



Aqueous two-phase extraction technique based on magnetic ionic liquid-nonionic surfactant for phenolic acids in grape, apple, pear, and banana

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Abstract: An aqueous two-phase extraction system was constituted using magnetic ionic liquid-nonionic surfactant for phenolic acids and the developed method was applied to apple, grape, pear, and banana samples. Ionic liquids based on imidazolium containing butyl, allyl, and benzyl groups were used for extraction. The parameters influencing the extraction efficiency such as volume of ionic liquids, type and amount of surfactant and salt, ultrasound extraction time, temperature, and sample pH were optimized. The extraction efficiencies of ionic liquids were compared for phenolic acids under optimized conditions. Limits of detection and limits of quantification were in the range of 1.34–1.56 $\mu\text{g L}^{-1}$ and 4.33–4.68 $\mu\text{g L}^{-1}$, respectively. The interday and intraday precision (RSD%) for the phenolic acids at 5 and 25 $\mu\text{g L}^{-1}$ were in the range of 4.48%–5.69% and 3.02%–4.51%, respectively. The spiked recoveries of phenolic acids were between 70.78% and 99.97% in real samples.

Key words: Aqueous two-phase extraction, magnetic ionic liquid, phenolic acid, high-performance liquid chromatography, surfactant

1. Introduction

Phenolic acids have natural antioxidant properties and also secondary metabolite properties [1]. These compounds are usually found in seeds, fruits, flowers, leaves, and branches of plants and they protect the plants from ultraviolet rays, diseases, and damages [2,3]. However, excess amounts of phenolic acids in food cause quality loss called enzymatic browning. The excessive consumption of these foods can create health problems such as nutritional disorders or cancer [4,5]. Therefore, it is necessary to control the amount of phenolic acids in foods. Chromatographic techniques such as high-performance liquid chromatography (HPLC) [6], gas chromatography (GC) [7], and high-performance thin-layer chromatography (HPTLC) [8] are used for the analysis of phenolic acids. These compounds must be separated and enriched from the complex matrix prior to their analysis for sensitive and selective determination [9,10].

Among these extraction methods, aqueous two-phase extraction (ATPE) is an economical and efficient technique. This method is superior to the traditional liquid-liquid extraction method with high water content of the lower and upper phases. In addition, the ATPE method is environmentally friendly due to the absence of volatile organic solvents and it enables the analyte to be highly enriched from the aqueous phase [11–13]. This extraction technique generally employs polymer/polymer [14], polymer/salt [15], surfactant/salt [16], and

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surfactant/ionic liquid [17] solutions. Ionic liquid-based aqueous two-phase extraction (IL-ATPE), due to the use of ionic liquid, has advantages such as little emulsion, quick phase separation, low viscosity, and environmental friendliness [18,19]. Thus, the IL-ATPE method has been applied in the extraction of various inorganic and organic analytes before their analysis [20,21]. Recently, magnetic room-temperature ionic liquids (MILs) have also been used in extraction techniques [22,23]. Phase separation efficiency in ATPE can be enhanced with the use of magnetic ionic liquid. Yao et al. used a guanidinium based MIL-ATPE system to determine trace amounts of chloramphenicol in water environments [18]. They achieved rapid extraction with MIL and showed that extraction efficiency was enhanced. Nie et al. prepared cholinium-based MIL and used ATPE for berberine hydrochloride in *Rhizoma coptidis* [24]. High extraction efficiency and rapid phase separation were obtained with this MIL.

In this work, an ATPE method was developed using MILs based on imidazolium and the nonionic surfactant Triton X-100 to separate trace amounts of phenolic acids in grape, apple, pear, and banana samples. HPLC was used to separate and determine trace amounts of phenolic acids in samples. Parameters such as volume of ionic liquid, type and amount of surfactant and salt, extraction time, temperature, and pH of the sample solution were optimized to examine the extraction efficiency. The effects on the extraction performances of the ionic liquids were compared. These results showed that the developed method is simple, practical, efficient, repeatable, inexpensive, and free of organic solvents. Moreover, the proposed method provided high recoveries and good repeatability for phenolic acids in real samples.

2. Results and discussion

2.1. Characterization of MILs

The structures of the synthesized MILs were illuminated by FTIR and UV-Vis region spectra (Figure 1). Thermal stabilities of MILs were investigated by TG/DTA curves. In FTIR spectra, $\sim 3137\text{--}3142\text{ cm}^{-1}$ were the aromatic $=\text{C-H}$ stretching vibrations, $\sim 2946\text{--}2868\text{ cm}^{-1}$ were aliphatic symmetric and asymmetric $-\text{C-H}$ stretching vibrations, $\sim 2352\text{--}2358\text{ cm}^{-1}$ were stretching vibrations of imidazolium rings, and $\sim 1446\text{--}1451$ and $\sim 1552\text{--}1558\text{ cm}^{-1}$ were $\text{C}=\text{C}$ and $\text{C}=\text{N}$ stretching vibrations, respectively [25]. Furthermore, $\sim 1331\text{--}1337\text{ cm}^{-1}$ were $-\text{C-H}$ bending vibrations, $\sim 1055\text{--}1160\text{ cm}^{-1}$ were $-\text{C-C}$ stretching vibrations, and $\sim 730\text{--}825\text{ cm}^{-1}$ were aromatic $=\text{C-H}$ out-of-plane bending vibrations. In the UV-Vis spectra, the peaks observed at 530–534 nm, 610–611 nm, and 680–688 nm in MILs were the characteristic peaks for FeCl_4^- ions [26,27]. In TG/DTG curves it was concluded that the MILs were very stable. The decomposition temperatures of MILs were 401, 374, and 392 °C for 1-butyl-3-methylimidazolium tetrachloroferrate, 1-allyl-3-methylimidazolium tetrachloroferrate, and 1-benzyl-3-methylimidazolium tetrachloroferrate, respectively.

2.2. Optimization of ATPE

2.2.1. Volume of MIL

As the volume of ionic liquid affects the formation of phase separation in IL-ATPE, the optimization of the volume of ionic liquid is an important parameter. The use of more ionic liquid can enhance the recovery of phenolic acid. The extraction efficiency can also decrease due to the higher viscosity of the ionic liquid [22]. For this, the optimization of a volume of MIL was carried out in the ranges given in Table 1. In experiments with three ionic liquids, the recoveries of phenolic acids first quickly increased, then quickly decreased with the increase of the volume of ionic liquid. This can be explained as follows: extraction efficiency rises with the

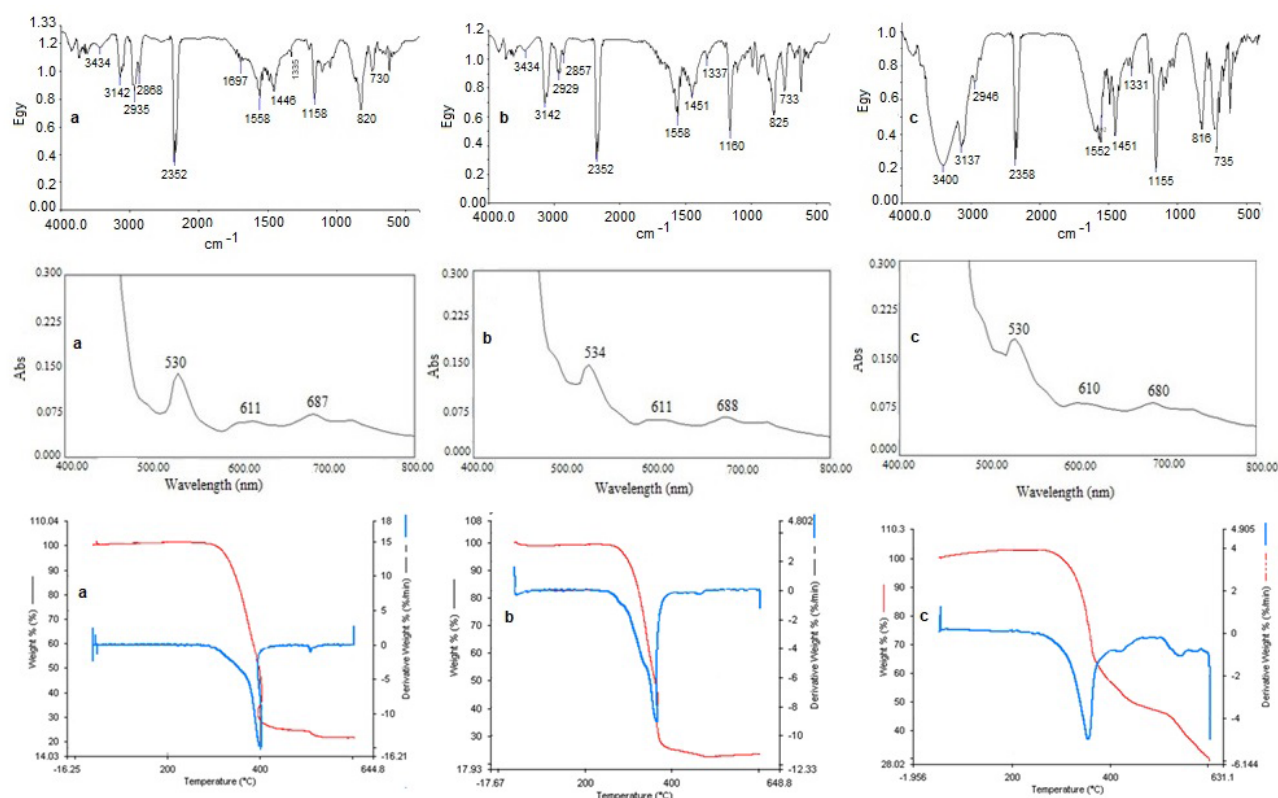


Figure 1. The FTIR and visible absorption spectra and TG/DTA curves of MILs: a) 1-butyl-3-methylimidazolium tetrachloroferrate, b) 1-allyl-3-methylimidazolium tetrachloroferrate, c) 1-benzyl-3-methylimidazolium tetrachloroferrate.

increasing amount of MIL until extraction saturation. Then an increase in the amount of ionic liquid causes an increase in the volume of the upper phase and a decrease in the volume of the lower phase. Therefore, the extraction efficiency begins to decrease [24]. The highest recovery was obtained with 200 μL of ionic liquid for three MILs. In Figure 2, the results of ATPE carried out with different volumes of 1-allyl-3-methylimidazolium tetrachloroferrate for phenolic acids are given. As seen, the recoveries of phenolic acids with 200 μL of ionic liquid were in the order of gallic acid protocatechuic acid = chlorogenic acid caffeic acid ferulic acid. Low recovery was achieved with gallic acid in the extraction with 50 and 100 μL of ionic liquid, but the extraction saturation was carried out with 200 μL of ionic liquid and the interaction of gallic acid and ionic liquid was observed at a maximum level. Then the recoveries were also reduced since interactions with increasing ionic liquid viscosity would be reduced. The optimum volume of MIL was taken as 200 μL for the next experiments.

Table 1. The optimized parameters and ranges with three MILs.

	Parameter						
	Volume of MIL	Amount of Triton X-100	Amount of Na_2CO_3	of	Extraction time	Extraction temperature	pH
Range	50–350 μL	0.05–0.3 g	0.05–0.6 g		5–25 min	25–60 $^\circ\text{C}$	3–9

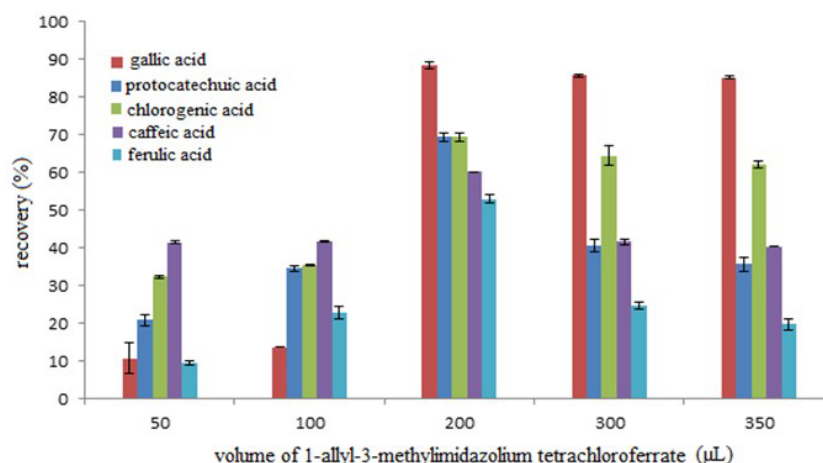


Figure 2. The effect of the amount of ionic liquid on the ATPE performed with different amounts of 1-allyl-3-methylimidazolium tetrachloroferrate ($n = 3$, 5 mL of $200 \mu\text{g L}^{-1}$ mixture, 0.2 g of Triton X-100, 0.5 g of Na_2CO_3 , pH 5.5, extraction time 15 min).

2.2.2. Type and amount of surfactant

Effects of the type and amount of surfactant on extraction recovery were investigated in detail. First, the extraction was carried out without surfactant, but phase formation could not be obtained with only ionic liquid and salt. The surfactants comprise two parts, the head and the tail. The head is polar or ionic and has a hydrophilic character. The tail is a hydrocarbon and has a hydrophobic property, and the surfactants are capable of forming self-assembling aggregates. These molecules indicate their tails in the interior for their micelle forms and their heads play a significant role in the interface between the aqueous solution and the aggregate's core [28]. Thus, MIL-ATPE was also carried out with anionic and cationic surfactant but the formation of the extraction phase was only achieved with Triton X-100 in the extractions carried out with three ionic liquids. This situation can be explained as follows: Triton X-100 is nonionic surfactant and a comparatively mild detergent. This surfactant forms a gentle environment for some molecules and so the use of Triton X-100 can be more effective for extraction studies [29]. For this, the amount of Triton X-100 was optimized for this study. On the other hand, low or high concentrations of surfactant negatively influenced the amount of solubilized phenolic acids [30]. The recoveries were decreased after 0.2 g of Triton X-100 in the extractions repeated with three ionic liquids. For this, 0.2 g of Triton X-100 was accepted as the optimum amount for MIL-ATPE carried out with three MILs. The extraction results with 1-benzyl-3-methylimidazolium tetrachloroferrate are given in Figure 3. As seen, the recoveries of phenolic acids with 0.2 g of Triton X-100 were in the order of protocatechuic acid ? gallic acid ? chlorogenic acid ? caffeic acid ? ferulic acid. Minor changes were observed with other amounts of Triton X-100 in this order. The chemical structure of the phenolic acids was similar. Therefore, it was similar in interactions during extraction. The amount of solubilized phenolic acids was affected with low and high amounts of Triton X-100. For this reason, the extraction efficiency of phenolic acids varied with increasing amounts of Triton X-100.

2.2.3. Type and amount of salt

As the inorganic salt may influence the extraction due to both intracellular and intercellular interactions, the type and amount of salt should be optimized in ATPE [31]. Therefore, MIL-ATPE was carried out with inorganic salts such as potassium carbonate (K_2CO_3), dipotassium hydrogen phosphate (K_2HPO_4), potassium

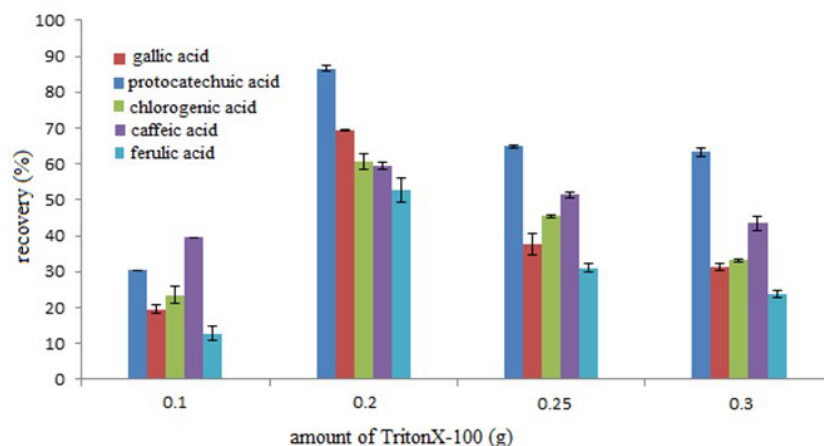


Figure 3. The effect of amount of Triton X-100 on the ATPE carried out with 1-benzyl-3-methylimidazolium tetrachloroferrate ($n = 3$, 5 mL of $200 \mu\text{g L}^{-1}$ mixture, 200 μL of MIL, 0.5 g of Na_2CO_3 , pH 5.5, extraction time 15 min).

dihydrogen phosphate (KH_2PO_4), sodium carbonate (Na_2CO_3), sodium bicarbonate (NaHCO_3), and sodium chloride (NaCl). The phase was not obtained for K_2CO_3 , K_2HPO_4 , KH_2PO_4 , NaHCO_3 , and NaCl after extraction was repeated with three ionic liquids. The effect of salting out was observed with only Na_2CO_3 for three MILs. Therefore, Na_2CO_3 was chosen as the optimum type of salt.

As the volume of the upper phase could be reduced by increasing the amount of Na_2CO_3 , the optimization of the salt was performed in the ranges given in Table 1. The phase was not observed with 0.05, 0.1, and 0.2 g of Na_2CO_3 . The highest recovery was obtained with 0.4 g of salt, while the recoveries decreased with 0.5 and 0.6 g of Na_2CO_3 . The same results were also acquired with three MILs. Therefore, 0.4 g of Na_2CO_3 was selected as the amount of salt. The extraction results obtained with 1-butyl-3-methylimidazolium tetrachloroferrate are given in Figure 4 as an example. As seen, the recoveries of phenolic acids with 0.4 g Na_2CO_3 were in the order of gallic acid > chlorogenic acid = protocatechuic acid > ferulic acid > caffeic acid. All interactions during extraction were high with 0.4 g of Na_2CO_3 . Therefore, the extraction reached saturation with 0.4 g of Na_2CO_3 . Then the interactions of phenolic acids with increasing amounts of salt changed, which resulted in a decrease in the extraction efficiency.

2.2.4. Ultrasound extraction time and temperature

The optimization of extraction time is an important parameter because ATPE is a type of equilibrium extraction. The partition equilibrium of analytes may be better at longer extraction times. This results in higher extraction efficiency [31]. Thus, to determine the optimum ultrasonic time for extraction efficiency, the extraction was carried out in the range of 5–25 min. The working frequency and power of the ultrasonic bath was adjusted to 35 kHz and 230 W. When the extraction time was 5 and 10 min, phase formation was not observed. The experimental results showed that the highest extraction efficiency was observed when the extraction time was 15 min with three MILs. The extraction efficiency decreased with 20 and 25 min of extraction time. Thus, 15 min was accepted as the optimum ultrasonic time for MIL-ATPE of phenolic acids. As the solubility and mass transfer of phenolic acids vary with temperature, the extraction temperature also has an important effect in ATPE [31]. To find the optimum extraction temperature, extraction was carried out at temperatures of 25, 30, 40, 50, and 60 °C in a heated ultrasonic bath and the temperature was controlled with a thermometer before

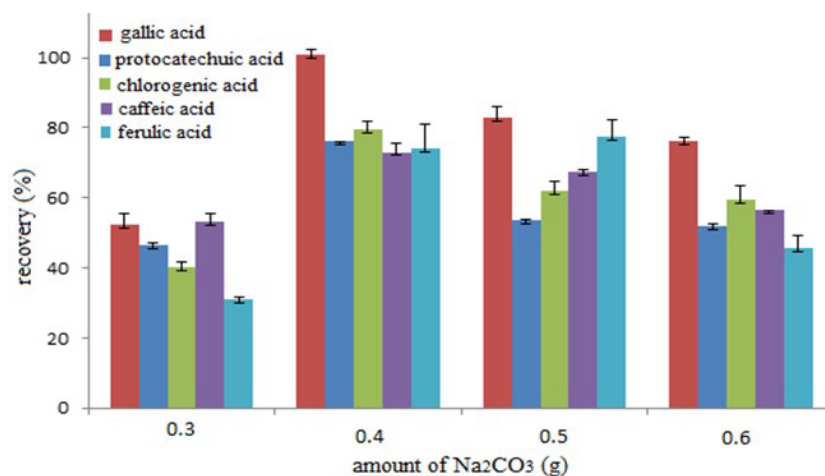


Figure 4. The effect of Na₂CO₃ on the ATPE carried out with 1-butyl-3-methylimidazolium tetrachloroferrate (n = 3, 5 mL of 200 µg L⁻¹ mixture, 200 µL of MIL, 0.2 g Triton X-100, pH 5.5, extraction time 15 min).

extraction. No phase difference was observed at 25 and 30 °C after extraction. Phase difference occurred at 60 °C, but the analyte peak was not on the chromatogram. Similar extraction efficiency was observed at 40 and 50 °C, so extraction was performed at ~40 °C.

2.2.5. pH of sample

As the solubility of phenolic acids depends on the pH of the medium, first the pH of the phenolic acid mixture was checked and it was observed that the pH was ~5.5–6. Then the influence of pH on the extraction recoveries was examined in the range of 3–9. pH adjustment was carried out by 0.1 mol L⁻¹ NaOH and HCl. The highest extraction recovery was obtained at pH 5.5–6, which was the pH of the solution. The pKa values of the studied phenolic acids were pKa = ~3.14–4.16 [32]. At pH 5.5–6, which was the pH of the solution, the uncharged phenolic acids migrated to the MIL-rich phase. When NaOH was added, the phenolic acids migrated to the Na₂CO₃-rich phase [33]. Thus, pH 5.5–6 was accepted as the optimum pH for extraction. The interaction between phenolic acids and ionic liquid could be defined as π-π interaction between the imidazolium ring of the ionic liquid and aromatic rings of phenolic acids [34]. The hydrophobic effect and van der Waals interactions such as with hydrogen bonds could also be observed between phenolic acids and ionic liquid in the aqueous medium.

2.3. Comparison of extraction efficiencies of MILs

In this study, extraction was carried out with three kinds of MILs containing butyl, allyl, and benzyl groups. After determination of the optimum parameters for extraction with three MILs, all the optimum values were seen to be the same in all three MILs. At these optimum values, extraction was repeated with three MILs and the extraction efficiencies of ionic liquids were compared. As shown in Figure 5, extraction recoveries were ≥75% for the three MILs and higher extraction efficiency was obtained with 1-butyl-3-methyl imidazolium tetrachloroferrate, while the highest recovery was obtained for gallic acid extraction with three MILs. The butyl group is more hydrophobic than the allyl and benzyl group. According to the extraction results, the interaction between ionic liquid and phenolic acids can be said to entail more hydrophobic effects and van der Waals interactions (hydrogen bonds) [35].

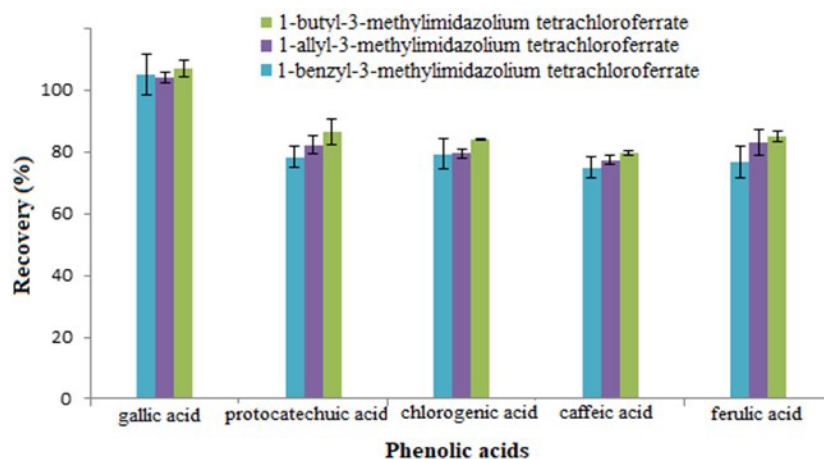


Figure 5. Comparison of extraction efficiencies of MILs in optimum conditions ($n = 3$, 5 mL of $200 \mu\text{g L}^{-1}$ mixture, 200 μL of MIL, 0.2 g of Triton X-100, 0.4 g of Na_2CO_3 , pH 5.5, extraction time 15 min).

To investigate the extraction efficiencies of MILs, the ATPE method was also carried out with 1-butyl-3-methylimidazolium chloride, 1-allyl-3-methylimidazolium chloride, and 1-benzyl-3-methylimidazolium chloride used as starting materials in the synthesis of MILs. The extraction was realized at optimum parameters determined by MILs. Although the phase was obtained with 1-butyl-3-methylimidazolium chloride and 1-benzyl-3-methylimidazolium chloride, no analyte peak was observed. Very low extraction efficiency was obtained with 1-allyl-3-methylimidazolium chloride for phenolic acids.

2.4. Method validation

As higher extraction efficiency was obtained with 1-butyl-3-methylimidazolium tetrachloroferrate for phenolic acids, method validation and the analytical performance of the method were determined out with this ionic liquid.

Under optimized conditions, linearity, limit of detection (LOD), limit of quantification (LOQ), and precision were examined. The calibration curve was established with a solution of standard phenolic acids at eight different concentrations with three replications. The linear ranges were found as $5\text{--}500 \mu\text{g L}^{-1}$ with coefficients of determination (R^2) ranging from 0.9947 to 0.9985 for each phenolic acid. LOD and LOQ were determined with $3 S_b/m$ and $10 S_b/m$ formulas based on the standard deviations of the blank signals (S_b), and the slope of the calibration curve (m). LOD and LOQ were found in the ranges of $1.34\text{--}1.56 \mu\text{g L}^{-1}$ and $4.33\text{--}4.68 \mu\text{g L}^{-1}$, respectively. The precision of the developed method was evaluated as % relative standard deviation (RSD%) and measured with 5 and $25 \mu\text{g L}^{-1}$ phenolic acid mixture in interday and intraday evaluations. RSD% values were found to be 3.02%–4.51% and 4.48%–5.69% for intraday precision and interday precision, respectively. The all results are given in Table 2.

2.5. Sample analysis

The developed MIL-ATPE technique was applied to grape, apple, pear, and banana samples for the investigation of the performance of the method. The sample analysis was carried out with 1-butyl-3-methylimidazolium tetrachloroferrate under optimum conditions. No phenolic acid residues were found in real samples. To demonstrate the accuracy of the proposed method a spiked experiment was conducted with standard mixtures of 5, 50, and $100 \mu\text{g L}^{-1}$ phenolic acid solutions and extraction recoveries were found in the range of 70.78%–99.97% (Table 3). The recoveries showed that the proposed MIL-ATPE technique was successfully applied to

Table 2. The parameters indicated the analytical performance of the developed method (n=3)

Parameters	Gallic acid	Protocatechuic acid	Chlorogenic acid	Caffeic acid	Ferulic acid
Linear range ($\mu\text{g L}^{-1}$)	5–500	5–500	5–500	5–500	5–500
Regression equation (n = 7)	0.9947	0.9956	0.9959	0.9967	0.9985
LOD ($\mu\text{g L}^{-1}$)	1.34	1.47	1.56	1.37	1.43
LOQ ($\mu\text{g L}^{-1}$)	4.47	4.33	4.68	4.55	4.45
Intraday precision (5 $\mu\text{g L}^{-1}$, RSD, %, n = 5)	3.37	3.99	3.20	3.66	3.65
Intraday precision (25 $\mu\text{g L}^{-1}$ RSD, %, n = 5)	3.73	3.02	3.77	4.51	3.51
Interday precision (5 $\mu\text{g L}^{-1}$ RSD, %, n = 5)	4.59	4.48	5.00	5.32	4.95
Interday precision (25 $\mu\text{g L}^{-1}$ RSD, %, n = 5)	5.16	4.63	5.69	4.50	4.96

grape, apple, pear and banana. Typical HPLC chromatograms of a blank and spiked apple and pear samples are shown in Figure 6.

2.6. Conclusions

An aqueous two-phase extraction technique based on magnetic ionic liquid-nonionic surfactant for phenolic acids in grape, apple, pear, and banana samples was developed. After extraction, separation was effectively performed with HPLC. High extraction efficiencies were achieved with magnetic ionic liquids. The extraction recovery obtained with 1-butyl-3-methylimidazolium tetrachloroferrate was higher than the recovery with 1-allyl-3-methylimidazolium tetrachloroferrate and 1-benzyl-3-methylimidazolium tetrachloroferrate. This result showed that the interaction entailed hydrophobic effects and van der Waals interactions between the ionic liquid and phenolic acid. The extraction efficiency was higher in gallic acid. As the chemical structures of the phenolic acids were similar, their extraction efficiencies were also close to each other with magnetic ionic liquid. The highest extraction efficiency was obtained for gallic acid with magnetic ionic liquids. The developed method also had small LOD and LOQ values and a good linear range for phenolic acids. High recoveries were obtained with spiked recovery experiments. Moreover, the proposed method for phenolic acids was a simple, repeatable, inexpensive, and rapid technique. The synthesized magnetic ionic liquids have inadequate magnetism; thus, a cloudy solution was centrifuged for phase separation. If the magnetism of the ionic liquids were sufficient, the extraction time could be shortened, as there would be no centrifugation step. For this reason, studies aiming at overcoming this issue can be planned in the future.

3. Experimental

3.1. Chemical and materials

1-Butyl-3-methylimidazolium chloride, 1-allyl-3-methylimidazolium chloride, and 1-benzyl-3-methylimidazolium chloride were of analytical grade and acquired from Sigma-Aldrich (Switzerland). $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, Triton X-100, Na_2CO_3 , and ethyl acetate of analytical purity and gallic acid, chlorogenic acid, caffeic acid, ferulic acid, protocatechuic acid, acetic acid, and methanol of HPLC purity were supplied by Sigma-Aldrich (Switzerland) or Merck (Germany). Ultrapure water was obtained from a Milli-Q water purification system (USA). The samples were filtered through 0.45- μm filter discs (Millipore Millex-HV, Hydrophilic PVDF) before analysis.

Table 3. The analytical results of real samples (n = 3).

Sample	Spiked ($\mu\text{g L}^{-1}$)	Gallic acid		Protocatechuic acid		Chlorogenic acid		Caffeic acid		Ferulic acid	
		Found ($\mu\text{g L}^{-1}$; mean \pm SD)	Recovery (%; mean \pm SD)	Found ($\mu\text{g L}^{-1}$; mean \pm SD)	Recovery (%; mean \pm SD)	Found ($\mu\text{g L}^{-1}$; mean \pm SD)	Recovery (%; mean \pm SD)	Found ($\mu\text{g L}^{-1}$; mean \pm SD)	Recovery (%; mean \pm SD)	Found ($\mu\text{g L}^{-1}$; mean \pm SD)	Recovery (%; mean \pm SD)
Apple	5	4.50 \pm 0.71	90.01 \pm 0.71	4.22 \pm 0.61	84.46 \pm 0.61	3.65 \pm 1.27	73.05 \pm 1.27	3.76 \pm 0.49	75.24 \pm 0.49	3.99 \pm 0.22	79.81 \pm 0.23
	50	38.84 \pm 0.32	77.68 \pm 0.32	35.95 \pm 0.62	71.91 \pm 0.62	36.66 \pm 1.11	73.35 \pm 1.11	38.90 \pm 1.71	77.80 \pm 1.72	35.39 \pm 0.58	70.78 \pm 0.58
	100	74.80 \pm 0.82	74.80 \pm 0.83	71.69 \pm 0.26	71.69 \pm 0.27	82.07 \pm 1.30	82.07 \pm 1.30	79.39 \pm 1.07	79.39 \pm 1.07	79.26 \pm 0.94	79.26 \pm 0.94
Pear	5	4.55 \pm 0.71	90.94 \pm 0.71	4.31 \pm 1.61	86.23 \pm 1.84	3.75 \pm 0.83	74.98 \pm 0.83	3.77 \pm 0.33	75.37 \pm 0.38	4.06 \pm 0.23	81.25 \pm 0.23
	50	43.01 \pm 0.71	86.01 \pm 0.71	39.28 \pm 1.06	78.59 \pm 1.07	40.91 \pm 1.01	81.81 \pm 1.01	42.57 \pm 1.04	85.15 \pm 1.05	38.96 \pm 0.67	77.93 \pm 0.67
	100	82.12 \pm 0.28	82.12 \pm 0.28	71.16 \pm 0.73	71.16 \pm 0.73	88.06 \pm 0.72	88.06 \pm 0.72	81.54 \pm 0.93	81.54 \pm 0.93	83.18 \pm 0.78	83.18 \pm 0.78
Banana	5	4.70 \pm 0.72	89.38 \pm 0.72	4.22 \pm 1.62	84.46 \pm 1.62	3.79 \pm 1.66	75.82 \pm 1.67	4.08 \pm 0.88	81.73 \pm 0.88	4.05 \pm 0.39	81.12 \pm 0.39
	50	39.35 \pm 1.57	78.70 \pm 1.58	39.29 \pm 1.29	78.59 \pm 1.30	41.55 \pm 1.75	83.11 \pm 1.75	39.44 \pm 0.72	78.89 \pm 0.72	39.34 \pm 1.23	78.69 \pm 1.23
	100	78.81 \pm 0.53	78.81 \pm 0.53	72.20 \pm 1.45	72.20 \pm 1.46	75.08 \pm 1.39	75.08 \pm 1.40	72.88 \pm 0.33	72.88 \pm 0.33	79.03 \pm 1.00	79.03 \pm 1.00
Grape	5	4.39 \pm 0.66	87.81 \pm 0.67	4.84 \pm 1.15	96.97 \pm 1.16	5.02 \pm 1.08	99.97 \pm 0.96	4.29 \pm 0.72	85.96 \pm 0.73	4.25 \pm 0.81	84.92 \pm 0.81
	50	43.43 \pm 0.88	86.87 \pm 0.95	45.14 \pm 0.86	90.28 \pm 0.87	49.86 \pm 1.86	99.73 \pm 1.87	50.05 \pm 1.45	99.90 \pm 0.92	41.88 \pm 0.71	83.77 \pm 0.72
	100	84.39 \pm 0.78	84.39 \pm 0.79	85.32 \pm 0.83	85.32 \pm 0.83	83.81 \pm 0.86	83.81 \pm 0.87	99.95 \pm 0.52	99.95 \pm 1.10	75.73 \pm 0.64	75.73 \pm 0.64

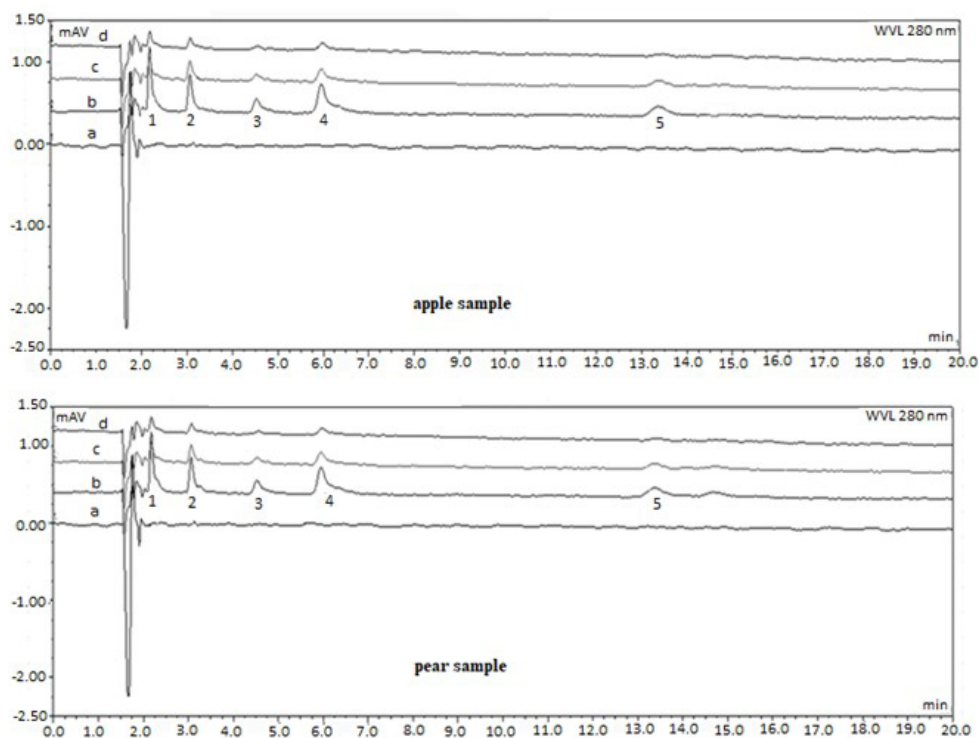


Figure 6. HPLC chromatograms: a) apple and pear samples after MIL-ATPE and b) $100 \mu\text{g L}^{-1}$, c) $50 \mu\text{g L}^{-1}$, and d) $5 \mu\text{g L}^{-1}$ standard phenolic acid spiked samples after MIL-ATPE (1- gallic acid, 2- protocatechuic acid, 3- chlorogenic acid, 4- caffeic acid, 5- ferulic acid).

Standard stock solutions ($100 \mu\text{g mL}^{-1}$) of phenolic acids were prepared in methanol and stored in the refrigerator at $4 \text{ }^\circ\text{C}$. Working solutions were obtained by appropriate dilution of stock phenolic acids with methanol.

3.2. Apparatus

A Thermo Scientific Dionex Ultimate 3000 HPLC system (Germany) equipped with a diode array detector (DAD) was used for the analysis of phenolic acids. The separation of phenolic acids was performed with a Thermo column ($15 \text{ cm} \times 4.6 \text{ mm} \times 3 \mu\text{m i.d.}$) at a column temperature of $50 \text{ }^\circ\text{C}$, and 2% (v/v) acetic acid:methanol (82:18) (v/v) in isocratic elution mode was used as the mobile phase at a flow rate of 1 mL min^{-1} . The wavelength of the DAD was 280 nm [4,36]. FTIR spectra of magnetic ionic liquids were recorded on a PerkinElmer Spectrum BX-II Fourier transform infrared spectrometer (USA). A T80 PG Instrument UV-Vis spectrophotometer (China) was used for absorption spectra. Thermal stabilities of ionic liquids were investigated with a PerkinElmer Diamond thermogravimetric/differential thermal analyzer (USA).

The centrifugation of real samples was carried out with a Nuve NF 200 type centrifuge device (Turkey). A Mettler Toledo model pH-meter (China) was used to optimize the pH of samples. The sonication of extraction mixture was performed with a Bandelin Sonorex model heated ultrasonic bath (Germany)

3.3. Samples

Grape, apple, pear, and banana samples were supplied by a commercial market in İzmir, Turkey. After the samples were homogenized by laboratory blender, the water of the samples was filtered on filter paper. A volume

of 1 mL of filtrate was diluted to 20 mL with ultrapure water and sonicated for 5 min at 0 °C in an ultrasonic bath. After centrifugation for 10 min at 5000 rpm, 15 mL of ethyl acetate was added to 15 mL of supernatant phase and mixed for 20 min. Ethyl acetate was evaporated and the residue was dissolved with methanol [4,37]. Five milliliters of this solution was used for the proposed MIL-ATPE.

Standard 5, 50, and 100 µg L⁻¹ phenolic acid solutions were spiked with 1 mL of sample filtrates for testing the accuracy of the developed method. The total volume was mixed at room temperature for 3 h after dilution to 20 mL by ultrapure water and the above extraction procedure was applied.

3.4. Preparation of MILs

MILs were synthesized by a reaction between 1-butyl-3-methylimidazolium chloride, 1-allyl-3-methylimidazolium chloride, or 1-benzyl-3-methylimidazolium chloride and iron(III) chloride hexahydrate (FeCl₃.6H₂O) [26,38]. For this, each of the starting ionic liquids was heated to 90 °C for 1 h. Then an equal molar amount of FeCl₃.6H₂O was added to the ionic liquids and the mixture was cooled to room temperature and stirred for 3 h. The mixture was taken into the tube and the water phase was decanted. 1-Butyl-3-methylimidazolium tetrachloroferrate, 1-allyl-3-methylimidazolium tetrachloroferrate, and 1-benzyl-3-methylimidazolium tetrachloroferrate were obtained in ~85%–90% yields.

3.5. MIL-ATPE procedure

A test solution of 5 mL containing standard phenolic acids or sample solution, 0.2 g of Triton X-100, 200 µL of MIL, and 0.5 g of Na₂CO₃ was put into a 15-mL centrifuge tube. This mixture was sonicated in an ultrasonic bath for 15 min at ~50 °C. As MILs have inadequate magnetism to be separated from the aqueous phase due to sample volume, the cloudy solution was centrifuged for 15 min at 5000 rpm to accelerate the separation. Then the upper MIL phase was taken and its volume was measured as about 350 µL. This upper phase was diluted with 100 µL of methanol in the HPLC vial and filtered with a 0.45-µm PTFE filter membrane, and 20 µL of the MIL phase was directly injected into HPLC for analysis of phenolic acids.

3.6. Optimization of extraction conditions

Experimental parameters such as volume of MIL, type and amount of surfactant and salt, ultrasound extraction time, temperature, and sample pH were optimized to obtain high extraction efficiency. All experimental parameters and ranges are shown in Table 1. Optimization was also carried out with three MILs. Results were given as the average of 3 parallel experimental results for all parameters. The recovery values were calculated using the following equation [39]:

$$\text{Recovery (\%)} = (100 C_t V_t) / (C_t V_t + C_b V_b)$$

C_t and C_b indicate the concentrations of phenolic acids in the top and bottom phase, respectively. V_t and V_b represent the volumes of the top and bottom phase, respectively.

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References

1. Bi W, Tian M, Row KH. Separation of phenolic acids from natural plant extracts using molecularly imprinted anion-exchange polymer confined ionic liquids. *Journal of Chromatography A* 2012; 1232: 37-42. doi: 10.1016/j.chroma.2011.08.054
2. Roche A, Ross E, Walsh N, O'Donnell K, Williams A et al. Representative literature on the phytonutrients category: phenolic acids. *Critical Reviews in Food Science and Nutrition* 2017; 57 (6): 1089-1096. doi: 10.1080/10408398.2013.865589
3. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry* 2006; 99 (1): 191-203. doi: 10.1016/j.foodchem.2005.07.042
4. Tashakkori P, Erdem P, Seyhan Bozkurt S. Molecularly imprinted polymer based on magnetic ionic liquid for solid-phase extraction of phenolic acids. *Journal of Liquid Chromatography & Related Technologies* 2017; 40 (13): 657-666. doi:10.1080/10826076.2017.1343732
5. Chen B, Xing R, Wang F, Zheng A, Wang L. Inhibitory effects of α - $\text{Na}_8\text{SiW}_{11}\text{CoO}_{40}$ on tyrosinase and its application in controlling browning of fresh-cut apples. *Food Chemistry* 2015; 188: 177-183. doi: 10.1016/j.foodchem.2015.05.003
6. Moreira GC, Dias FD. Mixture design and Doehlert matrix for optimization of the ultrasonic assisted extraction of caffeic acid, rutin, catechin and trans-cinnamic acid in *Physalis angulata* L. and determination by HPLC DAD. *Microchemical Journal* 2018; 141: 247-252. doi: 10.1016/j.microc.2018.04.035
7. Ivanovic M, Razborsk MI, Kolar M. Simultaneous GC-MS determination of free and bound phenolic acids in Slovenian red wines and chemometric characterization. *Acta Chimica Slovenica* 2016; 63 (3): 661-669. doi: 10.17344/acsi.2016.2534
8. Niranjana A, Verma S, Lehri A, Amla DV. High-performance thin-layer chromatographic analysis for the simultaneous quantification of four phenolic compounds in green, red, and black fruits of *Trapa natans* var. *bispinosa* Roxb. (Singhara). *JPC - Journal of Planar Chromatography - Modern TLC* 2013; 26 (4): 316-321. doi: 10.1556/JPC.26.2013.4.4
9. Ghani M, Cabello CP, Saraji M, Estela JM, Cerda V et al. Automated solid-phase extraction of phenolic acids using layered double hydroxide-alumina-polymer disk. *Journal of Separation Science* 2018; 41 (9): 2012-2019. doi: 10.1002/jssc.201701420
10. Xavier L, Freire MS, Vidal-Tato I, Gonzalez-Alvarez J. Aqueous two-phase systems for the extraction of phenolic compounds from eucalyptus (*Eucalyptus globulus*) wood industrial wastes. *Journal of Chemical Technology & Biotechnology* 2014; 89: 1772-1778. doi: 10.1002/jctb.4260
11. Wu H, Yao S, Qian G, Song H. Development of tropine-salt aqueous two-phase systems and removal of hydrophilic ionic liquids from aqueous solution. *Journal of Chromatography A* 2016; 1461: 1-9. doi: 10.1016/j.chroma.2016.06.081
12. Bulgariu, L, Bulgariu D. Selective extraction of Hg (II), Cd (II) and Zn (II) ions from aqueous media by a green chemistry procedure using aqueous two-phase systems. *Separation and Purification Technology* 2013; 118: 209-216. doi: 10.1016/j.seppur.2013.07.007
13. Li P, Zhao PY, Liu WJ, Jiang YF, Wang WJ et al. Determination of common ginsenosides in Kang'ai injection by aqueous two-phase extraction with deep eutectic solvents and HPLC-UV/DAD. *Microchemical Journal* 2018; 137: 302-308. doi: 10.1016/j.microc.2017.11.007
14. Lopes AM, Molino JVD, dos Santos-Ebinuma VC, Pessoa A Jr, Valentini SR et al. Effect of electrolytes as adjuvants in GFP and LPS partitioning on aqueous two-phase systems: 1. Polymer-polymer systems. *Separation and Purification Technology* 2018; 206: 39-49. doi: 10.1016/j.seppur.2018.04.090
15. Junqueira CM, Cabral DS, Penido JA, Mageste AB, Virtuoso LS. How does the use of surfactants in polymer-salt based aqueous two phase systems affect the annatto dye (*Bixa orellana* L.) partitioning? *Fluid Phase Equilibria* 2018; 478: 14-22. doi: 10.1016/j.fluid.2018.08.013

16. Liu LL, Liu Y, Du LP, Zhao YJ, Du H. (Liquid plus liquid) phase equilibrium of aqueous two-phase system containing (surfactant plus sodium sulfate plus water) at different temperatures. *Fluid Phase Equilibria* 2016; 415: 25-33. doi: 10.1016/j.fluid.2016.01.039
17. Gong A, Zhu X. Surfactant/ionic liquid aqueous two-phase system extraction coupled with spectrofluorimetry for the determination of dutasteride in pharmaceutical formulation and biological samples. *Fluid Phase Equilibria* 2014; 374: 70-78. doi: 10.1016/j.fluid.2014.04.022
18. Yao T, Yao S. Magnetic ionic liquid aqueous two-phase system coupled with high performance liquid chromatography: a rapid approach for determination of chloramphenicol in water environment. *Journal of Chromatography A* 2017; 1481: 12-22. doi: 10.1016/j.chroma.2016.12.039
19. Chang YK, Show PL, Lan JCW, Tsai JC, Huang CR. Isolation of C-phycoerythrin from *Spirulina platensis* microalga using ionic liquid based aqueous two phase system. *Bioresource Technology* 2018; 270: 320-327. doi: 10.1016/j.biortech.2018.07.138
20. Jiang Y, Ning Z, Li S. Extraction and purification of isochlorogenic acid C from *Chrysanthemum morifolium* using ionic liquid-based ultrasound-assisted extraction and aqueous two-phase system. *Food Science & Nutrition* 2018; 6 (8): 2113-2122. doi: 10.1002/fsn3.768
21. Chen YH, Wang HY, Pei YC, Wang JJ. A green separation strategy for neodymium (III) from cobalt (II) and nickel (II) using an ionic liquid-based aqueous two-phase system. *Talanta* 2018; 182: 450-455. doi: 10.1016/j.talanta.2018.02.018
22. Zhu S, Wang L, Su A, Zhang H. Dispersive liquid-liquid microextraction of phenolic compounds from vegetable oils using a magnetic ionic liquid. *Journal of Separation Science* 2017; 40 (15): 3130-3137. doi: 10.1002/jssc.201700274
23. Ma WW, Row KH. Solid-phase extraction of chlorophenols in seawater using a magnetic ionic liquid molecularly imprinted polymer with incorporated silicon dioxide as a sorbent. *Journal of Chromatography A* 2018; 1559: 78-85. doi: 10.1016/j.chroma.2018.01.013
24. Nie LR, Song H, Yohannes A, Liang SW, Yao S. Extraction in cholinium-based magnetic ionic liquid aqueous two-phase system for the determination of berberine hydrochloride in *Rhizoma coptidis*. *RSC Advances* 2018; 8: 25201-25209. doi: 10.1039/C8RA01745J
25. Jeon Y, Sung J, Seo C, Lim H, Cheong H et al. Structures of ionic liquids with different anions studied by infrared vibration spectroscopy. *Journal of Physical Chemistry B* 2008; 112 (15): 4735-4740. doi: 10.1021/jp7120752
26. Klee A, Prevost S, Kunz W, Schweins R, Kiefer K et al. Magnetic microemulsions based on magnetic ionic liquids. *Physical Chemistry Chemical Physics* 2012; 14 (44): 15355-15360. doi: 10.1039/C2CP43048G
27. Hayashi S, Hamaguchi H. Discovery of a magnetic ionic liquid [bmim]FeCl₄. *Chemistry Letters* 2004; 33: 1590-1591. doi: 10.1246/cl.2004.1590
28. Vicente FA, Malpiedi LP, Silva FAE, Pessoa A, Coutinho JAP et al. Design of novel aqueous micellar two-phase systems using ionic liquids as co-surfactants for the selective extraction of (bio)molecules. *Separation and Purification Technology* 2014; 135: 259-267. doi: 10.1016/j.seppur.2014.06.045
29. Salabat A, Moghadam ST, Far MR. Liquid-liquid equilibria of aqueous two-phase systems composed of TritonX-100 and sodium citrate or magnesium sulfate salts. *CALPHAD: Computer Coupling of Phase Diagrams and Thermochemistry* 2010; 34: 81-83. doi: 10.1016/j.calphad.2009.12.004
30. Amid M, Manap MY, Hussin M, Mustafa S. A novel aqueous two phase system composed of surfactant and xylitol for the purification of lipase from pumpkin (*Cucurbita moschata*) seeds and recycling of phase components. *Molecules* 2015; 20 (6): 11184-11201. doi: 10.3390/molecules200611184
31. Wei Y, Kai L, Zhongling L, Hangi Z, Xiangqun J. Novelty aqueous two phase extraction system based on ionic liquid for determination of sulfonamides in blood coupled with high-performance liquid chromatography. *Microchemical Journal* 2018; 136: 263-269. doi: 10.1016/j.microc.2017.03.053

32. Beltrán JL, Sanli N, Fonrodona G, Barrón D, Özkan G et al. Spectrophotometric, potentiometric and chromatographic pK_a values of polyphenolic acids in water and acetonitrile-water media. *Analytica Chimica. Acta* 2003; 484 (2): 253-264. doi: 10.1016/S0003-2670(03)00334-9
33. Claudio AFM, Ferreira AM, Freire CSR, Silvestre AJD, Freire MG et al. Optimization of the gallic acid extraction using ionic liquid-based aqueous two-phase systems. *Separation and Purification Technology* 2012; 97: 142-149. doi: 10.1016/j.seppur.2012.02.036
34. Antep HM, Mumcu T, BostancıK, Seyhan Bozkurt S, Merdivan M. Ultrasound-assisted surfactant/ionic liquid aqueous two-phase system extraction prior to high performance liquid chromatography for the determination of tetracyclines in milk and honey samples. *Turkish Journal of Chemistry* 2017; 41: 955-966. doi: 10.3906/kim-1611-51
35. Pei Y, Wang J, Wu K, Xuan X, Lu X. Ionic liquid-based aqueous two-phase extraction of selected proteins. *Separation and Purification Technology* 2009; 64 (3): 288-295. doi: 10.1016/j.seppur.2008.10.010
36. Tarnawski M, Depta K, Grejciun D, Szelepin B. HPLC determination of phenolic acids and antioxidant activity in concentrated peat extract - a natural immunomodulatory. *Journal of Pharmaceutical and Biomedical Analysis* 2006; 41(1): 182-188. doi:10.1016/j.jpba.2005.11.012
37. Wu J, Gao H, Zhao L, Liao X, Chen F et al. Chemical compositional characterization of some apple cultivars. *Food Chemistry* 2007; 103: 88-93. doi: 10.1016/j.foodchem.2006.07.030
38. Hayashi S, Saha S, Hamaguchi H. A new class of magnetic fluids: bmim[FeCl₄] and nbmim[FeCl₄] ionic liquids. *IEEE Transactions on Magnetics* 2006; 42 (1): 12-14. doi: 10.1109/TMAG.2005.854875
39. Qin B, Liu X, Cui H, Ma Y, Wang Z et al. Aqueous two-phase assisted by ultrasound for the extraction of anthocyanins from *Lycium ruthenicum* Murr. *Preparative Biochemistry and Biotechnology* 2017; 47 (9): 881-888. doi: 10.1080/10826068.2017.1350980