1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Ultrasound and salt-assisted liquid-liquid extraction as an efficient method for natural products extraction

Reza Rezaeepour [a], Rouhollah Heydari [b, *], Ahmad Ismaili[c]

[a] Department of Chemistry, Borujerd Branch, Islamic Azad University, Borujerd, Iran.

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

[c] Department of Agronomy and Plant Breeding, Faculty of Agriculture, Lorestan University, P.O. Box 68149-84896, Khorramabad, Iran.

[*] Correspondence to. Tel: +98-66-33225012; fax: +98-66-33204005.
E-mail address: rouhollahheydari@yahoo.com

1

Abstract

A simple, rapid and efficient ultrasound and salt-assisted liquid-liquid extraction (USALLE) method coupled with high-performance liquid chromatography (HPLC) has been introduced for extraction, clean-up and pre-concentration of oleuropein from olive leaves as model analyte. In this technique, plant sample was transferred to extraction solvent (consisting phosphate buffer and miscible organic solvents) and the mixture was exposed to ultrasonic waves. After ultrasonic-assisted extraction (UAE), phase separation was performed by addition of salt to liquid phase. In during salt-assisted liquid-liquid extraction (SALLE) analyte was transferred into the supernatant organic phase. Various parameters that affected the extraction efficiency such as ultrasonic time and temperature, sample amount, type and volume of miscible organic solvent, type and concentration of salt and pH were evaluated and optimized. The calibration curve shows good linearity (r^2=0.9934) and precision (RSD<5.5%) in the range of 2.5-50 µg mL^{-1}. The limit of detection (LOD) and limit of quantitation were 0.5 and 2.5 µg mL^{-1}, respectively. The recoveries were in the range of 90.0–97.0% with RSD values ranging from 4.0 to 6.5%. Unlike the conventional extraction methods for plant extract no evaporative and re-solubilize operations were needed in the proposed technique.

Keywords: Ultrasound; salt-assisted liquid-liquid extraction; olive; oleuropein; high-performance liquid chromatography

1. Introduction

Sample preparation techniques are used to improve the performance of an analysis. The most important aims of sample preparation are: to eliminate or reduce interferences, to enhance the sensitivity of the analysis by increasing the enrichment factor (EF) of the analyte and sometimes to convert the analytes of interest into a more appropriate form that can be easily separated, detected, and quantified [1-4]. The obtained sample in this step should have a high concentration of target analytes free of interfering compounds from the matrix. Therefore, extraction of target analytes is one of the most important steps in sample preparation [5].

Several solid–liquid extraction (SLE) techniques such as soaking (maceration) extraction, soxhlet extraction (SE), supercritical fluid extraction (SFE) and distillation are available for natural product extraction [6-9]. Choosing the extraction technique for extracting natural compounds depends on various process conditions such as temperature, pressure, shaking, and solvent type. Although applying heat, pressure and agitation usually lead to accelerate extraction process, but also their destructive effects on natural compounds must be considered.

Conventional extraction of natural compounds by using maceration, SE and distillation techniques are needed to large volume of organic solvent and longer extraction time. Also, SE and distillation techniques have destructive effects on natural compounds due to high temperature of process. In conventional solvent extraction (CSE) methods, due to large volume and incompatibility of extracting solvent with analytical instruments, evaporation to dryness and reconstitution of extract in a very small volume of appropriate solvent is essential [10-12]. As a result, an increasing demand for extraction of natural compounds by using an appropriate extraction method with safe solvents at low temperatures is needed. Ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE)

methods present several advantages such as: increasing the extraction efficiency of the target analytes, decreasing the volume of the solvent and extraction time in comparison with conventional methods [13-17].

Salt-assisted liquid-liquid extraction (SALLE) is based on the phase separation of water-miscible organic solvents from the aqueous solutions by using salt addition [18-21]. In this technique water-miscible organic solvents with low toxicity were used as extracting solvent. Compared to conventional liquid-liquid extraction (LLE) methods, in SALLE technique large volumes of immiscible organic solvents and vigorous mechanical shaking are not required. In the other hand, due to small volume of extracting solvent and compatibility of this solvent with analytical instruments, evaporation of solvent is not needed [22, 23]. There are several reports about the application of SALLE to determine the different compounds in various matrixes [24-28]. Therefore, coupling of SALLE with other extraction techniques such as UAE and MAE can be lead to good results in terms of efficiency, pre-concentration and clean-up. Recently, UAE coupled with a modified QuEChERS (Quick Easy Cheap Effective Rugged Safe) method was applied to extract of isoflavones from legume samples [29]. In previous reports, oleuropein was extracted from olive leaves by using UAE and pressurized liquid extraction (PLE) methods [30, 31]. These methods were used large solvent volume without pre-concentration and clean-up operations.

In this work, a new ultrasound and salt-assisted liquid-liquid extraction (USALLE) technique was developed for determination of oleuropein in olive leaves and fruits as a model analyte. Unlike conventional methods, in this technique extraction, pre-concentration and clean-up were performed together. After UAE, extract was transferred to a microtube and subjected to SALLE process. The SALLE is caused the clean-up and enrichment of analyte into organic phase. Finally, upper organic phase was removed and injected to high-

performance liquid chromatography (HPLC) system. The influences of the different experimental parameters on the extraction efficiency of oleuropein are studied and optimized.

2. Experimental

2.1 Chemicals

Oleuropein (purity≥ 98% by HPLC) was purchased from Indofine Chemical Company (Hillsbrough, USA). Acetonitrile (ACN), tetrahydrofuran (THF), methanol, ethanol, sodium chloride, sodium carbonate, sodium sulfate, ammonium acetate, potassium dihydrogen phosphate and orthophosphoric acid were purchased from Merck Chemical (Darmstadt, Germany). All solutions were prepared with deionized water from a Milli-Q system (Millipore, USA).

2.2 Samples

Olea europaea (variety: Sevillana) leaves and fruits were collected from Agricultural Research Garden, Khorramabad, Iran. The leaves and fruits (After removing the stones) were dried in shadow, milled, homogenized and kept at 4 $^{\circ}$C until analysis. The same sample (leaves) was used in the whole optimization study.

2.3 Standard solutions preparation

A stock standard solution (1000 μg mL^{-1}) was prepared by dissolving oleuropein in methanol. Working standard solutions at concentration of 0.5-100 μg mL^{-1} were prepared by diluting the suitable volume of the stock standard with deionized water.

2.4 Chromatographic conditions

The HPLC system (Shimadzu Corporation, Kyoto, Japan) which consisted 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., 5 µm, Shimadzu, Japan) was used.

The mobile phase consisted of phosphate buffer (50 mM and pH=3 adjusted with orthophosphoric acid) and acetonitrile (70:30, v/v). Prior to preparation of the mobile phase, buffer solution and acetonitrile were degassed separately using a Millipore vacuum pump. The UV detector was set at 254 nm. The flow rate and oven temperature were adjusted at 1.0 mL min^{-1} and ambient temperature, respectively.

2.5 Ultrasound and salt-assisted liquid-liquid (USALLE) extraction procedure

0.01 g of sample was transferred into a 15 mL conical polypropylene centrifuge tube. 10 mL of solvents mixture containing phosphate buffer (pH 3), ACN and THF (80:10:10, v/v/v) as extraction solvent was added to the tube and then the mixture was placed in an ultrasonic bath at 25 °C for 30 min. After this time period, phase separation was completed by centrifuging the solution at 4000 rpm for 5 min and 1 mL of liquid phase was transferred into a microtube. Then 0.2 g NaCl was added to the microtube and the mixture vortexes until dissolution of salt. Salt addition results in rapid separation of two phases without centrifugation. Finally, 10 µL of organic phase was withdrawn and injected into the HPLC system for analysis. The schematic diagram of sample preparation by using USALLE technique was illustrated in Fig. 1.

3. Results and discussion

Besides the extraction technique, extraction efficiency is also depends to extraction conditions. Therefore, several parameters affect the concentration of the desired component

in the extract, such as pH, extracting solvent, temperature, liquid phase to sample weight ratio, contact time and ionic strength were optimized. Each experiment in the USALLE optimization process was replicated three times.

3.1 The pH of aqueous phase

The effect of sample pH on extraction efficiency was studied in the range of 2-10. As seen from Fig. 2, an increase in pH lead to increase oleuropein extraction up to pH =3 and then decrease. This phenomenon is consistent with the isoelectric point (pI=3.23) of the oleuropein. In the isoelectric point net charge of oleuropein is zero and thus mass transfer to organic phase increases. Also, low pHs may be increasing the cell membrane permeability which lead to higher diffusion coefficient values. Low extraction efficiency in higher pHs can be attributed to decrease in the stability of oleuropein and increasing its surface charge.

3.2 Choice of organic solvent and its volume

In order to select an appropriate extracting solvent two important parameters, the solubility of the target compound and the penetrability into the matrix must be considered. Due to miscibility of extracting solvent in water, SALLE was applied for extraction, pre-concentration and clean-up of polar compounds from water or liquid samples. Therefore, it is important to choice an appropriate extraction solvent with suitable polarity for maximum analyte or analytes extraction during SLE and SALLE steps. Several water-miscible organic solvents such as methanol, ethanol, acetonitrile (ACN), tetrahydrofuran (THF) and their mixtures were examined as organic phase. No phase separation was observed by using methanol, ethanol and their mixtures with other solvents. The mixture of ACN/THF was shown higher extraction efficiency than pure ACN and THF (Fig. 3). Thus, different ratios of ACN/THF was examined (Fig. 4). As can be seen from Fig. 4, the ACN/THF (50:50 v/v)

exhibited the highest extraction efficiency for oleuropein. Therefore, ACN/THF (50:50 v/v) was selected as the extraction solvent for subsequent experiments.

Volume of organic solvent can be influenced the efficiency of SLE and also enrichment factor of SALLE. Therefore, optimization of this parameter is required. Various volumes of ACN/THF (50:50 v/v) in the range of 1.2 to 3.5 mL were investigated. In volumes less than 1.2 mL phase separation was not observed. As can be observed from Fig. 5 with increasing the organic solvent volume peak area of oleuropein was increased and then decreased. This behavior can be attributed to increase the organic phase volume after salting out phenomena which lead to dilution of the target analyte. Therefore, 2 mL was selected as the optimum volume.

3.3 Ultrasonic time and temperature

One of the main advantages of the UAE is the shorter extraction time compared to conventional techniques. In order to investigate the effect of ultrasonic time on extraction efficiency different times in the range of 1-50 min were examined. Fig. 6 shows the effect of ultrasonic time on the peak area of oleuropein. The results indicate the extraction efficiency increases with the increase of ultrasonic time in the range of 1 to 30 min. After 30 min the extraction efficiency was decreased. This behavior can be attributed to degradation of analyte due to prolong exposure to ultrasonic waves.

Since the stability of most natural compounds was affected by temperature variation. The optimization of extraction temperature must be achieved in order to obtain the highest extraction efficiency of analyte without degradation. Hence, a temperature control is essential to prevent the degradation of target compounds. In this work, the effect of temperature was studied in three different temperatures including 25, 35 and 45 °C. Fig. 7 illustrates the influence of temperature on the peak area of oleuropein. It is clear with increasing

temperature extraction amount was decreased. Therefore, 25 °C was chosen as the appropriate temperature.

3.4 Liquid phase to solid sample ratio

Generally, a higher solvent to sample ratio in extraction techniques can increase the recovery. In this study, the solvent volume was kept constant and the solid mass changed in the range of 0.001-0.1 g. As shown in Fig. 8, the peak area of oleuropein was increased with increasing the plant mass up to 0.01 g and then leveled off. This behavior can be attributed to saturation of the liquid phase, which prevents the more extraction with an increase in sample mass. Hence, 0.01 g was selected as the optimum sample mass in subsequent experiments.

3.5 Choice of salt and its concentration

In this study, four salts including sodium chloride, sodium carbonate, sodium sulfate and ammonium acetate were investigated as the salting-out reagents. Similar to previous reports [26, 32], it is clear from Fig. 9 that the sodium chloride is the most appropriate salt. In the next step, salt concentration effect in the range of 10-30 % w/v was investigated. The results were shown the peak area of oleuropein increases up to 20 %w/v and then decreased (Fig. 10). Increasing of salt concentration can lead to increase the organic phase volume. In the other hand, high salt concentration increases the viscosity of the aqueous phase which reduces the mass transfer of analyte from aqueous to organic phase. Hence, 20 %w/v was selected as the optimum salt concentration.

3.6 Method evaluation

Typical chromatograms of blank extract, standard solution and extracted oleuropein under the optimized conditions are shown in Fig. 11. It is observed which USALLE is an

9

effective method for extraction and pre-concentration of oleuropein. Under the optimized

conditions validation parameters of the proposed method such as linearity, limit of detection

(LOD), limit of quantitation (LOQ), precision and accuracy were determined. Linearity of the

USALLE-HPLC-UV method was evaluated by extracting and injecting standard solutions of

oleuropein at different concentrations after extraction under the optimized conditions. R-

square value of calibration curve was 0.9934 that approved the linearity of the technique. The

LOD and LOQ were 0.5 and 2.5 μg mL^{-1}, respectively.

Results of repeatability and reproducibility of the proposed method in three

concentration levels are detailed in Table 1. Intraday and interday relative standard deviation

(RSD) values for all concentration levels were less than 5.5 and 7.5 %, respectively.

The accuracy of the method was investigated by determining the relative recovery of

spiked oleuropein in plant samples at three concentration levels. Table 1 lists the obtained

relative recoveries from the analysis of spiked samples. As can be seen, relative recoveries

were in the range of 90.0–97.0%.

The analytical parameters of the proposed method were compared with several

reported methods in the literature (Table 2). The results show the LOD and LOQ were

improved by using the proposed USALLE-HPLC-UV method. In the other hand, analysis

time for this method was shorter than other methods. In this method, required organic solvent

volume and sample weight were reduced greatly. The main advantage of this method

compared to the conventional methods is the elimination of evaporation and reconstitution

operations in natural product sample preparation. The proposed method can be successfully

used to extract and quantify natural products.

In order to investigate of method performance oleuropein content of several olive

fruits and leaves was determined by using the proposed method under the optimized

conditions. The results of oleuropein content for fruits and leaves were in the range of 0.78-1.68 and 25.20-34.03 mg/g, respectively.

4. Conclusion

In this study, ultrasound and salt-assisted liquid-liquid extraction as an efficient sample preparation method for plant samples was introduced and optimized using oleuropein as model analyte. The proposed method is based on coupling two extraction techniques including solid-liquid and liquid-liquid extraction. Solid-liquid and liquid-liquid extraction methods were performed by using ultrasound-assisted extraction and salt-assisted liquid-liquid extraction, respectively. In this technique high efficiency extraction of UAE for solid samples and capability of pre-concentration and clean-up of SALLE were combined. Unlike conventional extraction methods for plant extract no evaporation and reconstitution operations were needed in the USALLE procedure. Organic phase can be directly injected to analytical instrument. In addition, centrifuging step was removed because the salt addition facilitated the phase separation.

Acknowledgments

Analytical Methods Accepted Manuscript

References

1 M. Rashidipour, A. Feizbakhsh, R. Heydari and P. Hashemi, *J. AOAC Int*., 2014, **97**, 1109-1113.

2 M. Hosseini, R. Heydari and M. Alimoradi, *Talanta,* 2014, **130**, 171–176.

3 Z. Pourghobadi, R. Heydari, R. Pourghobadi and M. Rashidipour, *Monatsh. Chem.*, 2013, **144**, 773–779.

4 M. Hosseini, R. Heydari and M. Alimoradi, *J. Sep. Sci*., 2015, **38**, 663–669.

5 M. A. Rostagno and J. M. Prado, Natural Product Extraction, Principles and Applications, The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK, 2013.

6 L. Niu, J. Li, M. S. Chen and Z. F. Xu, *Ind. Crop. Prod.*, 2014, **56**, 187-190.

7 P. S. Milić, K. M. Rajković, O. S. Stamenković and V. B. Veljković, *Ultrason. Sonochem*., 2013, **20**, 525-534.

8 H. Bagheri, M. Y. B. Abdul Manap and Z. Solati, *Talanta*, 2014, **121**, 220-228.

9 M. González and V. González, *Anal. Methods*, 2010, **2**, 1842-1866.

10 M. Contini, S. Baccelloni, R. Massantini and G. Anelli, *Food Chem*., 2008, **110**, 659–669.

11 S. Sahin, M. Bilgin and M. U. Dramur, *Sep. Sci. Technol*., 2011, **46**, 1829–1837.

12 S. Y. Cho, Y. N. Lee and H. J. Park, *Food Chem*., 2009, **117**, 312–317.

13 Y. Merdassa, J. F Liu and N. Megersa, *Anal. Methods*, 2014, **6**, 3025-3033.

14 Y. Wen, J. Nie, Z.G. Li, X. Y. Xu, D. Wei, and M. R. Lee, *Anal. Methods*, 2014, **6**, 3345-3352.

15 P. Fernández, M. Regenjo, A. M. Fernández, R. A. Lorenzo and A. M. Carro, *Anal. Methods*, 2014, **6**, 8239-8246.

16 M. D. R. Santos, M. R. F. Cerqueira, M. A. L. de Oliveira, R. C. Matos and M. A. C. Matos, *Anal. Methods*, 2014, **6**, 1650-1656.

17 A. Ghassempour, R. Heydari, Z. Talebpour, A. R. Fakhari, A. Rassouli, N. Davies and H. Y. Aboul-Enein, *J. Liq. Chromatogr. Related Technol.*, 2008, **31**, 2686–2703.

18 I. M. Valente, L. M. Gonçalves and J. A. Rodrigues, *J. Chromatogr. A*, 2013, **1308**, 58-62.

19 R. M. Ramos, I. M. Valente and J. A. Rodrigues, *Talanta*, 2014, **124**, 146-151.

20 F. Myasein, E. Kim, J. Zhang, H. Wu and T. A. El-Shourbagy, *Anal. Chim. Acta*, 2009, **651**, 112-116.

21 R. Heydari and S. Zarabi, *Anal. Methods*, 2014, **6**, 8469-8475.

22 Q. Wang, C.R. Yin and L. Xu, *J. Sep. Sci.*, 2013, **36**, 1007–1014.

23 T. Li, L. Zhang, L. Tong and Q. Liao, *Biomed. Chromatogr.*, 2014, **28**, 648–653.

24 H. Wu, J. Zhang, K. Norem and T. A. El-Shourbagy, *J. Pharm. Biomed. Anal.*, 2008, **48**, 1243-1248.

25 A. Gure, F. J. Lara, D. Moreno-González, N. Megersa, M. del Olmo-Iruela and A. M. García-Campaña, *Talanta*, 2014, **127**, 51-58.

26 H. Sereshti, M. Khosraviani and M. S. Amini-Fazl, *Talanta*, 2014, **121**, 199-204.

27 Y. Wen, J. Li, F. Yang, W. Zhang, W. Li, C. Liao and L. Chen, *Talanta*, 2013, **106**, 119-126.

28 Q. Xia, Y. Yang and M. Liu, *Spectrochim. Acta A*, 2012, **96**, 358-364.

29 M. Bustamante-Rangel, L. Pérez-Martín and M. M. Delgado-Zamarreño, *Phytochem. Anal.*, 2014, **25**, 170-177.

30 Z. İlbay, S. Şahin and K. Büyükkabasakal, *Korean J. Chem. Eng.*, 2014, **31**, 1661-1667.

31 N. Xynos, G. Papaefstathiou, E. Gikas, A. Argyropoulou, N. Aligiannis and A. L. Skaltsounis, *Sep. Purif. Technol.*, 2014, **122**, 323-330.

32 M. A. Farajzadeh, S. Sheykhizadeh and P. Khorram, *J. Sep. Sci.*, 2013, **36**, 939–946.

33 R. Japón-Luján and M. D. Luque de Castro, *J. Chromatogr. A*, 2006, **1136**, 185–191.

34 R. Japón-Luján, J.M. Luque-Rodríguez and M. D. Luque de Castro, *J. Chromatogr. A*, 2006, **1108**, 76–82.

35 K. Stamatopoulos, E. Katsoyannos, A. Chatzilazarou and S. J. Konteles, *Food Chem*., 2012, **133**, 344–351.

36 N. Xynos, G. Papaefstathiou, M. Psychis, A. Argyropoulou, N. Aligiannis and A. L. Skaltsounis, *J. Supercrit. Fluids*, 2012, **67**, 89–93.

37 F. Aouidia, N. Dupuy, J. Artaud, S. Roussos, M. Msallem, I. P. Gaime and M. Hamdi, *Ind. Crop. Prod.,* 2012, **37**, 292–297.

Figure Captions

Figure 1. Schematic diagram of proposed ultrasound and salt-assisted liquid–liquid extraction (USALLE) procedure.

Figure 2. Effect of aqueous phase pH on the extraction of oleuropein. Extraction conditions: organic phase, ACN; organic phase volume, 3 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 3. Effect of organic phase on the extraction of oleuropein. Extraction conditions: aqueous phase pH, 3; organic phase volume, 3 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 4. Effect of ACN/THF ratio on the extraction of oleuropein. Extraction conditions: aqueous phase pH, 3; organic phase volume, 3 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 5. Effect of organic phase volume on the extraction of oleuropein. Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 6. Effect of ultrasonic time on the extraction of oleuropein. Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 7. Effect of ultrasonic temperature on the extraction of oleuropein. . Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic time, 30 min; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

Figure 8. Effect of plant mass on the extraction of oleuropein. . Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; salt type, NaCl; salt concentration, 10 %w/v.

Figure 9. Effect of salt on the extraction of oleuropein. . Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt concentration, 20 %w/v.

Figure 10. Effect of salt concentration on the extraction of oleuropein. . Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl.

Figure 11. HPLC chromatograms of blank extract (a), direct injection of oleuropein standard solution (b) and oleuropein standard solution after extraction by USALLE method (c). Concentration of oleuropein in standard was 200 μg mL^{-1}. Extraction conditions: aqueous phase pH, 3; organic phase, ACN/THF (50:50 v/v); organic phase volume, 2 mL; ultrasonic time, 30 min; ultrasonic temperature, 25 °C; plant mass, 0.01 g; salt type, NaCl; salt concentration, 10 %w/v.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Obtained precision and accuracy data for oleuropein spiked in plant sample by using USALLE method.

Concentration (μg mL^{-1})	Accuracy (n=3)			Precision (RSD %)	
	Concentration found (μg mL^{-1})	Recovery (%)	RSD (%)	Intraday (n=3)	Interday (n=9)
5	4.6	92.0	6.5	5.2	7.5
10	9.0	90.0	5.2	5.5	6.7
50	48.5	97.0	4.0	4.8	5.4

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Table 2. Comparison between extraction parameters of the proposed method and other methods in the literature for oleuropein

Extraction method	Extraction time (min)	Extraction temperature (°C)	Extraction solvent	Extraction solvent volume (mL)	Sample amount (g)	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)	RSD (%)	Ref.
SHLE [a]	13	140	Ethanol–water (70:30, v/v)	11	1.0	11.59	29.79	6.89	[33]
DUAE [b]	25	40	Ethanol–water (59:41, v/v)	NR [c]	1.0	11.46	30.67	5.49	[34]
Steam blanching and maceration	50	40	Ethanol–water (70:30, v/v)	80	10.0	NR [c]	NR	NR	[35]
SFE [d]	NR	50	CO_2-ethanol (80:20, v/v)	NR	275.0	NR	NR	NR	[36]
PLE [e]	32	150	Water	NR	6.0	NR	NR	NR	[36]
Maceration	24 h	Room temperature	Methanol-water (80:20, v/v)	30	1.0	NR	NR	NR	[37]
USALLE	30	25	phosphate buffer (pH 3)/ACN/THF (80:10:10, v/v/v)	10	0.01	0.5	2.5	6.5	This work

[a] Superheated liquid extraction, [b] Dynamic ultrasound-assisted extraction, [c] Not reported, [d] Supercritical fluid extraction, [e] Pressurized liquid extraction
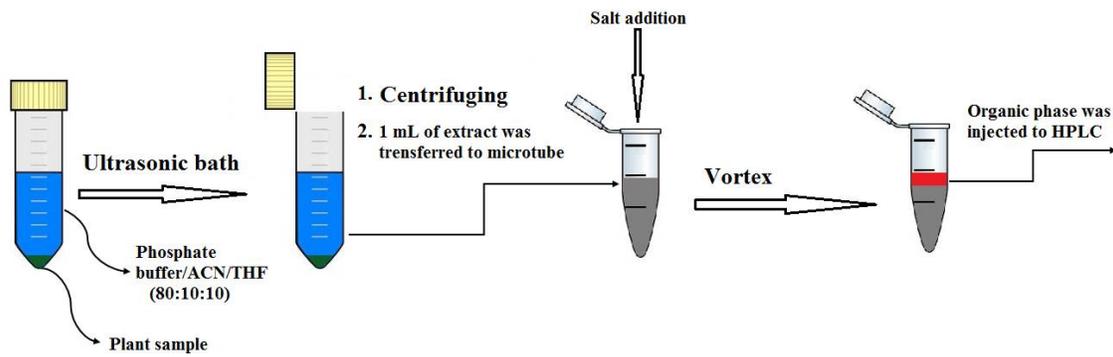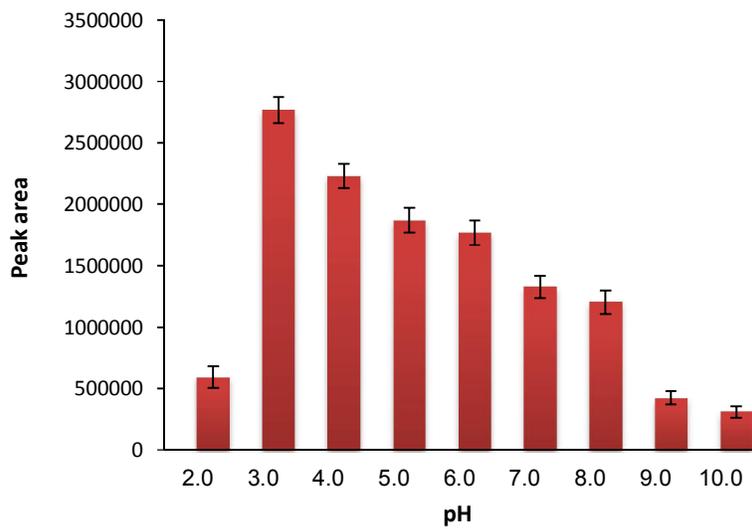
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Fig. 1

Fig. 2

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
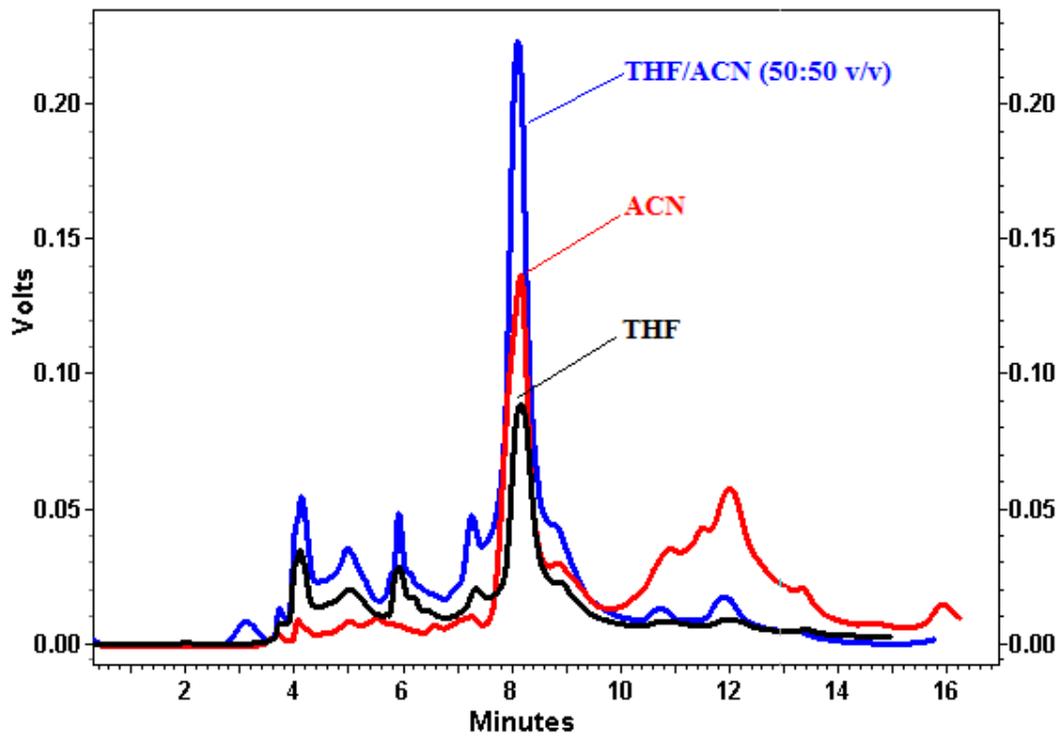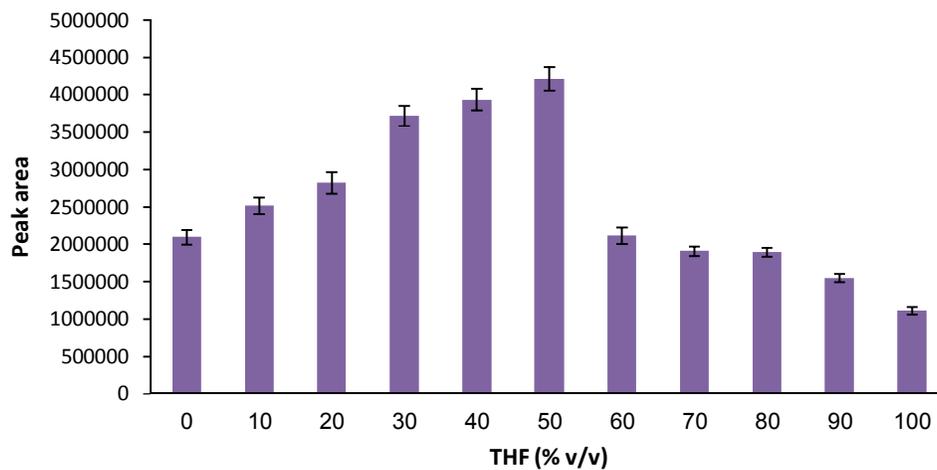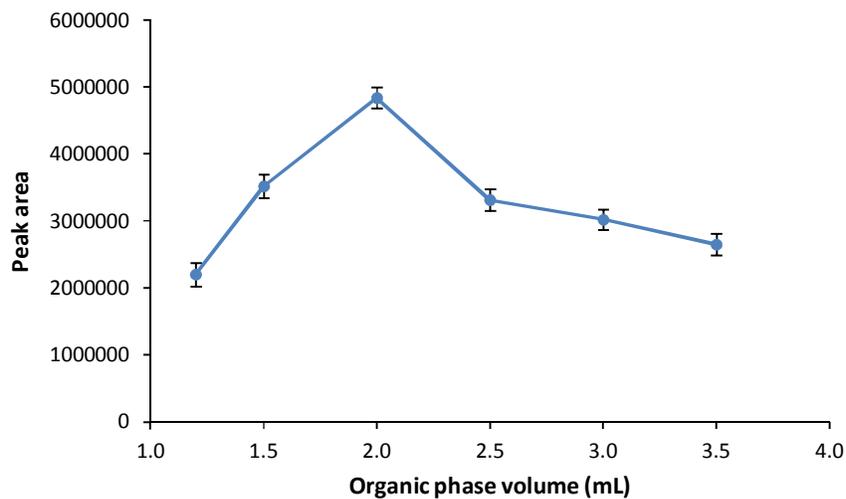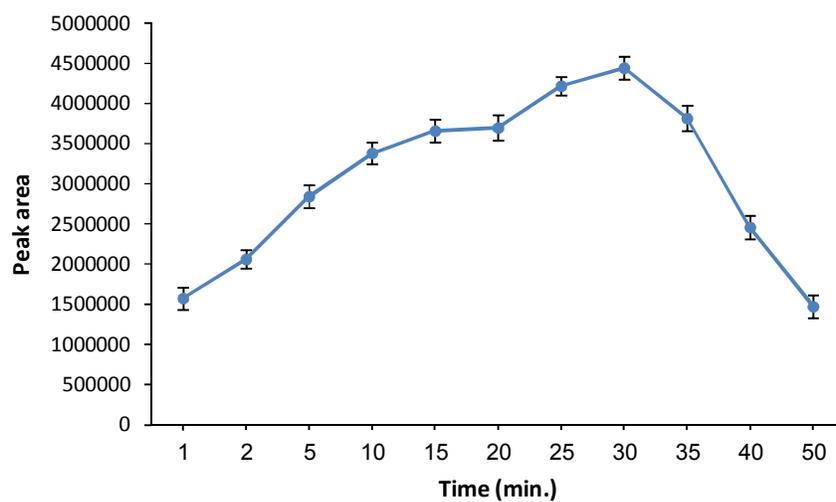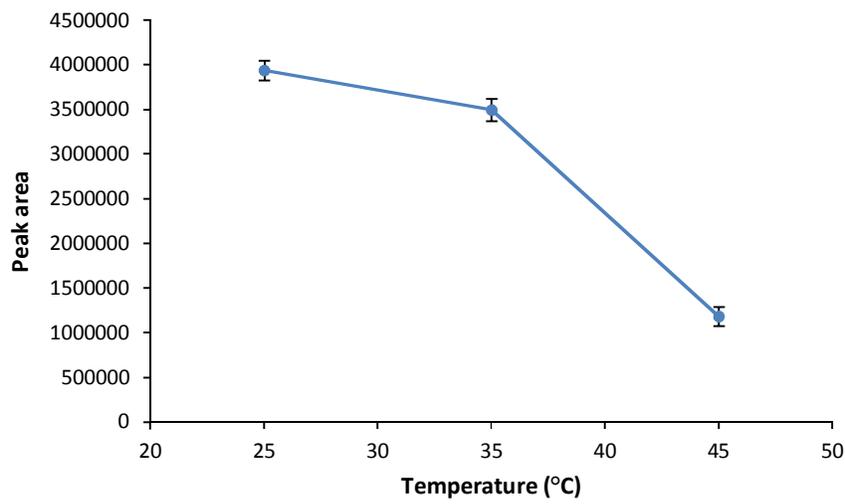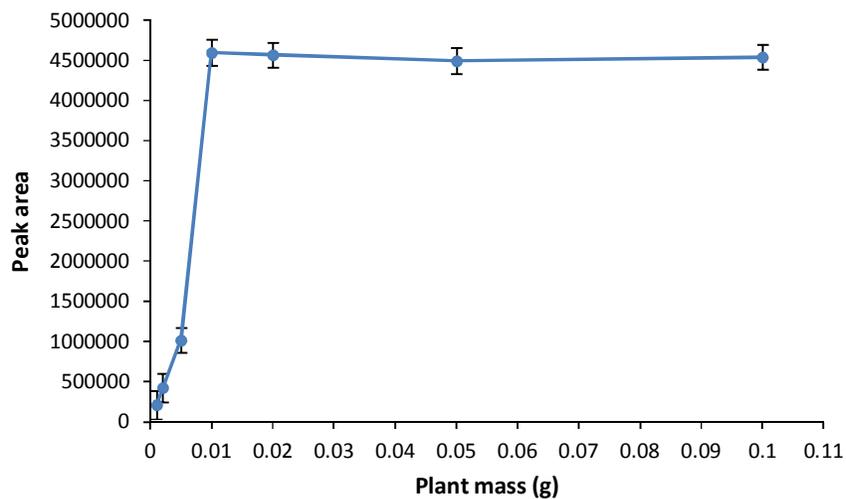42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Fig. 3

Fig. 4

Fig. 5

Fig. 6

24

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
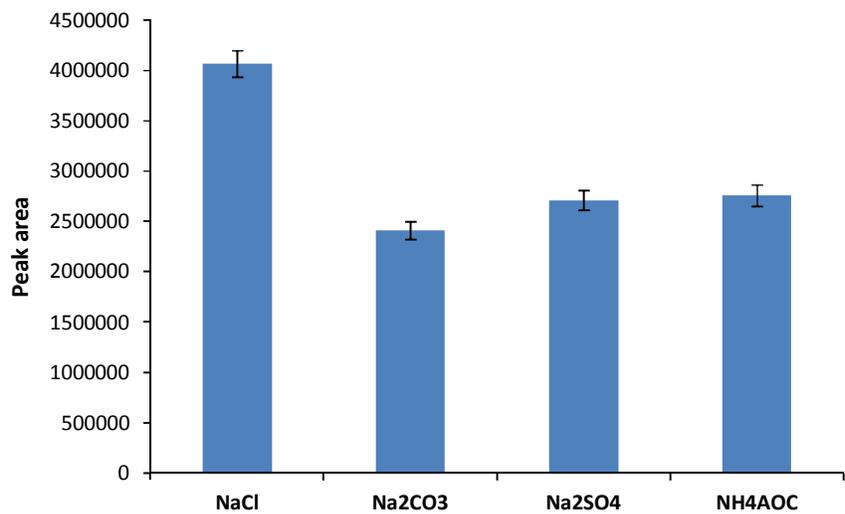51
52
53
54
55
56
57
58
59
60

Fig. 7

Fig. 8

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
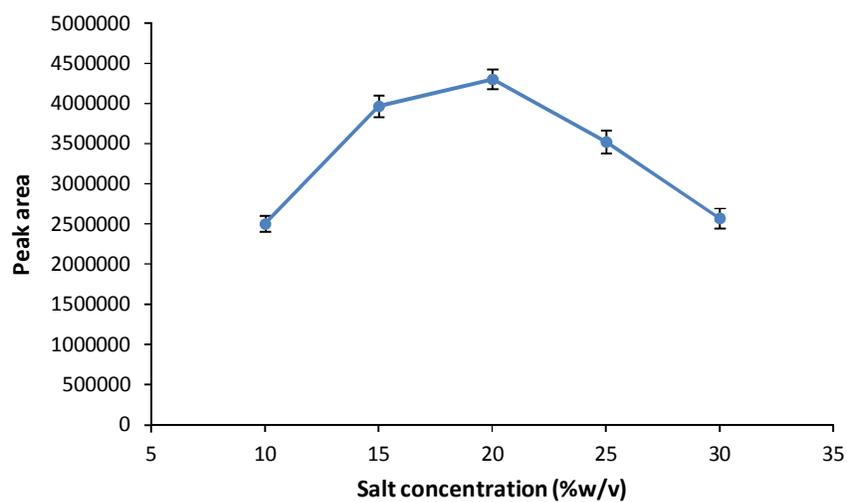48
49
50
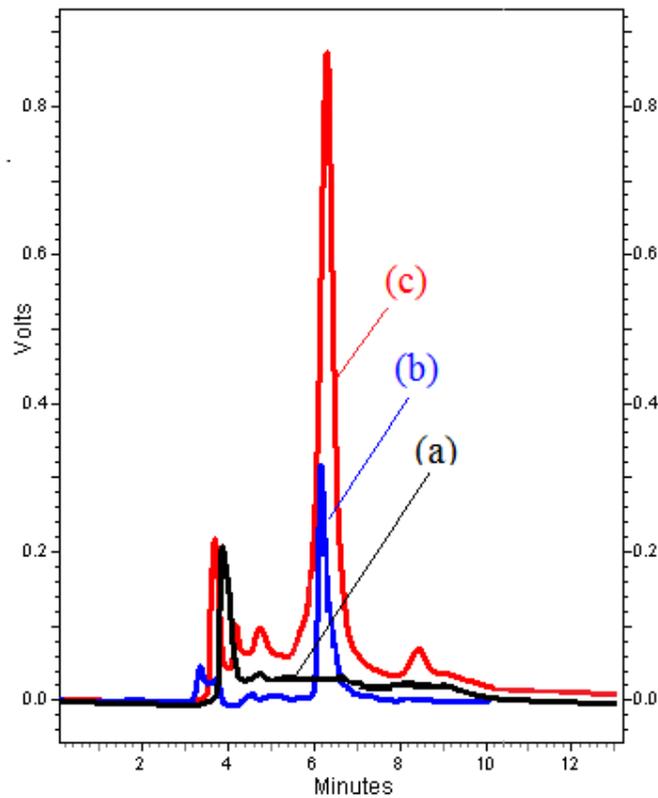51
52
53
54
55
56
57
58
59
60

Fig. 9

Fig. 10

Fig. 11