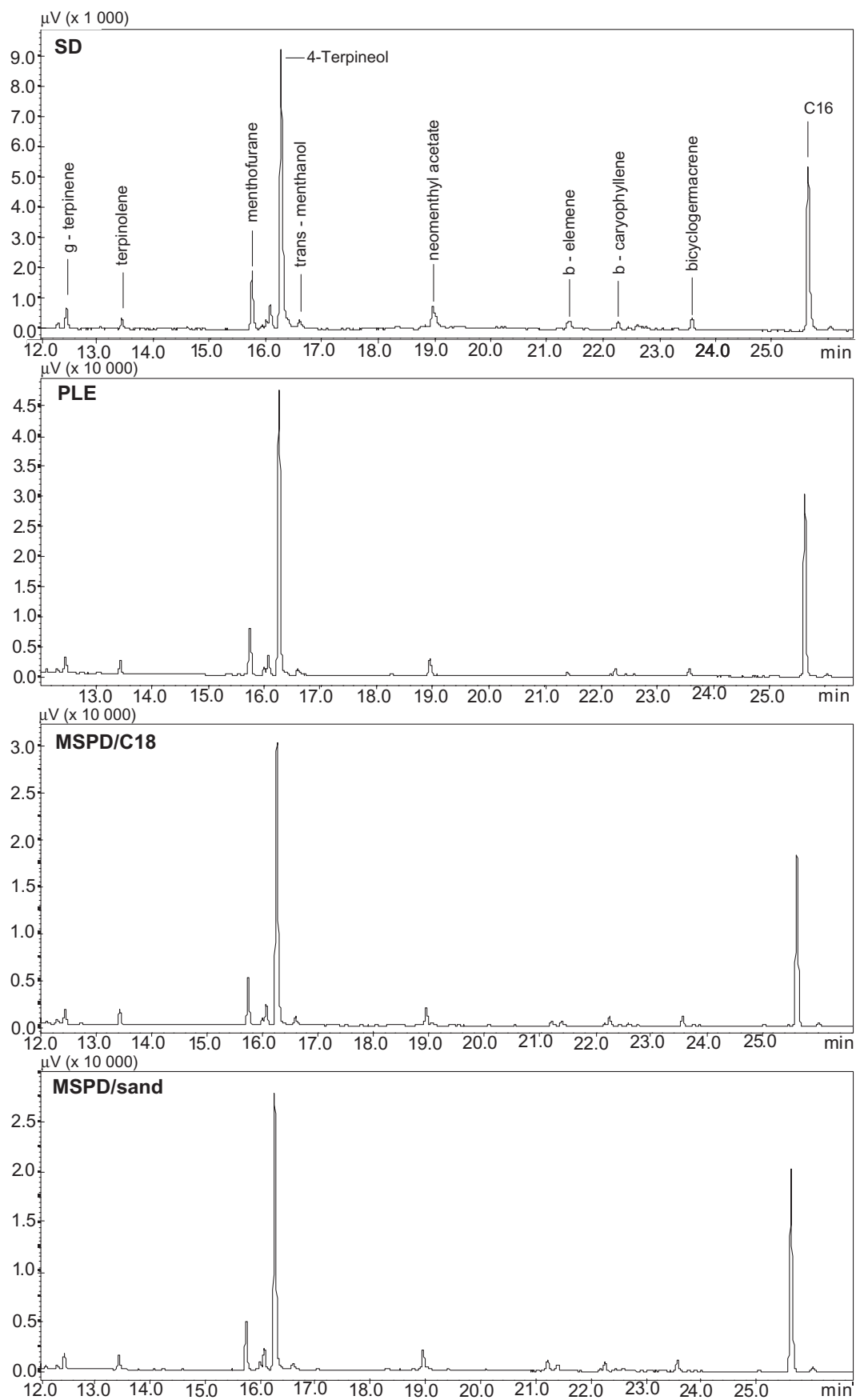


Figure 1. Gas chromatograms of SD, PLE, MSPD/C₁₈ and MSPD/sand extracts from mint.

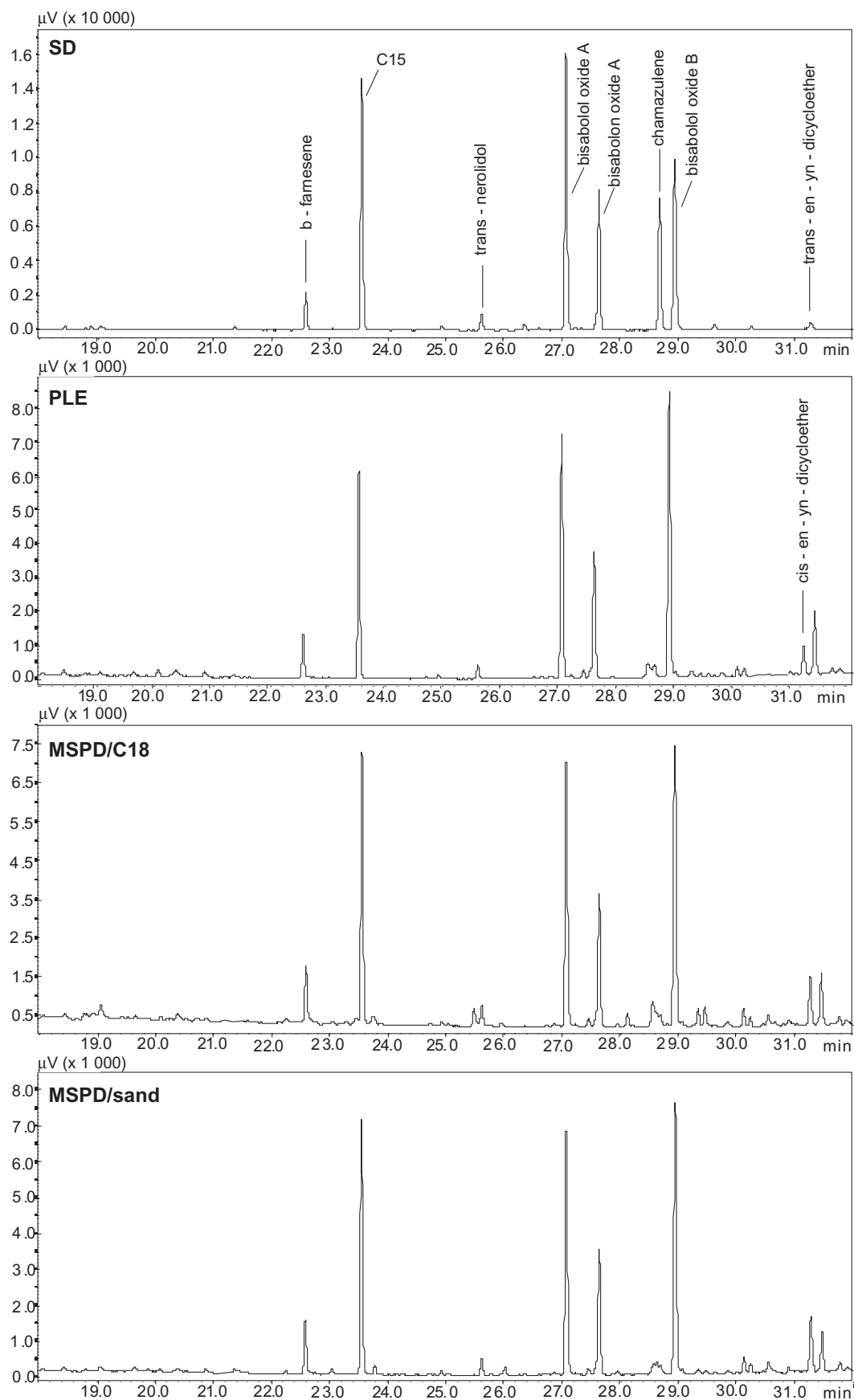


Figure 2. Gas chromatograms of SD essential oil, PLE, MSPD/C₁₈ and MSPD/sand extracts from chamomile.

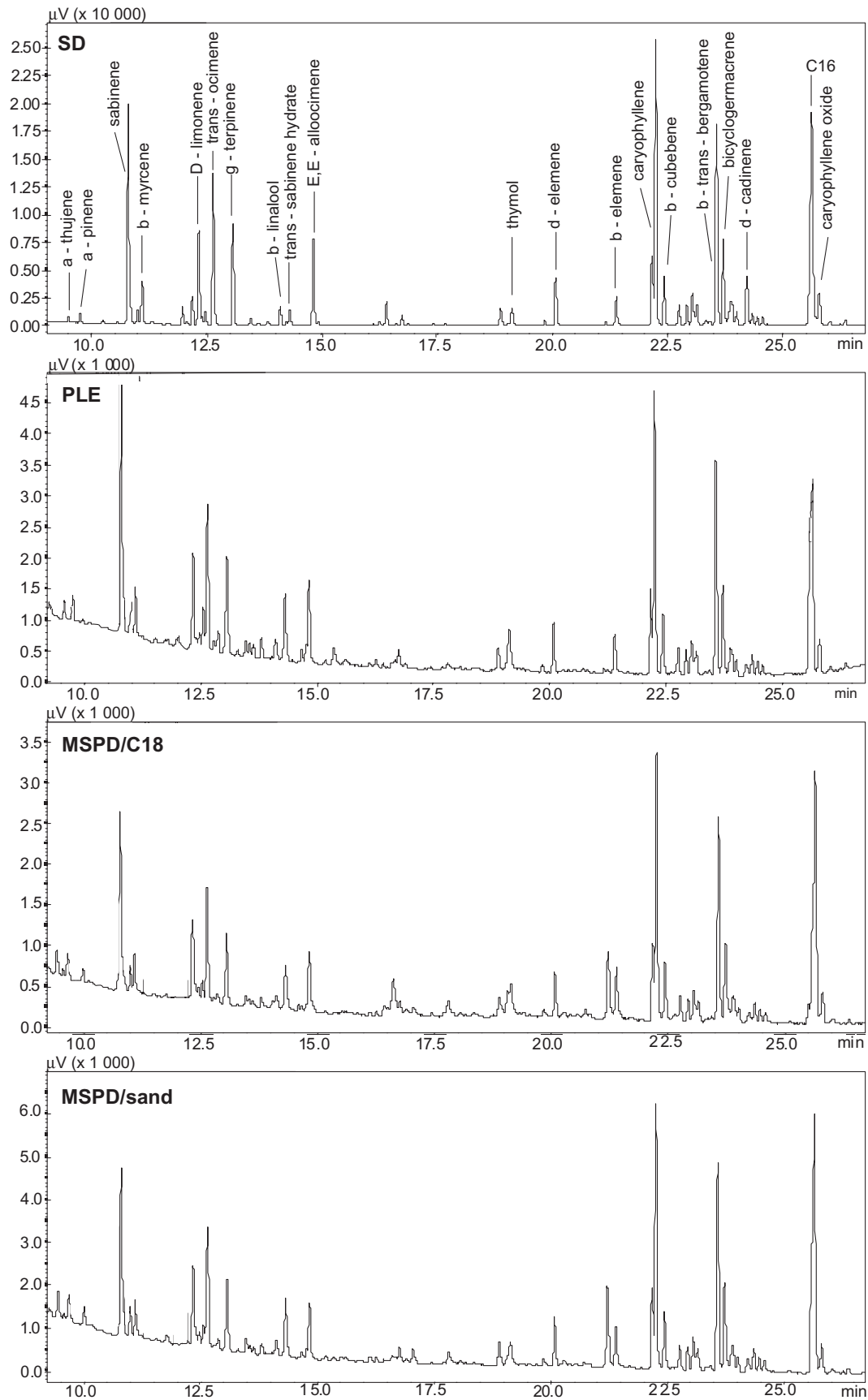


Figure 3. Gas chromatograms of SD, PLE, MSPD/C₁₈ and MSPD/sand extracts from oregano.

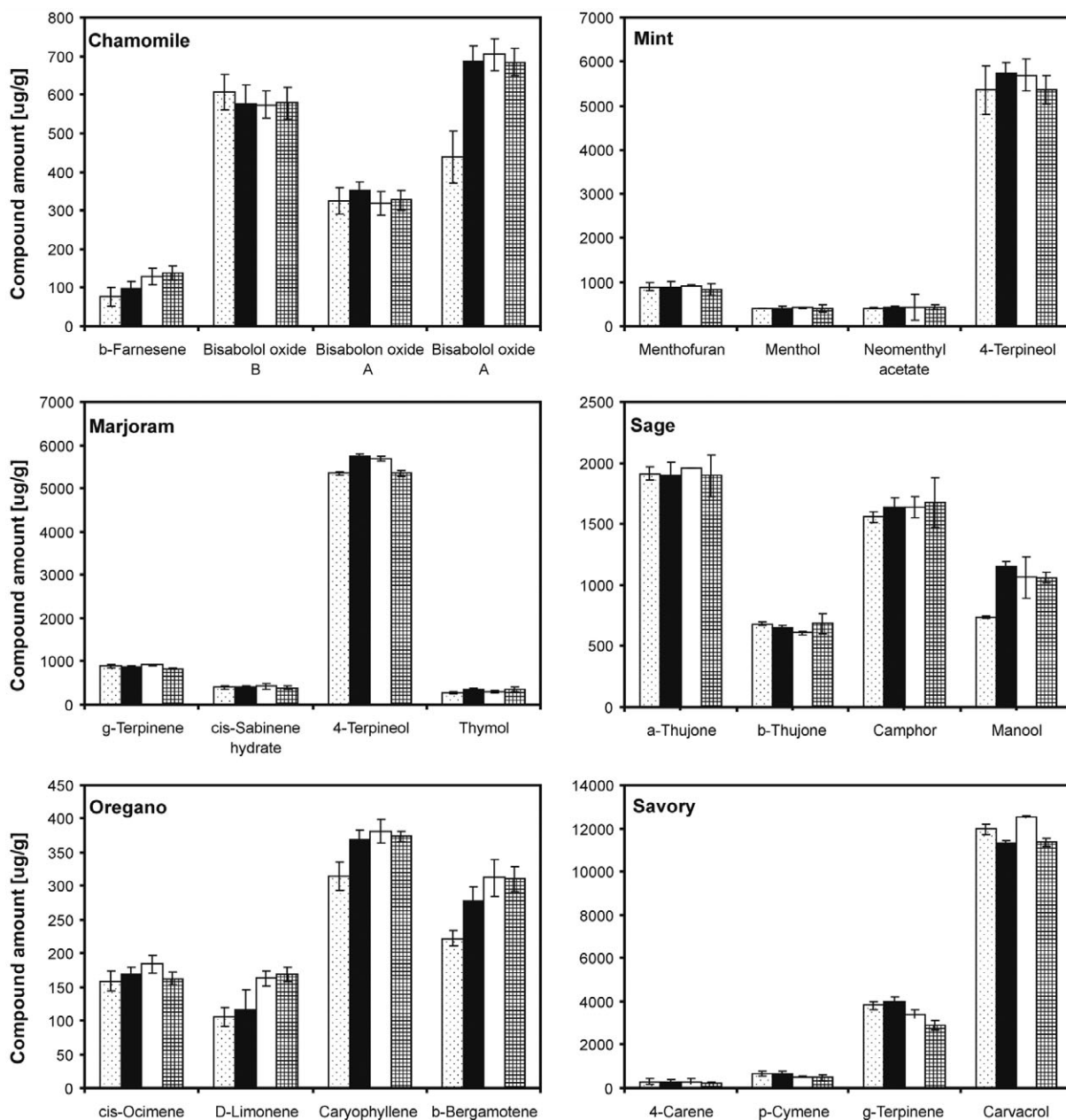


Figure 4. Amounts of main aroma compounds (µg/g) estimated in the examined herbs using SD (dotted bars), PLE (black bars), MSPD/C₁₈ (blank bars) and MSPD/sand procedure (checked bars).

Table 2. The influence of MSPD dispersing liquid on extraction efficiency on total oregano essential oil amount

		MSPD/sand Type of solvent				PLE Hexane
1,4-Dioxane	Methanol	<i>n</i> -Propanol	Propanol-2	Butanol	C8	
3.08 ± 0.12	2.01 ± 0.34	2.90 ± 0.29	2.69 ± 0.41	2.93 ± 0.35	2.64 ± 0.27	3.04 ± 0.12

the examined herbs. For better comparison of the data, the last column of Table 2 also contains the total amount of essential oil components estimated for an exemplary herb by means of PLE. As appears from the table, the total amount of the essential oil components depends on the type of the MSPD dispersing liquid used. The lowest amount is for methanol and the highest, com-

parable with the PLE data, for 1,4-dioxane. The visible variations among the used MSPD liquids should be connected with the evaporation velocity of individual liquids but not with their ability to dissolve essential oil components. It should be stressed that all the MSPD dispersing liquids used in the experiments are good solvents of essential oil components. In the case of 1,4-dioxane,

Table 3. Exemplary experimental data showing the precision of MSPD/sand method for the estimation of total mass essential oil in herbs—values for oregano

No	Mass ($\mu\text{g/g}$)
1	3213.8
2	3093.5
3	3006.5
4	2907.2
5	2981.6
6	2950.6
Mean (6)	3025.5
RSD (%)	3.68

Table 4. Exemplary experimental data showing the recovery of MSPD/sand method for the estimation of essential oil components in herbs—values for oregano spiked with known amount of pentadecane and hexadecane ($n = 3$)

Compound	Concentration level ($\mu\text{g/g}$)	Recovery (%)	Mean recovery (%)
C15	1292	95.12	95.47
	2584	95.70	
	3876	95.59	
C16	1279	96.87	97.03
	2558	97.34	
	3837	96.87	

the MSPD mixture desiccates most slowly, which prevents losses of essential oil components and explains the obtained results. Similar variations, showing the influence of the MSPD liquid type on the total amount of essential oil components, were observed for other examined herbs. Each time 1,4-dioxane appeared to be the best MSPD dispersing liquid of all the used solvents.

Additional opinion about applicability of MSPD/sand method for the isolation of essential oil components from herbs can be formulated considering the data collected in Tables 3 and 4 which were obtained during simplified validation of the method. As results from Table 3, repeatability of the MSPD/sand method is acceptable from analytical point of view in the case of essential oil analysis. A similar conclusion can be drawn analyzing recovery of the examined method. The recoveries estimated for two internal standards exceed 95%, which is satisfactory for isolation of essential oils for analytical purposes.

The results presented in the paper reveal that MSPD, a very simple and cheap sample preparation procedure, is suitable for the isolation of essential oil components from herbs. Its extraction efficiency is equivalent to PLE, which unlike MSPD, is a technically advanced and complex method recognised as one of the most efficient extraction techniques. Moreover, the discussed results prove that essential oil components belong to easily recoverable analytes. In consequence, the C_{18} sorbent, commonly employed in MSPD processes, can be substituted with sand in the analysis of essential oil in herbs, thus significantly diminishing the cost of the analytical procedure. However, such replacement requires the employment of a properly selected MSPD dispersing liquid.

References

- ASE 200. 1995. *Accelerated Solvent Extractor Operator's Manual*. Document No. 031149, Revision 01, Dionex, Sunnyvale, CA, Sect. 3–5.
- Baker SA. 2000. Applications of matrix solid-phase dispersion in food analysis. *J Chromatogr A* **880**: 63–68.
- Baker SA. 2007. Matrix solid phase dispersion (MSPD)—a review. *J Biochem Biophys Meth* **70**: 151–162.
- Baker SA, Long AR, Short CR. 1989. Isolation of drug residues from tissues by solid phase dispersion. *J Chromatogr A* **475**: 353–361.
- Carabias-Martinez R, Rodrigues-Gonzalo E, Revilla-Ruiz P, Hernandez-Mendez J. 2005. Pressurized liquid extraction in the analysis of food and biological samples. *J Chromatogr A* **1089**: 1–17.
- Chiacchierini E, Restuccia D, Vinci G. 2006. Evaluation of two different extraction methods for chromatographic determination of bioactive amines in tomato products. *Talanta* **69**: 548–555.
- Corey EJ, Mitra RB, Uda H. 1964. Total synthesis of d,l-caryophyllene and d,l-isocaryophyllene. *J Am Chem Soc* **86**: 485–492.
- Dawidowicz AL, Rado E. 2010. Matrix solid-phase dispersion (MSPD) in chromatographic analysis of essential oils in herbs. *J Pharm Bioanal Anal* **52**: 79–85.
- Dawidowicz AL, Rado E, Wianowska D, Gawdzik J, Mardarowicz M. 2008. Application of PLE for the determination of essential oil components from *Thymus vulgaris* L. *Talanta* **76**: 878–884.
- European Pharmacopoeia. 2009. 6th edn. European Directorate for the Quality of Medicines and Health Care.
- Kristenson EM, Brinkman UATH, Ramos L. 2006. Recent advances in matrix solid-phase dispersion. *Trends Anal Chem* **25**: 96–111.
- Larsen SD, Monti SA. 1977. Total synthesis of racemic α -trans- and α -cis-bergamotene and α -pinene. *J Am Chem Soc* **99**: 8015–8020.
- Popa DP, Salei LA. 1974. Manool from *Salvia sclarea*. *Chem Nat Comp* **10**: 409.
- Waleczek KJ, Cabral Marques HM, Hempel B, Schmidt PC. 2003. Phase solubility studies of pure (–)- α -bisabolol and camomile essential oil with β -cyclodextrin. *Eur J Pharm Biopharm* **55**: 247–251.
- Ziakova A, Brandstecerova E, Blahova E. 2003. Matrix solid-phase dispersion for the liquid chromatographic determination of phenolic acids in *Melissa officinalis*. *J Chromatogr A* **983**: 271–275.