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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.