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Short communication

Matrix solid-phase dispersion for the liquid chromatographic determination of phenolic acids in *Melissa officinalis*

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Abstract

Matrix solid-phase dispersion (MSPD) was used for sample preparation of plant material (*Melissa officinalis*, Lemon Balm) prior to liquid chromatography of rosmarinic, caffeic and protocatechuic acids, phenolic compounds present in this herb. Different MSPD sorbents and various elution agents were tested and the optimal extraction conditions determined with the aim to obtain extraction recoveries greater than 90% for all analytes.

Keywords: *Melissa officinalis*; Matrix solid-phase dispersion; Phenolic acids

1. Introduction

Melissa officinalis belongs to the group of native medicinal plants *Lamiaceae*. It can be used for the treatment of several diseases, and extracts have significant antioxidative activity. Published results confirm that the activity is partly due to the content of phenolic acids, mainly rosmarinic acid, which has been found in large amounts in many medicinal plants [1–3].

Plant material contains a huge variety of different compounds, such as waxes, oils, sterols and chlorophyll, which may interfere with the analyzed compounds. Various methods are recommended for the sample handling of plant material before HPLC analysis [liquid–liquid extraction (LLE), solid-phase extraction (SPE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE)] [4,5].

Matrix solid-phase dispersion (MSPD) is a sample preparation technique that combines both sample homogenization and extraction of the analysed compounds in one step. The application of MSPD is based on the blending of a viscous, solid or semi-solid sample with an abrasive solid support material. The bound organic phase acts as a solvent or detergent that dissolves and disperses the sample components into the bound phase. MSPD columns prepared with reversed-phase supports are most frequently eluted with a sequence of solvents beginning with the least polar (hexane) and then with those of increasing polarity (ethyl acetate, acetonitrile, methanol), then water [6,7].

Only a few reports have been published dealing with MSPD as an isolation technique for biologically active compounds (herbicides, pesticides and other pollutants) in fruits, vegetables and plant material

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[8–11], but none of these reports has described the application of this preparation technique for the extraction of phenolic compounds from medicinal plant material.

The aim of this work was to use the MSPD method for the extraction of the phenolic acids present in *Melissa officinalis* and to determine the optimal conditions for the sample preparation step.

2. Experimental

2.1. Chemicals and reagents

Standards of rosmarinic, caffeic and protocatechuic acids and plant samples of *Melissa officinalis* L. grown in Slovakia were obtained from the Research Institute of the Food Industry, Biocentrum Modra (Slovakia). Stock solutions of standards (ca. 1 mg/ml) were prepared in methanol and stored in a freezer at −20 °C. The stability of the stock solutions was controlled and no change in concentrations was observed. Working solutions were prepared by diluting the stock solutions with mobile phase.

HPLC-grade methanol was obtained from Merck (Slovakia), and *n*-hexane, dichloromethane, formic acid and ethyl acetate, all p.a., were supplied by Lachema (Czech Republic).

The solid phases used for MSPD were:

- Polygoprep C_{18}, 40 μm (Macherey-Nagel, Duren, Germany), non-end-capped, 14% C;
- Bakerbond C_{18}, 40 μm (J.T. Baker, Deventer, Netherlands), end-capped, 17.5% C;
- Silasorb C_{18}, 30 μm (Lachema, Brno, Czech Republic), non end-capped, 15% C;
- Alltech bulk high capacity C_{18} sorbent, 50 μm (Alltech, Deerfield, IL, USA), end-capped, 17% C;
- SGX C_{18}, 60 μm (Tessek, Prague, Czech Republic), slightly end-capped, 16% C;
- Florisil, 60–100 mesh (Merck, Darmstadt, Germany).

2.2. MSPD of plant material

Dried plant tops were ground to powder. A 0.5 g aliquot of the sample was placed in a mortar and mixed with 2 g of previously cleaned sorbent and 1 ml of *n*-hexane. The mixture was then homogenized in the agate mortar using an agate pestle to obtain an homogenous mixture. The blend was then transferred into a 10 ml syringe with a paper frit on the bottom. The sample was covered with another paper frit and compressed using the syringe plunger. Interfering compounds were washed with 10 ml *n*-hexane and 10 ml dichloromethane. The syringe was then dried for 5 min under vacuum. Phenolic compounds were eluted directly with the elution mixture and the residue after evaporation to dryness was dissolved in methanol–water, pH 2.5 (80:20). Eluents were filtered through a Teflon microfilter and injected into the HPLC system.

2.3. LC analysis

An HP 1100 system (Hewlett-Packard, Waldbronn, Germany) consisting of a pump with degasser, a diode-array detector (DAD) and an HP ChemStation was used. The analytical column was a reversed-phase Symmetry C_{18}, 150×3.9 mm I.D., 5 μm particle size, with a Symmetry C_{18}, 20×3.9 mm I.D. guard column, from both Waters (Milford, MA, USA). The mobile phase was methanol–water (pH 2.5), delivered at a flow-rate of 0.4 ml/min, with a linear gradient composition increasing from 15% methanol to 75% methanol over 40 min. All analyses were carried out at ambient temperature.

3. Results and discussion

In our previous work a simple HPLC method was developed for the simultaneous separation of phenolic acids in *Melissa officinalis* and the extraction conditions for LLE sample preparation were optimized [1]. Different sample preparation and clean-up assays (LLE-SPE, ASE, SFE) were compared for analyte isolation from the same plant material [12]. The optimized LLE technique has also been applied for other herbs of the *Lamiaceae* family (*Rosmarinus officinalis*, *Salvia officinalis*, *Thymus serpyllum*, *Origanum vulgares*) and yields of phenolic acids were determined [13].

In this work, the MSPD technique was examined as a preparation technique for the isolation of phenolic acids from *Melissa officinalis* (rosmarinic,

caffeic and protocatechuic acids). Sorbents with different physical and chemical properties and their combinations were used and, for various elution media, the optimal elution volumes were determined. From our previous experience with plant sample preparation, the following elution agents were tested: methanol, methanol acidified with 0.2% formic acid, methanol–water, pH 2.5, and ethyl acetate. Yields of phenolic acids from *Melissa officinalis* obtained with the use of these elution media were evaluated and the results are illustrated in Fig. 1. The same volumes of elution agents (10 ml) were used in all experiments. It is clear that the highest yields of all the analyzed phenolic acids were achieved with methanol–acidified water, pH 2.5 (60:40 and 20:80), as the solubility of polar phenolic acids in pure organic elution agents is much less than in water-containing mixtures. Of course, regulation of the pH to 2.5 before the isolation step is also a very important factor, increasing the extraction recoveries of acidic analytes (the p*K* values of the analyzed compounds are about 4.5).

The extraction recoveries of all analyzed compounds were evaluated for various volumes of elution solvents. Although a volume of 10 ml is often reported to be sufficient for MSPD procedures, we found that this was not always true for all tested elution solvents and all studied analytes. A typical example is shown in Fig. 2: for methanol–water, pH

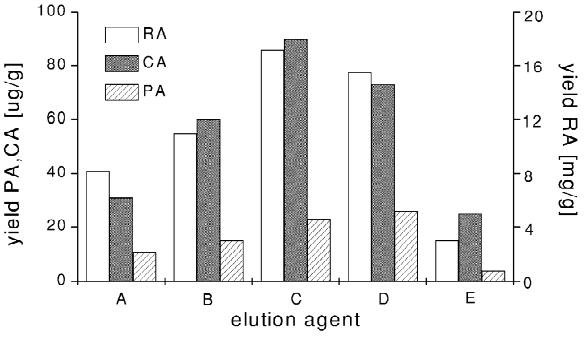

Fig. 1. Yields of phenolic acids [rosmarinic (RA), caffeic (CA) and protocatechuic (CA)] from plant material using different elution agents for MSPD. Sorbent, Alltech C_{18}; elution volume, 10 ml. Elution solvents: A, MeOH; B, MeOH + 0.2% HCOOH; C, MeOH–water, pH 2.5 (80:20); D, MeOH–water, pH 2.5 (60:40); E, ethyl acetate.

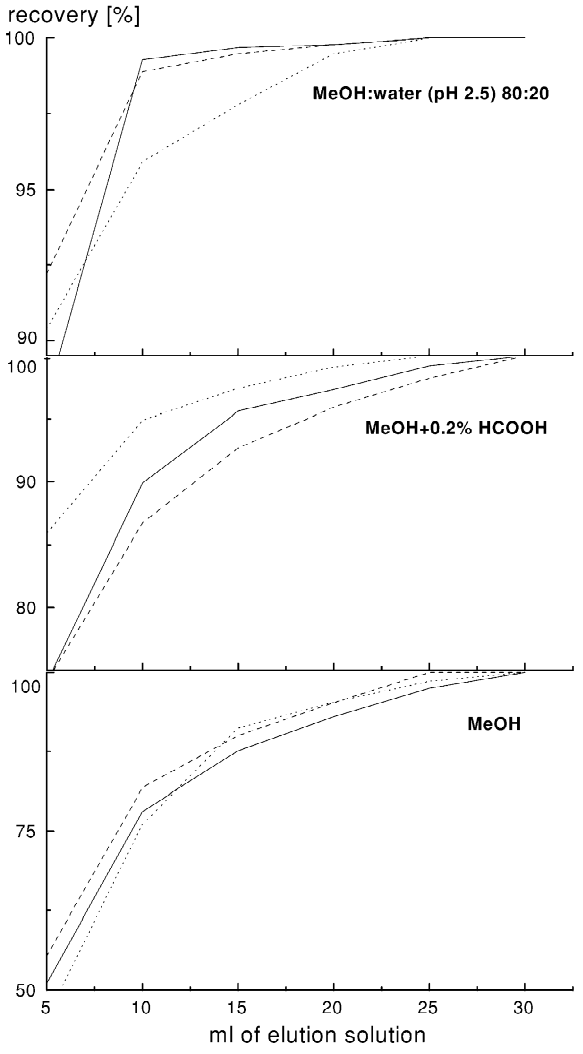

Fig. 2. Dependence of MSPD recovery on the volume of the applied elution solvent.

2.5 (80:20), 10 ml was sufficient for two of the phenolic acids, but 25 ml was needed for protocatechuic acid. With methanol and acidified methanol, up to 20–25 ml was required for quantitative recovery of all analytes.

Different C_{18} bulk materials were tested as solid supports for the MSPD assay. The results were evaluated for methanolic–acidic water, pH 2.5, as the best elution agent and an elution volume of 10 ml (Table 1). No significant differences in analyte yields

Table 1
Yields of phenolic acids extracted from *Melissa officinalis* using MSPD with various sorbents

Sorbent	RA (mg/g)	CA (μg/g)	PA (μg/g)
Alltech C_{18}	16.8	88.8	23.1
SGX C_{18}	14.4	72.1	13.7
Silasorb C_{18}	15.5	79.0	20.0
Polygoprep C_{18}	14.7	60.0	6.5
Bakerbond C_{18}	16.0	67.2	23.2
Florisil + Alltech	15.1	71.0	16.6
Silasorb + Alltech	15.5	57.1	13.1

Extraction conditions: elution with 10 ml MeOH–water, pH 2.5 (80:20) ($n=3$). RSDs: RA, 0.4–1.2%; CA, 0.2– 5.0%; PA, 1.6–4.3%.

were found for the different sorbents. Both end-capped and non-end-capped sorbent materials are suitable for MSPD of phenolic acids from plant material. Also, there were no significant differences between materials with different particle sizes. These results are in accordance with those already published [7]. The best overall results were obtained for Alltech C_{18}. Mixed sorbents were also tested for the MSPD assay. The homogenized plant sample with the Alltech C_{18} sorbent was introduced into MSPD syringes containing 0.5 g Florisil or Silasorb at the bottom of the column, but yields did not differ much from those obtained using one sorbent, and if they did, the differences were not significant (Table 1).

A LC–UV chromatogram of MSPD extracts obtained using the Alltech C_{18} sorbent and methanol–water, pH 2.5 (80:20), as elution solvent is shown in Fig. 3. Calibration curves were constructed for the analyzed compounds: RA, $y = -1.996 + 1.016x$, $r=0.9981$; CA, $y = 0.7159 + 0.9414x$, $r=0.9988$; PA, $y = 0.0004 + 0.5819x$, $r=0.9986$. It is clear that PA and CA are present in the plant samples at concentrations 1000 lower than RA (Figs. 1 and 3).

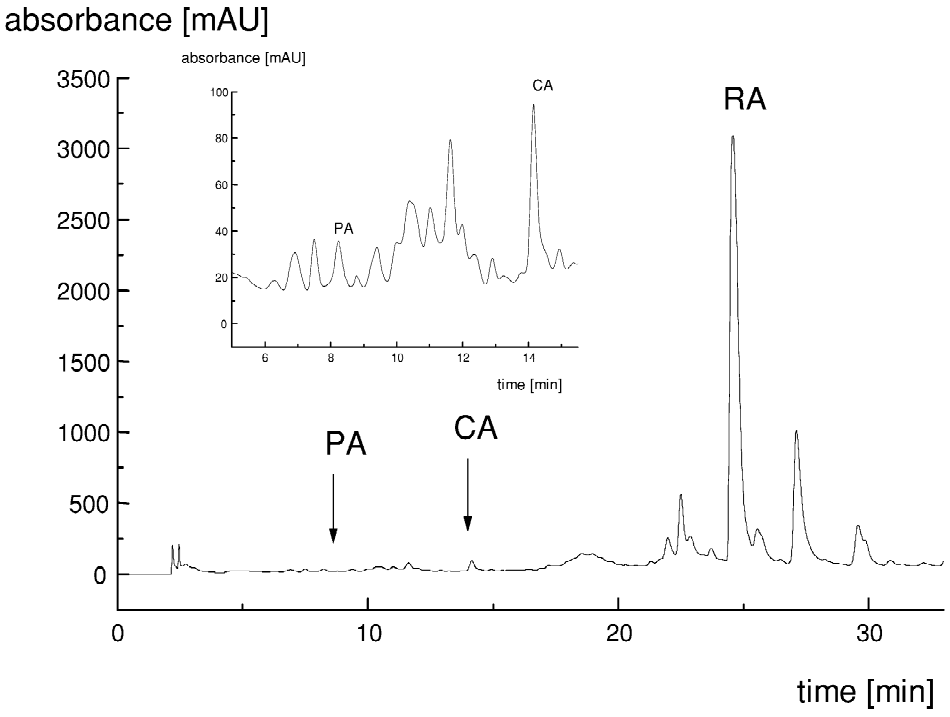

Fig. 3. LC–UV chromatogram of *Melissa officinalis* extracts after MSPD. Sorbent, Alltech C_{18}; elution agent, 10 ml methanol–water, pH 2.5 (80:20). Chromatographic conditions: chromatographic column, Symmetry C_{18} (150×3.9 mm, 5 μm) with a Symmetry C_{18} (20×3.9 mm) guard column; mobile phase, MeOH–water (pH 2.5) gradient elution; flow-rate, 0.4 ml/min; detection, 280 nm; injection volume, 20 μl. PA, protocatechuic acid; CA, caffeic acid; RA, rosmarinic acid.

4. Conclusion

MSPD has been demonstrated to be a suitable preparation technique, a simple alternative to LLE, SPE and SFE, for the isolation of phenolics from plant material. No homogenization, grinding or milling steps are necessary. It is only necessary to select suitable elution agents giving the highest yields of the analytes and to optimize the volume of the elution medium. Washing steps can be changed according to the amounts of interfering and co-eluting compounds. The MSPD procedure developed here can be modified very simply for the isolation of phenolics in other plant materials.

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