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Research Article

Quantitation of antioxidants in water samples using ionic liquid dispersive liquid–liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection

A simple and efficient method, ionic liquid-based dispersive liquid–liquid microextraction combined with high-performance liquid chromatography-ultraviolet detection (HPLC-UV), has been applied for the extraction and determination of some antioxidants (Irganox 1010, Irganox 1076 and Irgafos 168) in water samples. The microextraction efficiency factors were investigated and optimized: 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6] (0.06 g) as extracting solvent, methanol (0.5 mL) as disperser solvent without salt addition. Under the selected conditions, enrichment factors up to 48-fold, limits of detection (LODs) of 5.0–10.0 ng/mL and dynamic linear ranges of 25–1500 ng/mL were obtained. A reasonable repeatability (RSD $\leq$ 11.8%, $n = 5$) with satisfactory linearity ($r^2 \geq 0.9954$) of the results illustrated a good performance of the presented method. The accuracy of the method was tested by the relative recovery experiments on spiked samples, with results ranging from 85 to 118%. Finally, the method was successfully applied for determination of the analytes in several real water samples.

Keywords: Antioxidants / High-performance liquid chromatography-ultraviolet detection / Ionic liquid dispersive liquid–liquid microextraction / Water samples
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1 Introduction

Ionic liquids (ILs) are considered as environmentally benign replacements for traditional organic solvents for their unique chemical and physical properties such as negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions [1–5].

Sample preparation is the first and most tedious step in the whole process of analysis and its main aims are separation and preconcentration of the target analytes from complex matrixes [6–8]. Liquid–liquid extraction (LLE) is probably the most widely used sample preparation method for aqueous samples. ILs have been widely applied in LLE of various compounds such as metal ions, small organic molecules and biological compounds [9–13]. However, it is time consuming and labor intensive. In addition, large amounts of potentially toxic and expensive organic solvents are needed [14, 15].

Relatively new solvent-minimized techniques generally known as liquid-phase microextraction (LPME), as an alternative to LLE, have been developed. Compared to LLE, LPME is faster and less expensive since only a few microliters of organic solvent are required [15, 16]. Jiang and coworkers were among the first who applied ILs as extracting solvents in LPME [17–19].

In 2006, Rezaee *et al.* reported a novel LPME method named dispersive liquid–liquid microextraction (DLLME) [20], which has attracted much attention in the recent years [21–23]. DLLME is based on a ternary component solvent system including disperser solvent, extracting solvent and aqueous samples containing analytes of interest. Rapidity, high enrichment factor, operation simplicity and low cost are some of the advantages of this method. However, the commonly used high-density extracting solvents, such as chlorobenzene, chloroform, carbon tetrachloride, *etc.*, are typically highly toxic. So, ILs can be used as alternative extracting solvents, due to their unique physicochemical properties. Application of ILs in DLLME has been reported in the literature [24–28].

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Abbreviations: **DLLME,** dispersive liquid–liquid microextraction; **IL,** ionic liquid; **IL-DLLME,** ionic liquid-based DLLME; **LLE,** liquid–liquid extraction; **LPME,** liquid-phase microextraction; **PF,** preconcentration factor

Plastic additives such as antioxidants, stabilizers and plasticizers have a major influence in the processing and shelf life of plastics, and are responsible for many properties of these materials [29]. The compounds have relatively low molecular weight and their migration mechanisms into water, food and environment are often of great concern [30]. Therefore, their determination in the above-mentioned media, at trace level, is of great importance.

The aim of this study is to assess the ionic liquid-based DLLME (IL-DLLME) technique suitability for the determination of three antioxidants in water samples. The factors affecting the microextraction efficiency were studied in detail and the optimal conditions were established. The resulting method was validated for quantitative purposes in combination with high-performance liquid chromatography-ultraviolet detection (HPLC-UV).

2 Materials and methods

2.1 Chemicals

All reagents used were of analytical grade. Irganox 1076 (m.p.: 50–55°C; flash point: 273°C; solubility in water $<0.01\%$ w/w) (www.epa.gov/hpv/pubs/summaries/cibaspec/12667b2.pdf) was obtained from Sigma (St. Louis, MO, USA). Irganox 1010 m.p.: 110–125°C; flash point: 297°C; solubility in water $<0.01\%$ w/w) (www.epa.gov/hpv/pubs/summaries/cibaspec/c12667b1rs.pdf) and Irgafos 168 (m.p.: 181–184°C; b.p.: 619.8°C; solubility in water: insoluble) (www.epa.gov/hpv/pubs/summaries/cibaspec/c12667b3rs.pdf) were received as gift from Petrochemical Research and Technology (Tehran, Iran). The structures of the mentioned antioxidants are shown in Fig. 1 (www.epa.gov/hpv/pubs/summaries/cibaspec/12667b2.pdf; www.epa.gov/hpv/pubs/summaries/cibaspec/c12667b1rs.pdf; www.epa.gov/hpv/pubs/summaries/cibaspec/c12667b3rs.pdf). Other chemicals such as 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6], methanol, acetone, ethanol and sodium chloride were obtained from Merck (Darmstadt, Germany). Double distilled water used for preparing the working solutions was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Standard solutions and real samples

Due to the limited solubility of Irgafos 168 in methanol, the polymer additives were initially dissolved in carbon tetrachloride to prepare a stock solution (each 1000 mg/L) [30]. This solution is stable for at least 2 months at room temperature. A standard solution of antioxidants in methanol was prepared daily. This solution was injected to the separation system each day (three times) for the quality control and the obtained peak areas were used in the calculation of preconcentration factors (PFs) and relative recoveries. Working standard solutions were prepared by spiking the above standard solutions in reagent water (400 ng/mL). Tap water sample was collected freshly from our laboratory (Tehran Payamenoor University, Tehran, Iran) and packed mineral waters were purchased from a local shop (Tehran, Iran).

Figure 1. The structure of studied antioxidants.

2.3 Instrumentation

The HPLC system consisted of a Shimadzu (Tokyo, Japan) LC-10 AV pump, a Rheodyne 7725 injector equipped with 20 μL sample loop combined with a SPD-10 AV UV–Vis detector. Chromatographic separation was made on a Knauer C18 (250 mm × 4.6 mm; 5 μm) column under isocratic elution condition. The mobile phase was pure methanol with a flow rate of 1.5 mL/min. UV detection at 210 nm was used for quantification.

2.4 Extraction procedure

A proper amount of double distilled water (5 mL) was placed in a 10 mL screw-capped glass test tube with conical bottom and then it was spiked into at the level of 400 ng/mL of the antioxidants. A solution of 0.06 g [C_6MIM][PF_6] (extracting solvent) in 0.5 mL methanol (disperser solvent) was quickly injected into the above sample solution. Cloudy solution was quickly formed as a result. The analytes in the aqueous sample were extracted into the fine IL droplets at this step. Then the water–methanol–[C_6MIM][PF_6] mixture was centrifuged at 4000 rpm for 5 min. Soon afterwards, the phase containing the IL settled at the bottom of the conical test tube. The upper aqueous phase was removed with a syringe, and the IL phase (about 25 μL) was dissolved in 75 μL methanol to lower the viscosity of the concerning IL phase. Of which, 25 μL was injected into the HPLC system for the final analysis. All experiments were performed three times and the mean value of extraction percent (EX%) was used as the analytical signal.

3 Results and discussion

There are various parameters affecting the DLLME efficiency, including the amounts of extracting solvent, the type and volume of the disperser solvent and the addition of salt. These parameters were investigated and the optimal conditions were then established. ''One at a time'' strategy was employed to optimize the influential factors in this

method. To screen the mentioned parameters, PF and EX% were calculated (as indices of extraction efficiency) using the following expressions:

$$PF = C_{sed}/C_o \quad (1)$$

where C_{sed} and C_o are the concentration of analyte in sedimented phase and initial concentration of analyte in aqueous sample solution, respectively. These parameters were known, except for C_{sed}. The C_{sed} calculation was conducted by the direct injection of the antioxidant standard solutions at the concentrations in the range of 0.5–5.0 mg/L.

$$EX\% = (C_{sed} \times V_{sed}/C_o \times V_{aq}) \times 100 = PF \times (V_{sed} \times V_{aq}) \times 100 \quad (2)$$

where EX%, V_{sed} and V_{aq} are the extraction percent, the volume of the settled phase and the volume of the aqueous sample, respectively. It is worth noting that in the following optimization process the concentration of the analytes in the aqueous sample was kept constant at the level of 400 ng/mL.

3.1 Selection of extracting solvent

Suitable IL for the extraction in water samples should meet some requirements such as low solubility in water, good extraction capability for the target analytes and higher density than water and also it should exist in the form of liquid during the experiment. For these reasons, we focused on the inexpensive imidazolium-ILs containing $[PF_6]^-$ and side hydrophobic alkyl chain [31]. In this study, 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) was selected.

3.2 Selection of disperser solvent

The miscibility of the disperser solvent in the IL phase (extracting solvent) and the aqueous phase (sample solution) is the main point for its selection [32]. Acetone, methanol and ethanol illustrating the above ability were initially chosen for this purpose. A series of sample solutions were studied using 0.5 mL of each disperser solvent containing 0.06 g $[C_6MIM][PF_6]$. Based on the results shown in Fig. 2, methanol was selected as the optimal disperser solvent for further studies, due to giving higher responses for the target analytes.

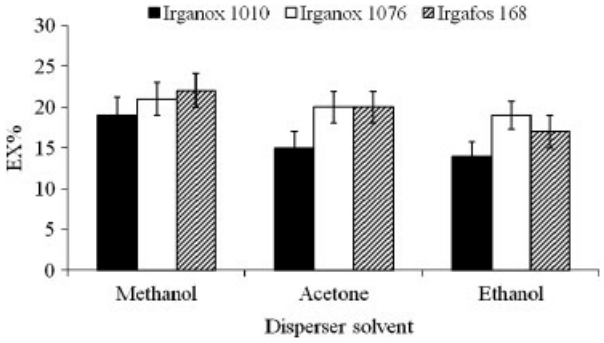

Figure 2. Effect of type of disperser solvent on the extraction efficiency. Extraction conditions: aqueous sample volume, 5.0 mL at 400 ng/mL of the targets; disperser solvent volume, 0.5 mL; extracting solvent ($[C_6MIM][PF_6]$) 0.06 g.

3.3 Effect of the extracting solvent amounts

In order to evaluate the effect of the amounts of extracting solvent on the extraction efficiency, additional experiments were performed using 0.5 mL methanol containing different $[C_6MIM][PF_6]$ amounts (0.04, 0.06, 0.08 and 0.1 g). It is clear that by increasing the amounts of extracting solvent, the volume of the settled phase increases (nearly up to 32 μL). As shown in Fig. 3, by increasing the amount of $[C_6MIM][PF_6]$ to 0.06 g, EX% for the three antioxidants reached a relatively constant level. Therefore, the exact amount (0.06 g) of the IL was applied for the rest of studies.

3.4 Effect of the disperser solvent volume

The variation of methanol volume (as disperser solvent) causes changes in the volume of the settled phase. To avoid

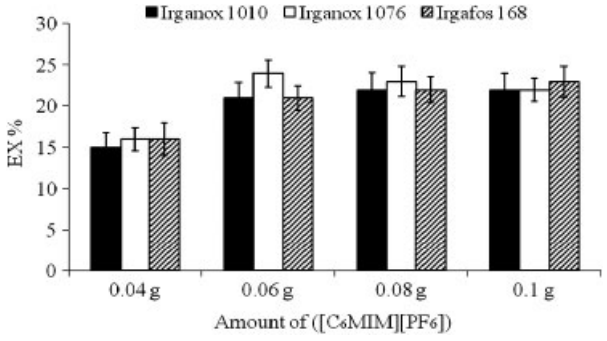

Figure 3. Effect of the amounts of extracting solvent ($[C_6MIM][PF_6]$) on the extraction efficiency. Extraction conditions: aqueous sample volume, 5.0 mL at 400 ng/mL of the targets; disperser solvent (methanol) volume, 0.5 mL.

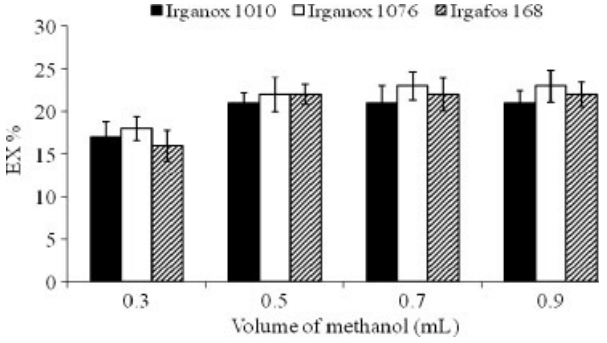

Figure 4. Effect of the volume of disperser solvent (methanol) on the extraction efficiency. Extraction conditions: aqueous sample volume, 5.0 mL at 400 ng/mL of the targets; extracting solvent ($[C_6MIM][PF_6]$) 0.06 g.

this problem and also achieving a constant volume of the settled phase, the volume of methanol and also the amounts of the IL were changed simultaneously. The experimental conditions were fixed and included the use of different methanol volumes (0.3, 0.5, 0.7 and 0.9 mL), each of which contained 0.054, 0.060, 0.068 and 0.072 g $[C_6MIM][PF_6]$, respectively. At this step, the volume of settled phase was relatively constant (~25 μL). It seems that at low volumes of methanol the cloudy state is not fully formed, and thus EX% is low, whereas at higher volumes of methanol the solubility of the analytes in aqueous samples increases slightly. Taking all the mentioned points into consideration, a constant volume of methanol (0.5 mL) has been selected as the optimal value (Fig. 4).

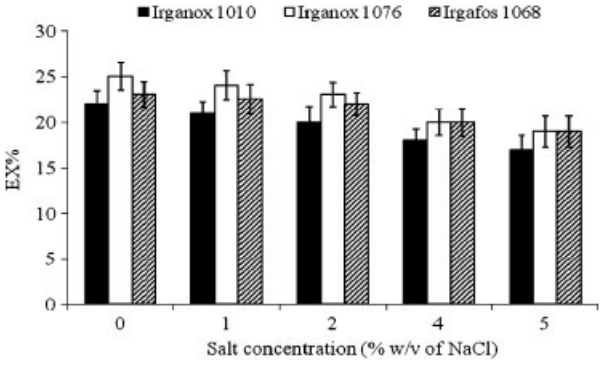

Figure 5. Effect of the addition of NaCl on the extraction efficiency. Extraction conditions are as in Fig. 2.

3.5 Effect of salt addition

The addition of salt to the aqueous sample may have several effects on the extraction efficiency. To investigate that influence, various experiments were performed by adding different amounts of NaCl (0–5% w/v) into aqueous sample solutions. However, the rest of experimental conditions were kept constant. The plot of EX% *versus* the salt concentration is shown in Fig. 5. As NaCl concentration increases, the volume of the settled phase decreases from 25 to 16 μL. The result could be explained that the salt addition enhanced the solubility of the IL in the aqueous sample leading to a decrease in EX%. Thus, no addition of salt was employed for the further experiments.

3.6 Effect of extraction time

According to the literature [20, 32], time has no influence on the extraction efficiency. It has been revealed that the surface area between extracting solvent and aqueous phase (sample solution) is infinitely large. Thereby, the transfer of analytes from the aqueous to the extracting phase is fast. Subsequently, the equilibrium state is achieved quickly. As a result, the extraction time is very short. This is the most important advantage of DLLME over the other microextraction techniques. In this method, time-consuming step is centrifuging of the sample solution in extraction procedure, which is about 5.0 min.

Table 1. Quantitative results of IL-DLLME-HPLC-UV of the antioxidants

Analyte	Linear range (ng/mL)	Regression equation	r^2	LOD (ng/mL)	PF
Irganox 1010	25–1500	$Y^{a)} = 1395.5\ X^{b)} + 35\,415$	0.997	10	43
Irganox 1076	25–1500	$Y = 705.4\ X + 16\,799$	0.9975	10	48
Irgafos 168	25–1500	$Y = 1754.1\ X + 156\,483$	0.9954	5	46

a) Peak area.
b) Concentration, ng/mL.

Table 2. Results obtained for analysis of the antioxidants in three different spiked real water samples

Sample		Irganox 1010	Irganox 1076	Irgafos 168
Tap water (100 ng/mL added)	Found (ng/mL)	89	111	94
	Relative recovery (%)	89	111	94
	RSD% ($n = 5$)	10.7	9.6	9
Polur mineral water (50 ng/mL added)	Found (ng/mL)	42	58	59
	Relative recovery (%)	85	117	118
	RSD% ($n = 5$)	10	9.1	7.5
Vata mineral water (50 ng/mL added)	Found (ng/mL)	46	47	52
	Relative recovery (%)	91	94	105
	RSD% ($n = 5$)	7.6	6.6	11.8

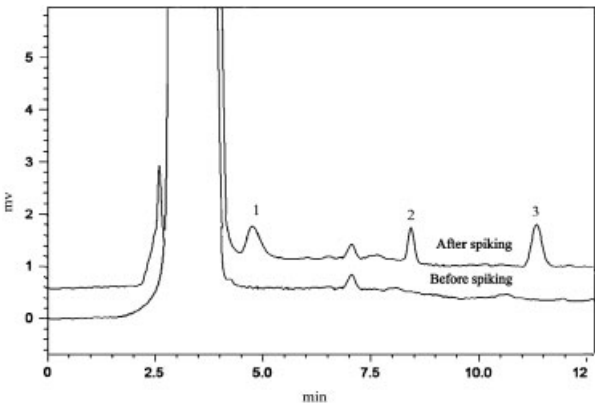

Figure 6. The IL-DLLME-HPLC-UV chromatograms of the antioxidants at the concentration level of 50 ng/mL in the mineral water sample before and after spiking. Extraction conditions are as in Fig. 2. The peak number corresponds to (1) Irganox 1010; (2) Irganox 1076; (3) Irgafos 168.

3.7 Evaluation of the method performance

Under the optimum experimental conditions, the proposed method was applied to a series of standard solutions containing various concentrations of the analytes, in order to develop the respective calibration curves. For each level, three similar extractions were performed. The limits of detection (LODs), defined as the analytical signal which is larger than the blank by multiple three of the variation in the blank, correlation coefficients (r^2), linear ranges and PFs were calculated and the results are summarized in Table 1. As summarized in this table, LODs for the tested antioxidants were less than 10 ng/mL. Linearity values varied in the range of 25–1500 ng/mL with correlation coefficients in the range of 0.9954–0.9975. Furthermore, the PFs varied from 43 to 48.

3.8 Analysis of real samples

Applicability of the extraction method was investigated in three different spiked water samples. The initial results proved that the samples were free of the analytes contamination. Then, all the real water samples were spiked into with the antioxidant standards at different concentration levels to assess the matrix effects. The results of relative standard deviations (RSDs) based on five similar determinations were within the range of 6.6–11.8%, as summarized in Table 2. The data also demonstrated a good relative recovery in the range of 85–118%, indicating that the real water matrices had almost little effect on the extraction efficiency. Figure 6 shows the IL-DLLME-HPLC-UV chromatograms of the antioxidants at the concentration level of 50 ng/mL in the mineral water sample before and after spiking.

4 Concluding remarks

This study shows that IL-DLLME technique in combination with HPLC-UV is a valid means of preconcentration and quantification of the antioxidants at trace level in water samples. The method seems to be precise, reproducible, time independent and linear over a wide concentration range. The nonvolatile IL reduces the exposure danger in comparison with the conventional organic solvent. Moreover, the entire analytical process presents a simple, economical and rapid way for screening purposes.

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The authors have declared no conflict of interest.

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