Regular Article

ANALYTICAL AND BIOANALYTICAL CHEMISTRY RESEARCH

Published by the Iranian Chemical Society

Anal. Bioanal. Chem. Res., Vol. 5, No. 2, 307-316, December 2018.

Ultrasonic-Assisted Matrix Solid-Phase Dispersion and High-Performance Liquid Chromatography as an Improved Methodology for Determination of Oleuropein from Olive Leaves

Marzieh Rashidipour[a] and Rouhollah Heydari[b,]*

[a]*Environmental Health Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran*

[b]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, P. O Box: 68149-89468, Khorramabad, Iran*

(Received 18 February 2018, Accepted 7 May 2018)

In this study, ultrasonic-assisted matrix solid-phase dispersion (UA-MSPD) as a new sample preparation method for natural product analysis was developed. Influential parameters of the UA-MSPD method were optimized using oleuropein content of olive leaves as a model analyte. Main parameters of the proposed technique such as ultrasonic time, ultrasonic amplitude and pulse, sorbent material, the ratio of sample to sorbent material, elution solvent and its volume have been fully evaluated and optimized. In the proposed method several steps in classical MSPD including transfer of sample and sorbent mixture to cartridge, packing and elution under vacuum conditions were removed. Also, ultrasound waves were applied to the sample and sorbent mixture in elution step for effective analyte desorption. Oleuropein was successfully extracted by silica gel and acetone as the sorbent and elution solvent, respectively. The calibration curve shows good linearity (R^2 = 0.9979) and precision (RSD < 6.8) in the concentration range of 0.1-200 μg ml^{-1} for oleuropein. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.03 and 0.1 μg ml^{-1}, respectively. The recovery values were in the range of 90.2-96.7% with RSD values ranging from 5.5-7.2%.

Keywords: Matrix solid-phase dispersion, Ultrasonic probe, Oleuropein, Olive leave

INTRODUCTION

Phenolic compounds are the most abundant and important compounds in plants. Phenolic compounds have shown several biological activities such as antimicrobial [1-3], anti-inflammatory [4], antioxidant [1,5] and anti-cancer [6,7]. Oleuropein is the main phenolic compound in olive leaves. Various procedures including static-dynamic superheated liquid extraction [8], dynamic ultrasound-assisted extraction (UAE) [9], microwave-assisted extraction (MAE) [10], reversed-phase dispersive liquid-liquid microextraction (RP-DLLME) [11], supercritical fluid extraction (SFE) [12], pressurized liquid extraction (PLE) [13], steam and hot water blanching [14], solid-phase extraction (SPE) coupled with gas chromatography-mass spectrometry (GC-MS) [15], mid-infrared (MIR) spectroscopy combined with chemometric analysis [16] and ultrasound and salt-assisted liquid-liquid extraction (USALLE) [17,18] have been reported for the extraction and determination of oleuropein individually or together with other phenolic compounds from natural sources.

Almost all of the above mentioned methods require the complicated and expensive instruments for sample preparation. In addition, these methods are unable to extract analyte selectively. Therefore, a clean-up step is often needed.

Extraction of organic compounds from solid samples using conventional extraction methods usually is tedious, require sample clean-up and trace enrichment. Also, large amounts of sample and organic solvent are consumed [19-23].

Matrix solid-phase dispersion (MSPD) enables the

*Corresponding author. E-mail: rouhollahheydari@yahoo.com

simultaneous extraction and clean-up of analytes from solid, semi-solid and highly viscous samples [24]. In the last decade, MSPD as a suitable sample preparation technique is used for the extraction and purification of various analytes from different matrices [25-30]. Most of the solid-liquid extraction (SLE) procedures such as MAE and PLE provide high extraction yields due to combination of high temperatures and pressures. Although MAE and PLE have many advantages than other extraction techniques, clean-up of MAE and PLE extracts are required due to the large amounts of co-extracted compounds. On the other hand, high temperatures can lead to analyte loss. In the MSPD technique, extraction conditions are mild and analyte is not decomposed during the extraction process [31]. Other advantages of MSPD over conventional methods of sample treatment consist in short extraction time, use of smaller amounts of sample and organic solvents and simultaneous extraction and clean-up steps. Recently, MSPD technique has been successfully developed for monitoring the oleuropein content in olive leaves using silica gel and dichloromethane-methanol as sorbent and elution solvent, respectively [31]. In order to desorption of analyte from the sorbent surface, the selection of elution solvent and its volume have been of particular importance in this method. Incomplete desorption of analyte from sorbent material leads to low recovery values. In this study, to enhance analyte desorption from sorbent material, ultrasound waves were applied to the sample and the sorbent mixture in elution step. On the other hand, ultrasound waves enhanced the extraction efficiency of analyte from the sample matrix simultaneously to increase the analyte desorption from the sorbent. However, ultrasound-assisted matrix solid-phase dispersion (UA-MSPD) using an ultrasonic bath was applied to determine several analytes in various matrices [32-36]. To the best our knowledge, this is the first report of ultrasonic-assisted matrix solid-phase dispersion (UA-MSPD) method used for sample preparation.

The aim of this study was to optimize a new sample preparation method, namely ultrasonic-assisted matrix solid-phase dispersion (UA-MSPD), for the determination of oleuropein content of olive leaves as a model analyte. Influential parameters on the extraction efficiency such as ultrasonic time, amplitude and pulse, sorbent material and its amount, elution solvent and its volume and the ratio of sample to sorbent were evaluated and optimized.

EXPERIMENTS

Chemicals and materials

Oleuropein (purity ≥ 98% by HPLC) was purchased from Indofine Chemical Co. (Hillsborough, USA). Acetonitrile (HPLC grade), methanol, ethanol, dichloromethane, acetone, orthophosphoric acid, octadecyl-functionalized silica (C_{18}) and silica gel 60 (15-40 μm) were purchased from Merck Chemical Company (Darmstadt, Germany). Disposable polytetrafluoroethylene (PTFE) filters (0.45 μm pore size) were supplied by MACHEREY-NAGEL (Düren, Germany). All solutions were prepared with deionized water from a Milli-Q system (Millipore, USA). Diatomaceous earth (DE, 95% SiO_2) was obtained from Aldrich Chemical Company (Milwaukee, WI, USA).

Samples

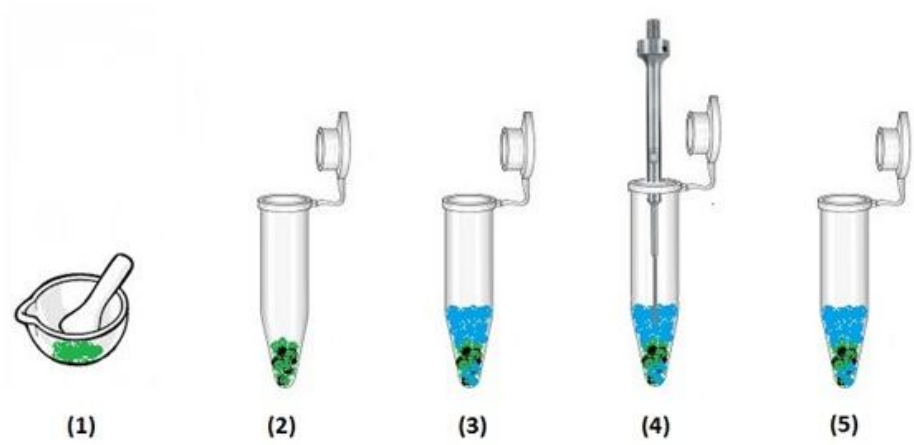
Olea europaea (variety Sevillana) leaves were collected from Agricultural Research Garden, Khorramabad, Iran, in July 2015. Before the extraction, the leaves were dried in shadow, milled, homogenized and kept at 4 °C until analysis. The same sample was used in the whole optimization study.

Preparation of Standard Solutions

A stock standard solution (1000 μg ml^{-1}) was prepared by dissolving oleuropein in methanol. Working standard solutions at different concentrations in the range of 0.1-200 μg ml^{-1} were prepared by diluting the suitable volume of the stock standard solution using ethyl acetate.

Chromatographic Conditions

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a quaternary pump (LC-10ATvp), UV-Vis detector (SPD-M10Avp), vacuum degasser and system controller (SCL-10Avp) was used. A manual injector with a 10 μl sample loop was applied for loading the sample. Class VP-LC workstation was employed to acquire and process chromatographic data. A reversed-phase C_{18} analytical column (Shim-Pack VP-ODS, 250 mm × 4.6 mm i.d., Shimadzu, Japan) was used.



Scheme 1. Steps in the proposed ultrasonic-assisted matrix solid phase dispersion (UA-MSPD) method. 1) Plant sample and sorbent material are manually blended together using a pestle; 2) the mixture is transferred into a microtube; 3) adding organic solvent in order to analyte desorption; 4) the mixture of sample, sorbent and organic solvent is exposed to ultrasonic waves; 5) microtube is centrifuged and 10 µl of supernatant is injected to HPLC system.

The mobile phase consisted of water (pH 2.9 adjusted with orthophosphoric acid) and acetonitrile (70:30, v/v). Prior to preparation of the mobile phase, the acidic water and acetonitrile were degassed separately using a Millipore vacuum pump. The UV detector was set at 254 nm. Flow rate was adjusted at 1.2 ml min^{-1} and column oven set on 30 °C.

Ultrasonic-assisted Matrix Solid-phase Dispersion Procedure

Powder (0.01 g) of Olea europaea leaves was weighted and then blended thoroughly with 0.08 g of silica gel in an agate mortar for 5 min using an agate pestle to obtain a homogeneous mixture. The mixture was quantitatively transferred to a microtube. 300 µl of acetone was added to the microtube as elution solvent and the mixture was exposed to ultrasonic waves using an ultrasonic probe (hielscher, Model UP 200H, Germany). After the ultrasonic process, microtube was centrifuged (hettich zentrifugen d 78532 tuttlingen, Germany) at 7500 rpm for 5 min. Finally, 10 µl of the supernatant was removed and injected onto the HPLC column for analysis (Scheme 1). Three replicates were performed for each set of experiments during the method optimization.

RESULTS AND DISCUSSIONS

Influential parameters of the MSPD technique such as sorbent material, the ratio of sorbent to sample, elution solvent and its volume and ultrasonic parameters including ultrasonic time, ultrasonic amplitude and pulse were investigated and optimized.

Optimization of MSPD Parameters

Several sorbents with different properties including silica gel, DE and C_{18} were examined. Figure 1 shows the effect of three sorbents on the extraction efficiency of oleuropein from solid samples. As can be seen, the maximum oleuropein extraction was obtained using silica gel as sorbent.

The elution solvent should be compatible with the analytical instrument and capable to remove the adsorbed analyte from the sorbent material quantitatively. Also, the analyte must be soluble in elution solvent. Several solvents with different polarities including methanol, acetonitrile, acetone, dichloromethane and ethanol were used as elution solvent. Among these solvents, acetone is shown the maximum extraction efficiency.

A minimum volume of elution solvent leads to analyte

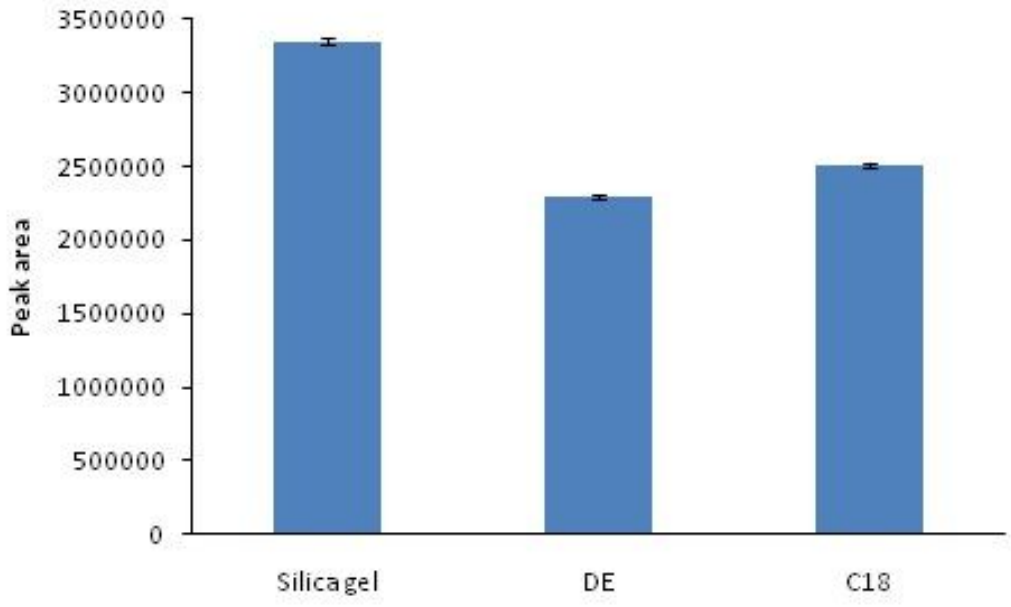


Fig. 1. Effect of sorbent on the extraction of oleuropein from olive leaves. Extraction conditions: elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g; ultrasonic time, 30 s; ultrasonic amplitude, 50 %; ultrasonic pulse, 0.7.

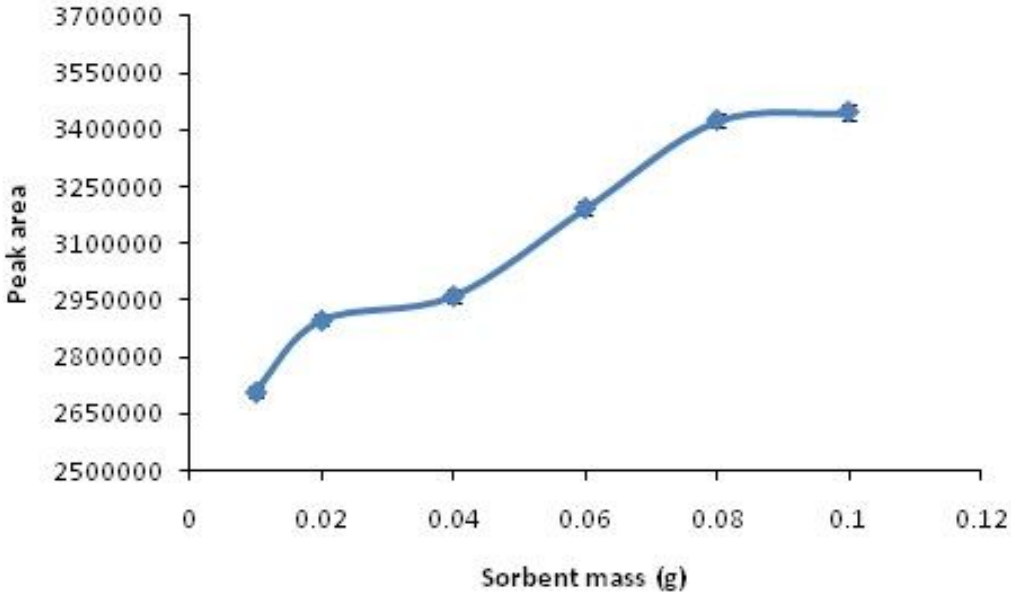


Fig. 2. Effect of sorbent mass on the extraction of oleuropein from olive leaves. Extraction conditions: sorbent, silica gel; elution solvent, acetone; elution solvent volume, 300 µl; ultrasonic time, 30 s; ultrasonic amplitude, 50%; ultrasonic pulse, 0.7.

pre-concentration and signal enhancement. On the other hand, there is a possibility that low volume of elution solvent cannot quantitatively elute analyte from sorbent material. Consequently, optimization of elution solvent volume is a key parameter in the MSPD method development. Different volumes of acetone as elution solvent in the range of 200-450 µl were examined. The results showed that increasing the volume of elution solvent up to 300 µl increases the analyte signal. At higher elution solvent volumes, analyte signal due to dilution phenomenon was decreased. Therefore, 300 µl of acetone was selected as the optimum elution solvent volume for further investigations.

The ratio of sorbent to sample is a main factor in the MSPD method. The effect of sorbent amount on analyte extraction is illustrated in Fig. 2. As observed, extraction efficiency of oleuropein increases with increase of the sorbent amount up to 0.08 g and then remains constant. Increasing of extraction efficiency with an increase in sorbent amount can be attributed to increase the available active site for analyte sorption. After 0.08 g, increasing the amount of sorbent has no significant effect on the extraction efficiency. This phenomenon can be explained by the limited amount of analyte. Therefore, 0.08 g was selected as the optimum sorbent amount for subsequent experiments.

Optimization of Ultrasonic Parameters

Ultrasonic time was investigated in the range of 15-60 s. As can be seen from Fig. 3, increase of ultrasonic time leads to increase of extraction efficiency up to 30 s and then a decrease. At the beginning of desorption step, increase of ultrasonic time can lead to analyte desorption efficiently. Decreasing of extraction efficiency at higher ultrasonic times (>30 s) can be attributed to oleuropein decomposition by ultrasonic waves. Consequently, 30 s was chosen as the optimum ultrasonic time in further experiments.

The intensity of sonication is proportional to the amplitude of ultrasonic probe vibration. Therefore, higher amplitudes of vibrations lead to an increase in the intensity of vibrations and an increase in the analyte desorption from sorbent. However, the intensity of sonication can be adjusted using ultrasonic amplitude. The effect of ultrasonic amplitude on the extraction efficiency was investigated in the range of 20-100%. Figure 4 shows the effect of ultrasonic amplitude on the peak area of oleuropein. According to these results, 50% was selected as the optimum amplitude.

Acoustic irradiation time in ultrasonic probe instrument is adjustable using pulse mode. Pulse optimization was performed in the range of 0.1-1 cycle. The maximum extraction efficiency was achieved at 0.7 cycles (Fig. 5). This behavior can be explained similarly by the mentioned reasons for the influence of ultrasonic time on the extraction efficiency. Therefore, 0.7 cycles were chosen as the optimum pulse in the subsequent experiments.

Method Evaluation

Under the optimized conditions, the developed UA-MSPD method was evaluated in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy. The analytical figures of merit of the proposed sample preparation technique are summarized in Table 1.

Precision and accuracy data were obtained using spiked real samples containing oleuropein standard solution in three concentration levels. Relative standard deviation (RSD) values for oleuropein in three concentration levels were in the range of 5.5-7.2% (Table 2). In order to validate the method accuracy, the recovery tests were performed by the analysis of the spiked samples with three different concentrations of oleuropein. Relative recovery values were in the range of 90.2-96.7%. The results in Tables 1 and 2 indicate that this method can be successfully applied for the determination of oleuropein in the olive leave samples. Typical chromatograms of blank, oleuropein standard solution and extracted oleuropein using the UA-MSPD method are shown in Fig. 6. As can be seen, blank chromatogram has no interference peak at the oleuropein retention time.

Comparison of the Proposed Method with MSPD Method

To study the effect of ultrasonic on the extraction efficiency, extraction of oleuropein from olive leaves was performed under the optimized conditions by UA-MSPD and MSPD methods. The results are shown in Table 3. The means of two methods are compared using Student's t-test. The two-tailed P value is less than 0.0001 indicating that

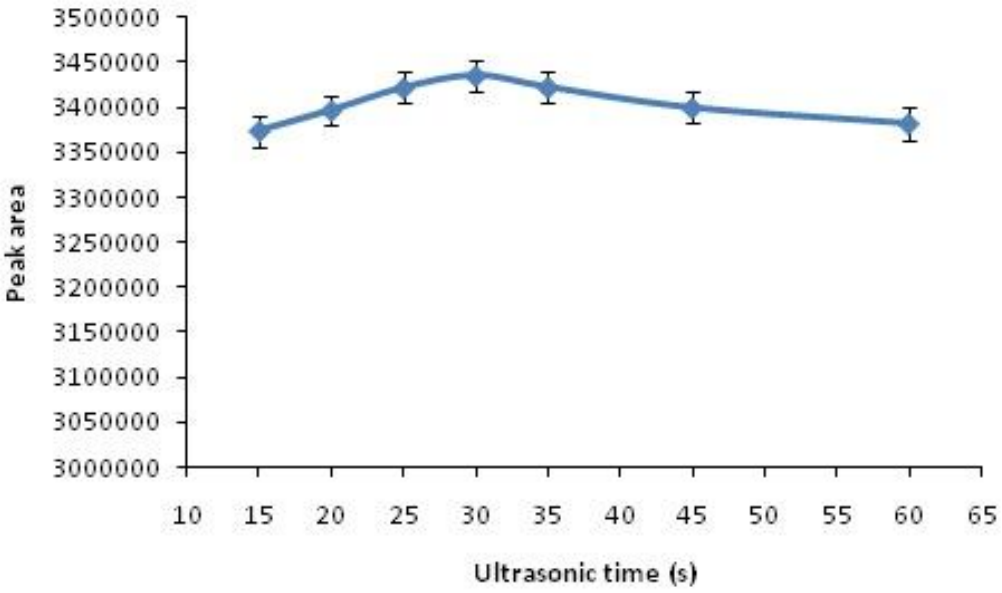


Fig. 3. Effect of ultrasonic time on the extraction of oleuropein from olive leaves. Extraction conditions: sorbent, Silica gel; elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g; ultrasonic amplitude, 50%; ultrasonic pulse, 0.7.

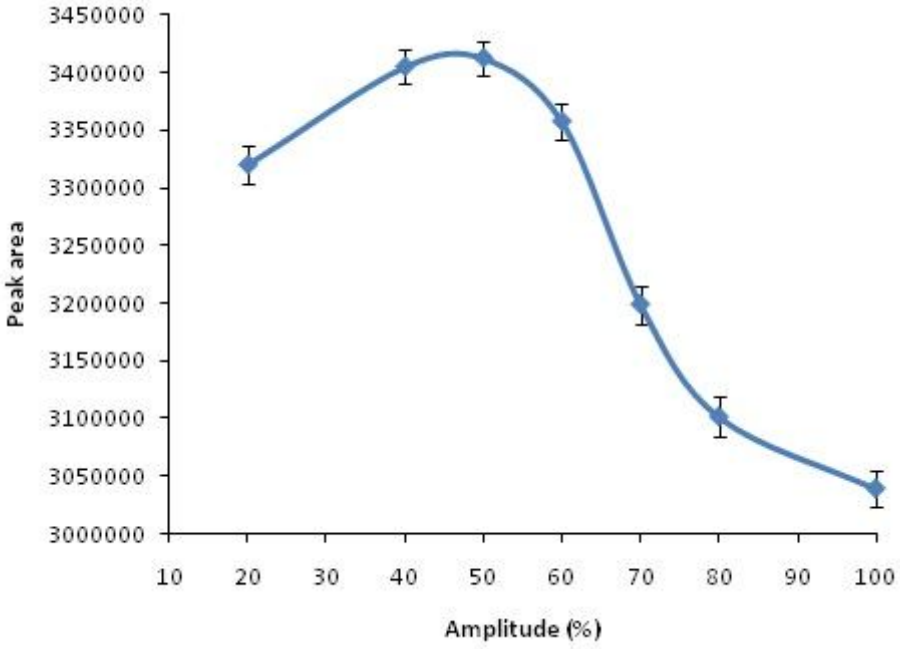


Fig. 4. Effect of ultrasonic amplitude on the extraction of oleuropein from olive leaves. Extraction conditions: sorbent, silica gel; elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g; ultrasonic time, 30 s; ultrasonic pulse, 0.7.

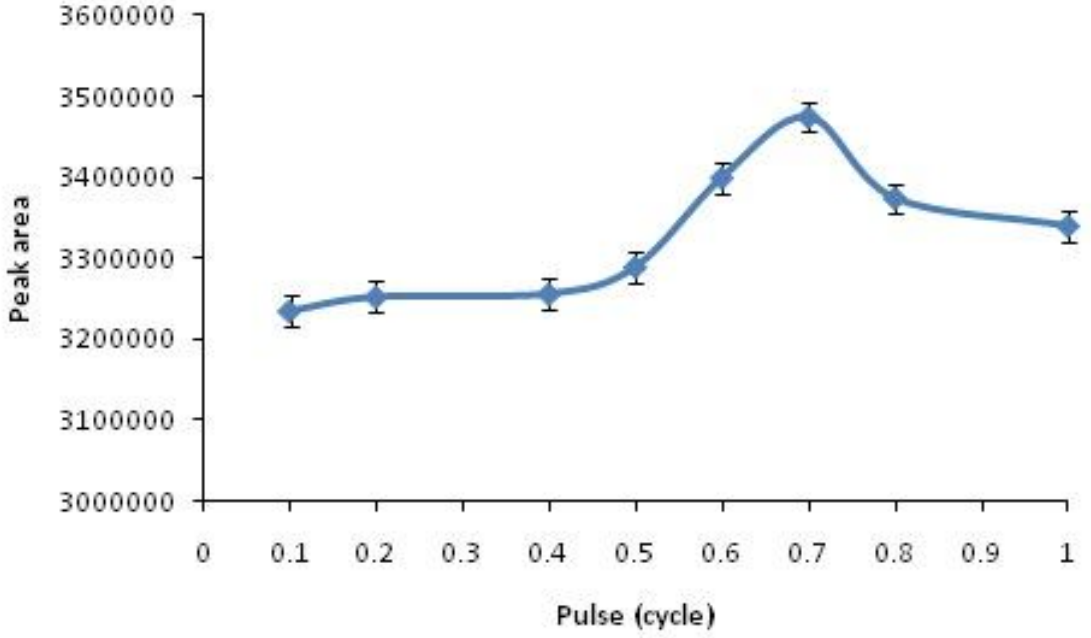


Fig. 5. Effect of ultrasonic pulse on the extraction of oleuropein from olive leaves. Extraction conditions: sorbent, silica gel; elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g; ultrasonic amplitude, 50 %; ultrasonic time, 30 s.

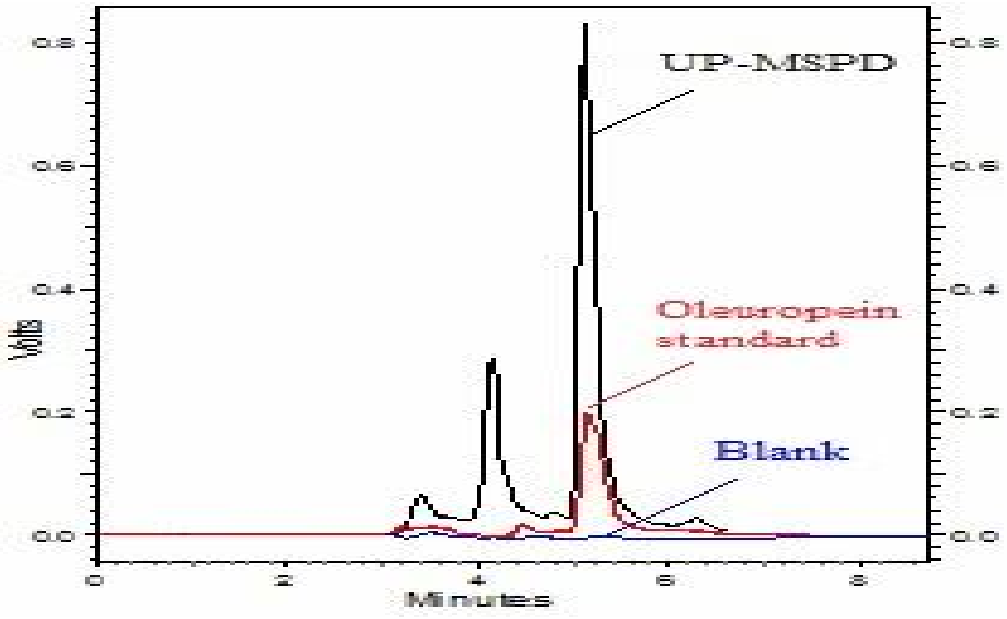


Fig. 6. Typical chromatograms of blank, oleuropein standard solution (300 µg ml^{-1}) and extracted oleuropein using the UA-MSPD method.

Table 1. Some of Analytical Parameters for the Proposed Method

Analytical parameter	Numerical value
LOD ($\mu g\ ml^{-1}$)	0.03
LOQ ($\mu g\ ml^{-1}$)	0.1
R^2	0.9979
Slope	27870
Linear range ($\mu g\ ml^{-1}$)	0.1-200

Table 2. Recovery and Precision Data Obtained with the Proposed UA-MSPD Method for Spiked Samples

Spiked concentration ($\mu g\ ml^{-1}$)	Recovery (%), (n = 6)	Precision (RSD%)	
		Within day (n = 3)	Between day (n = 9)
5	90.2	6.8	7.2
10	95.1	5.5	7.0
20	96.7	6.2	7.1

the difference is statistically significant. In the proposed technique, elution step was performed in the presence of sonication using an ultrasonic probe. Sound waves are used to agitate sorbent and sample particles simultaneously. This phenomenon leads to increase the analyte desorption from sorbent material and decrease the analyte desorption time. On the other hand, in this step desorption process analyte and extraction of analyte from solid sample occured simultaneously using ultrasound-assisted extraction (UAE) process. Therefore the amount of extracted analyte by using UA-MSPD is significantly higher than MSPD method.

CONCLUSIONS

For the first time, UA-MSPD as a sample preparation method was applied to determine the oleuropein content of olive leaves. Enhancement of analyte desorption from sorbent material was achieved by applying ultrasound waves to the sample and sorbent mixture in elution step. Also, ultrasound waves increased analyte extraction from the sample matrix, and simultaneously analyte desorption from the sorbent. The proposed method has several advantages compared to the classical MSPD method such as shorter sample preparation time, higher extraction efficiency, and no need to transfer the sample and sorbent mixture to the cartridge, packing and elution under vacuum conditions. These advantages facilitate the extraction process and reduce the analysis time. Based on the results presented, the proposed method has a higher extraction efficiency compared to classical MSPD. The UA-MSPD method can be examined for the extraction of other analytes from solid and semi-solid matrices.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the support of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences.

Table 3. The Results of Analyzed Samples Using MSPD and UA-MSPD Methods

Sample no.	Extraction yield of oleuropein (%)[a]	
	MSPD	UA-MSPD
1	3.51	6.75
2	3.48	6.98
3	3.59	7.01
4	3.64	7.11
5	3.45	6.80
6	3.60	7.08
Mean	3.545	6.955
SD[b]	0.0756	0.148
SEM[c]	0.0308	0.0604
N[d]	6	6

[a]Extraction yield, % = (weight of the oleuropein in extract × 100)/(weight of the plant sample). [b]SD; standard deviation. [c]SEM; standard error of mean. [d]N; number of samples MSPD conditions: sorbent, silica gel; elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g. UA-MSPD conditions: sorbent, silica gel; elution solvent, acetone; elution solvent volume, 300 µl; sorbent mass, 0.08 g; ultrasonic amplitude, 50%; ultrasonic time, 30 s; ultrasonic pulse, 0.7.

REFERENCES

[1] C. Proestos, N. Chorianopoulos, G.J.E. Nychas, M. Komaitis, J. Agric. Food Chem. 53 (2005) 1190.

[2] E. Medina, A. de Castro, C. Romero, M. Brenes, J. Agric. Food Chem. 54 (2006) 4954.

[3] M. Gañan, A.J. Martínez-Rodríguez, A.V. Carrascosa, Food Control 20 (2009) 739.

[4] T.D. Cuong, T.M. Hung, J.S. Lee, K.Y. Weon, M.H. Woo, B.S. Min, Bioorg. Med. Chem. Lett. 25 (2015) 1129.

[5] Z. Zhanga, L. Liao, J. Moore, T. Wu, Z. Wang, Food Chem. 113 (2009) 160.

[6] E.A. Hillard, P. Pigeon, A. Vessières, C. Amatore, G. Jaouen, Dalton Trans. 43 (2007) 5073.

[7] V. Mendes, V. Cost, N. Mateus, RSC Adv. 5 (2015) 1.

[8] R. Japón-Luján, M.D. Luque de Castro, J. Chromatogr. A 1136 (2006) 185.

[9] R. Japón-Luján, J.M. Luque-Rodríguez, M.D. Luque de Castro, J. Chromatogr. A 1108 (2006) 76.

[10] R. Japón-Luján, J.M. Luque-Rodríguez, M.D. Luque de Castro, Anal. Bioanal. Chem. 385 (2006) 753.

[11] P. Hashemi, F. Raeisi, A.R. Ghiasvand, A. Rahimi, Talanta 80 (2010) 1926.

[12] N. Xynos, G. Papaefstathiou, E. Gikas, A. Argyropoulou, N. Aligiannis, A.L. Skaltsounis, Sep. Purif. Technol. 122 (2014) 323.

[13] N. Xynos, G. Papaefstathiou, M. Psychis, A.

Argyropoulou, N. Aligiannis, A.L. Skaltsounis, J. Supercrit. Fluids 67 (2012) 89.

[14] K. Stamatopoulos, E. Katsoyannos, A. Chatzilazarou, S.J. Konteles, Food Chem. 133 (2012) 344.

[15] J.J. Ríos, M.J. Gil, F. Gutiérrez-Rosales, J. Chromatogr. A 1093 (2005) 167.

[16] F. Aouidi, N. Dupuy, J. Artaud, S. Roussos, M. Msallem, I.P. Gaime, M. Hamdi, Ind. Crop. Prod. 37 (2012) 292.

[17] R. Rezaeepour, R. Heydari, A. Ismaili, Anal. Methods 7 (2015) 3253.

[18] A. Ismaili, R. Heydari, R. Rezaeepour, J. Sep. Sci. 39 (2016) 405.

[19] P. Salehi, A.R. Fakhari, S.N. Ebrahimi, R. Heydari, Flavour Frag. J. 22 (2007) 280

[20] A. Ghassempour, R. Heydari, Z. Talebpour, A.R. Fakhari, A. Rassouli, N. Davies, H.Y. Aboul-Enein, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 2686

[21] R. Heydari, Anal. Lett. 45 (2012) 1875

[22] R. Heydari, M. Hosseini, S. Zarabi, Spectrochim. Acta A 150 (2015) 786.

[23] R. Heydari, S. Zarabi, Anal. Methods 6 (2014) 8469

[24] S.A. Barker, J. Biochem. Biophys. Methods 70 (2007) 151.

[25] J.H. Chen, G.M. Zhou, H.Y. Qin, Y. Gao, G.L. Peng, Anal. Methods 7 (2015) 6537.

[26] Y. Zhang, X. Xu, H. Liu, Y. Zhai, Y. Sun, S. Sun, H. Zhang, A. Yu, Y. Wang, J. Chromatogr. Sci. 50 (2012) 131.

[27] [27] F.A. Duarte, B.M. Soares, A.A. Vieira, E.R. Pereira, J.V. Maciel, S.S. Caldas, E.G. Primel, Anal. Chem. 85 (2013) 5015.

[28] A. Oniszczuk, M. Waksmundzka-Hajnos, R. Podgórski, R. Nowak, W. Żukiewicz-Sobczak, T. Blicharski, Acta Chromatographica 27 (2015) 687.

[29] C.S. Binellas, C.D. Stalikas, J. Sep. Sci. 38 (2015) 3575.

[30] Q. Zhang, B. Hong, L. Zheng, X. Wang, D. Cai, J. Sep. Sci. 35 (2012) 2453.

[31] M. Rashidipour, R. Heydari, A. Feizbakhsh, P. Hashemi, J. AOAC Int. 97 (2014) 1109.

[32] H. Liu, J. Shao, T. Lin, Q. Li, Chromatographia 76 (2013) 1785.

[33] M. Manoochehri, A.A. Asgharinezhad, M. Safaei, J. Chromatogr. Sci. 53 (2015) 189.

[34] E.G. Karageorgou, V.F. Samanidou, J. Sep. Sci. 33 (2010) 2862.

[35] E.G. Karageorgou, V.F. Samanidou, I.N. Papadoyannis, J. Sep. Sci. 35 (2012) 2599.

[36] B. Barfi, A. Asghari, M. Rajabi, A. Barfi, I. Saeidi, J. Chromatogr. A 1311 (2013) 30.