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Article

Matrix Solid-Phase Dispersion *versus* Ultrasound Assisted Extraction with Solid-Phase Extraction in the HPLC Analysis of Furanocoumarins from Fruits of *Archangelica officinalis* Hoffm.

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Neste estudo, um eficiente método, a dispersão da matriz em fase sólida (DMFS), foi usado para a determinação simultânea de furanocoumarinas de frutos de *Archangelica officinalis* Hoffm. Amostras da planta foram preparadas pelo procedimento MSPD otimizado, utilizando C18 como sorvente. A análise foi realizada por cromatografia líquida de alta eficiência com detector de arranjo de diodos (HPLC-DAD). A eficiência do método DMFS também foi comparada com a extração assistida por ultra-som associada à extração em fase sólida (SPE com USAE). O MSPD extraiu furanocoumarins (isoimperatorin, imperatorin, bergapteno, isopimpinellin, xantotoxina, umbeliferona e xanthotoxol) de *Archangelica officinalis* com recuperações satisfatórias que variam de 91,43 a 96,07% e desvio padrão relativo menor do que 4,34% . O limite de detecção das várias furanocoumarinas, encontra-se na faixa de 0,37 μg mL^{-1} para a xanthotoxol e 10,82 μg mL^{-1} para imperatorin. Os resultados apresentados no manuscrito revelam que o método DMFS é eficiente, simples, rápido e de fácil execuçaõ, e é adequado para o isolamento de furanocoumarins a partir da plantas.

In this study an efficient matrix solid-phase dispersion (MSPD) method for the simultaneous HPLC analysis of furanocoumarins from fruits of *Archangelica officinalis* Hoffm. was performed. Herbal samples were prepared by an optimized MSPD procedure using C18 as sorbent. The analysis was performed by high performance liquid chromatography with diode array detector (HPLC-DAD). The efficiency of the MSPD method was also compared with ultrasound assisted extraction with solid-phase extraction (USAE with SPE). The MSPD extracted furanocoumarins (isoimperatorin, imperatorin, bergapten, isopimpinellin, xanthotoxin, umbelliferone and xanthotoxol) from *Archangelica officinalis* with satisfactory recoveries ranging from 91.43% to 96.07% and relative standard deviations lower than 4.34%. The detection limit of various furanocoumarins was found to be in the range of 0.37 μg mL^{-1} for xanthotoxol to 10.82 μg mL^{-1} for imperatorin. The results presented in the paper reveal that MSPD is efficient, fast, simple and easy to perform method suitable for the isolation of furanocoumarins from herbs.

Keywords: *Archangelica officinalis* Hoffm., furanocoumarins, extraction techniques, high performance liquid chromatography

Introduction

The goal of every extraction process is rapid and effective isolation of compounds from matrix by use of minimum amount of solvent. Traditional liquid-solid extraction (LSE) procedures (e.g. Soxhlet extraction) are generally labour-intensive and time and solvent-consuming. New extraction methods such as microwave assisted solvent extraction (MASE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE) or ultrasound assisted

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extraction (USAE) require shorter extraction time, they use low amount of solvents, allow for simultaneous parallel processing of several samples and are automatic but more expensive. The important step in sample preparation is purification of crude extract. The most commonly used purification technique is the solid-phase extraction (SPE).[1]

An attractive alternative, introduced for sample preparation of complex matrices, is matrix solid-phase dispersion (MSPD).[2-4] This method can eliminate many complicated steps in classical solid-liquid and/or SPE, reduce time and solvent consumption.[1,5,6]

Matrix solid-phase dispersion has been widely used in the last year for the isolation of a wide range of natural compounds, drugs, pesticides, and other analytes from different biological matrices providing, in many cases, equivalent or superior results to other extraction methods coupled with SPE techniques.[7-10]

The aim of this paper was the suitability comparison of matrix solid-phase dispersion as alternative method to ultrasound assisted extraction coupled with solid-phase extraction for the analysis of furanocoumarins (isoimperatorin, imperatorin, bergapten, isopimpinellin, xanthotoxin, umbelliferone and xanthotoxol) from fruits of *Archangelica officinalis* Hoffm.

Furanocoumarins have pharmacological activities including cytostatic, anti-tumor, antiinflamatory, and anti-fungal.[11-13] They are important drugs in vitiligo and psoriaris therapy,[14,15] and are also used in therapy of cutaneous T-cell lymphoma and chronic graft-*versus*-host disease.[16] For this reason it is important to develop rapid, simple and inexpensive method of extraction and purification of furanocoumarins from plant material.

Experimental

Sampling

Fruits of *Archangelica officinalis* Hoffm. were collected in 2009 in Medicinal Plant Garden, Department of Pharmacognosy, Medical University in Lublin, Poland. Fruits were dried at room temperature and powdered to a homogenous size. A voucher specimen was deposited in the Herbarium of Pharmacognosy Department. 1 g and 0.25 g of dried *Archangelica officinalis* fruits powder was used to USAE and MSPD extraction respectively.

Materials and chemicals

Certified analytical standards of all furanocoumarins, purity >98%, were purchased from Sigma Aldrich. Methanol, dichloromethane, and petroleum ether used for preparation and purification of the extracts were of analytical grade and purchased from the Polish Reagents (POCH, Gliwice, Poland). Methanol used for HPLC was of chromatographic grade (J.T. Baker Inc., Netherlands), water was purified using a Millipore laboratory ultra pure water system (Simplicity™ system, Millipore, Molsheim, France). Solid phase used for MSPD was Alltech bulk high capacity C18 sorbent, 50 µm (Alltech, Deerfield, IL, USA), end-capped, 17% C. Columns used for SPE were Bakerbond C18 3 mL columns, packed with 500 mg reversed phase, 40 µm (J.T. Baker, Deventer, Netherlands), end-capped, 17.5% C.

Ultrasound assisted extraction and solid-phase extraction

1 g amount of dried *Archangelica officinalis* fruits powder was extracted with 20 mL of applicable solvent (80% aqueous solution of methanol, dichloromethane, petroleum ether and petroleum ether/methanol 50:50 v/v) in ultrasonic bath with temperature regulation (Bandelin electronic, Sonorex RK 100H, Germany) at 65 °C for 30 min. Extract was filtered and plant material was afterwards extracted with two portions of solvent by the same way. Extracts were filtered, combined and evaporated to dryness. The residues were dissolved in 10 mL of 80% aqueous solution of methanol and 2.5 mL of this solution was passed through a Bakerbond C18 SPE column (previously conditioned with 10 mL of methanol, 10 mL of water and 80% aqueous solution of methanol in sequence). Then the retained furanocoumarins were eluted with 5 mL of 80% aqueous solution of methanol. The collected fractions were transferred into 10 mL volume flask, filled up to their volume with 80% aqueous solution of methanol and analyzed by HPLC. The whole procedure was repeated three times for each solvent.

Matrix solid-phase dispersion

0.25 g of exactly weighted dried *Archangelica officinalis* fruits powder was placed in a glass mortar and mixed with 0.5 g of sorbent (previously conditioned with 10 mL of methanol, 10 mL of water and 80% aqueous solution of methanol in sequence) and 1 mL of applicable solvent (80% aqueous solution of methanol, dichloromethane, petroleum ether or petroleum ether/methanol 50:50 v/v). The mixture was then homogenized in the glass mortar using a pestle to obtain a homogenous mixture. The blend was then transferred into a 3 mL syringe with a paper frit on the bottom. The sample was covered with another paper frit and compressed using the syringe plunger. Coumarins were eluted with 5 mL of 80% aqueous solution of methanol. The collected fractions were evaporated to dryness, dissolved

in 10 mL of 80% aqueous solution of methanol in volume flask and analyzed by HPLC. The whole procedure was repeated three times for each solvent.

Chromatographic analysis

Agilent 1100 system coupled with diode-array detector (DAD) with the stainless steel column (250 mm × 4.6 mm), packed with 5 μm Hypersil BDS C18 (Shandon, UK) was used. The sample injection volume was 10 μL. The mobile phases were methanol (A) and water (B) in a stepwise gradient as follow: 0 min, 50% A in B; 5 min, 60% A in B; 25 min, 80% A in B; 30-40 min, 100% A. The flow rate was 1 mL min^{-1}, the column temperature was 25 °C.

The identification was performed by comparing retention times and UV-DAD spectra with those analyzed under the same conditions for appropriate standards. The qualitative and quantitative determination was performed in following wavelengths: λ = 254, 280 and 320 nm.

The proposed analytical method for the determination was carefully evaluated in terms of accuracy, repeatability and precision.[17]

The accuracy of the SPE method was evaluated through recovery studies by adding already known amounts of the each standard solution (three concentration levels) to the extracts and SPE method was performed. Also 10 mL of water - methanol solutions of pure standards (three concentration levels) were filtered through the SPE columns.

The recovery tests for MSPD was assessed by measuring the recovery of each standard solution (three concentration levels) after it was added to the mortar, mixed with sorbent and herb and extracted in the same way as described above. The amount of the spiked standard was calculated by subtracting the total amount of standard after spiking from the amount in the fruits of *Archangelica officinalis* before spiking. Also pure standards (three concentration levels) were mixed with sorbent without herb and extracted.

Injection repeatability was validated by injecting a mixed reference solution six times during one day. The relative standard deviation (RSD) was a measure of repeatability. The method precision was evaluated by intra-day and inter-day tests. Intra-day experiments were performed by replicate analysis of six aliquots of the same sample within one day. Inter-day tests were carried out on three consecutive working days in the same way as intra-assay experiments. Three measurements of every peak area for the extract components were carried out. Limit of detection (LOD) and limit of quantitation (LOQ) values were determined by calculation of the signal-to-noise (S/N) ratio. S/N ratios of approximately 3:1 and 10:1 were used for estimating the LOD and LOQ, respectively.

Calibration curves were obtained by injecting in the chromatographic system solutions of the standards. Each calibration curve was analyzed three times with five different concentrations as follow: 100; 75; 50; 25; 10 μg mL^{-1}.

Results and Discussion

Method assessment

The calibration curves for all standards were linear over the concentration range 10-100 μg mL^{-1}. The correlation coefficients of all calibration curves were $R^2 > 0.9990$. LOD and LOQ values ranged from 0.37 μg mL^{-1} (for xanthotoxol) to 10.82 μg mL^{-1} (for imperatorin), and from 1.05 μg mL^{-1} (for xanthotoxol) to 38.32 μg mL^{-1} (for imperatorin) respectively.

The recoveries of the SPE and MSPD method were in the range of 94.89-102.00% and 91.43 to 96.07%, respectively. The relative standard deviation (RSD%) was lower than 4.34% for MSPD and 4.64% for SPE. The RSD% of intra- and inter-day precision was less than 3% for all compounds. Recoveries for each furanocoumarin's standards and fortified extracts calculated in both SPE and MSPD method are presented in Table 1.

Table 1. Values of LOD, LOQ, concentrations of the standards added to the sample and recoveries for each furanocoumarins standards (I) and fortified extracts (II) calculated in both SPE and MSPD method (n = 3)

Compound	Concentrations of the standards added / (μg mL^{-1})	Recovery / % SPE (I)	Recovery / % SPE(II)	Recovery / % MSPD (I)	Recoveries / % MSPD (II)	LOD / (μg mL^{-1})	LOQ / (μg mL^{-1})
Umbelliferone	4.13; 5.50; 6.88	97.21	95.78	93.55	94.78	1.04	3.12
Xanthotoxol	1.73; 2.30; 2.88	94.99	96.12	95.74	96.07	0.37	1.05
Xanthotoxin	45.38; 60.50; 75.63	94.89	95.34	91.85	92.90	7.17	21.12
Isopimpinellin	24.00; 32.00, 40.00	96.34	98.45	93.78	95.65	4.31	12.64
Bergapten	51.00; 68.00, 85.00	95.78	96.43	94.72	92.67	7.52	22.35
Imperatorin	373.88; 498.50; 623.13	99.54	102.00	91.43	93.79	10.82	38.32
Isoimperatoirin	33.00; 44.00; 55.00	97.76	99.90	92.21	93.82	6.35	18.82

Optimization of MSPD and USAE - SPE procedure

In this work for the first time the MSPD method was examined as a preparation technique for the isolation of furanocoumarins from Angelica officinalis fruits.

The first aim of the optimization procedure was evaluation of plant matrix to sorbent mass ratio. The following Angelica officinalis sample to sorbent ratio were examined 1:2, 1:4 and 1:8. 80% aqueous solution of methanol was used in this experiment as dispersing agent. 1:2 plants to sorbent ratio were the most appropriate.

In the second step the optimal elution volumes were determined. The coumarins were eluted with 2.5, 5, 7.5 and 10 mL of 80% aqueous solution of methanol. The experiment revealed that 5 mL of solvent was sufficient for effective elution of furanocoumarins.

Another important task in the MSPD procedure is the elution profile. Several different solvents for furanocoumarins were investigated, including 80% aqueous solution of methanol dichloromethane, petroleum ether and petroleum ether/ methanol 50:50 v/v. The results of MSPD procedure, presented in Table 2, indicated that the highest yield for most of analyzed furanocoumarins (for isoimperatorin, imperatorin, bergapten, xanthotoxin and umbelliferone) gives 80% aqueous solution of methanol.

The same plant to sorbent ratio (1:2), type and volumes (5 mL) of elution agents were used in USAE with SPE method. In this procedure the best results were obtained also for 80% aqueous solution of methanol.

HPLC chromatogram of furanocoumarins isolated by MSPD technique where petroleum ether was used for homogenization is shown in Figure 1.

Comparison of MSPD and USAE - SPE

The MSPD method was compared with ultrasound assisted extraction coupled with SPE technique for the isolation of furanocoumarins from *Archangelica officinalis* fruits. MSPD proved to be an effective and precise technique. It can be seen, that MSPD and USAE - SPE gives similar yield of investigated furanocoumarins. In addition, MSPD allows extraction of more compounds than ultrasonification with SPE (e.g. extraction of umbelliferone with petroleum ether and dichloromethane and extraction of xanthotoxol with petroleum ether).

RSD% values for both procedures is comparable (0.43-4.34% for MSPD and 0.19-4.64% for USAE with SPE, Table 2). MSPD technique is also accurate, as indicated the value of recoveries. Values of recoveries (Table 1) for SPE were slightly higher than those of MSPD. MSPD method exhibited acceptable reproducibility, recovery, extraction

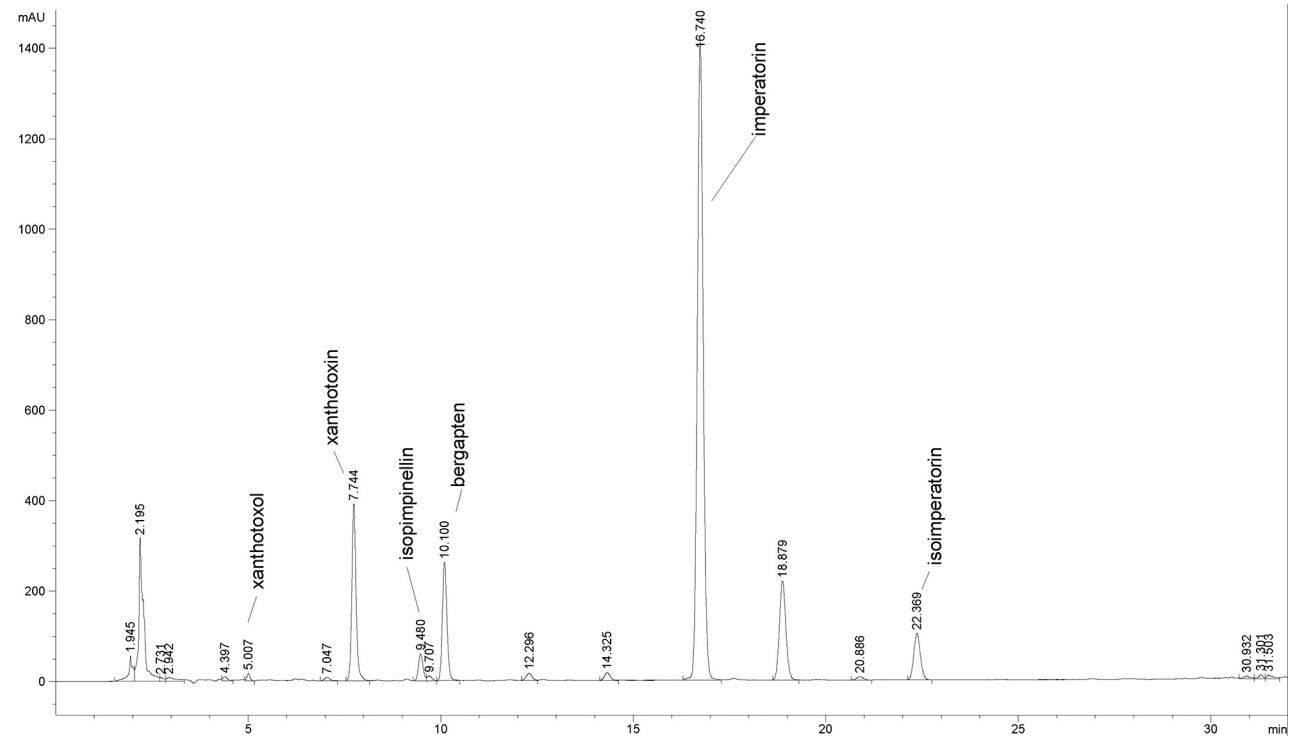

Figure 1. HPLC chromatogram of furanocoumarins isolated by MSPD technique. HPLC condition: λ = 254 nm, column - Hypersil BDS C18, (250 mm × 4.6 mm I.D., 5 µm), column temperature 25 °C, flow rate was 1 mL min^{-1}, methanol (A) and water (B) gradient: 0 min, 50% A in B; 5 min, 60% A in B; 25 min, 80% A in B; 30-40 min, 100% A.

Table 2. Yield of extraction of investigated furanocoumarins from *Archangelica officinalis* fruits by different methods and extractant (n = 3)

Compound	Method	Extractant	Yield / mg 100 g^{-1}	Yield / (μg mL^{-1})	RSD / %
Umbelliferone	MSPD	petroleum ether	15.27	3.82	2.67
		80% aqueous methanol	17.56	4.39	2.22
		dichloromethane	17.55	4.39	2.03
		ether/methanol 50:50 v/v	12.29	3.07	3.19
	USAE - SPE	petroleum ether	-	-	-
		80% aqueous methanol	22.05	5.51	3.77
		dichloromethane	-	-	-
		ether/methanol 50:50 v/v	12.76	3.19	3.87
Xanthotoxol	MSPD	petroleum ether	6.43	1.61	3.22
		80% aqueous methanol	4.32	1.08	4.08
		dichloromethane	5.61	1.40	4.22
		ether/methanol 50:50 v/v	5.41	1.35	4.34
	USAE - SPE	petroleum ether	-	-	-
		80% aqueous methanol	6.83	1.71	1.22
		dichloromethane	4.29	1.07	4.64
		ether/methanol 50:50 v/v	9.33	2.33	3.26
Xanthotoxin	MSPD	petroleum ether	228.99	57.25	1.03
		80% aqueous methanol	236.02	59.01	3.28
		dichloromethane	217.12	54.28	2.09
		ether/methanol 50:50 v/v	193.81	48.45	1.54
	USAE - SPE	petroleum ether	132.96	33.24	0.63
		80% aqueous methanol	241.50	60.38	2.16
		dichloromethane	156.90	29.23	0.51
		ether/methanol 50:50 v/v	209.24	52.31	2.67
Isopimpinellin	MSPD	petroleum ether	83.88	20.97	2.11
		80% aqueous methanol	66.83	16.71	2.28
		dichloromethane	70.75	17.68	3.92
		ether/methanol 50:50 v/v	59.75	14.94	3.79
	USAE - SPE	petroleum ether	66.94	16.74	1.99
		80% aqueous methanol	95.49	23.87	1.87
		dichloromethane	128.95	32.24	0.92
		ether/methanol 50:50 v/v	89.95	22.49	1.04
Bergapten	MSPD	petroleum ether	270.18	67.55	0.76
		80% aqueous methanol	271.96	67.99	0.72
		dichloromethane	258.04	64.51	1.49
		ether/methanol 50:50 v/v	222.75	55.69	0.82
	USAE - SPE	petroleum ether	225.21	56.30	0.80
		80% aqueous methanol	267.07	66.77	2.31
		dichloromethane	233.07	58.27	2.21
		ether/methanol 50:50 v/v	262.80	65.7	3.31
Imperatorin	MSPD	petroleum ether	1903.76	475.94	0.73
		80% aqueous methanol	1994.48	498.62	2.37
		dichloromethane	1849.27	462.32	1.26
		ether/methanol 50:50 v/v	1636.63	409.16	2.74
	USAE - SPE	petroleum ether	1580.09	395.02	0.33
		80% aqueous methanol	1910.29	477.57	0.45
		dichloromethane	1784.49	446.12	0.84
		ether/methanol 50:50 v/v	1815.73	453.94	1.86
Isoimperatoirin	MSPD	petroleum ether	166.21	41.55	0.43
		80% aqueous methanol	155.82	38.96	3.73
		dichloromethane	161.18	40.30	1.44
		ether/methanol 50:50 v/v	151.28	37.82	0.94
	USAE - SPE	petroleum ether	137.50	34.38	0.19
		80% aqueous methanol	174.79	43.70	1.38
		dichloromethane	143.87	35.98	1.79
		ether/methanol 50:50 v/v	149.97	37.49	2.06

efficiency relative to ultrasound assisted extraction with SPE.

Conclusions

MSPD has been demonstrated to be a suitable preparation technique, a simple alternative to conventional extraction methods, for the isolation of furanocoumarins from *Archangelica officinalis* fruits. This method exhibited acceptable reproducibility, recovery and extraction efficiency. Moreover MSPD requires lower solvent volumes and time and involves less steps in the determination of furanocoumarins than USAE with SPE method. No homogenization, grinding or milling steps are necessary. The proposed procedure does not require heating during the extraction, avoiding the possible degradation of thermolabile compounds.

Matrix solid-phase dispersion could therefore be useful to extract and purify furanocoumarins from plant material as advantageous alternative procedure to routine extraction methods.

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