Talanta 118 (2014) 129–136

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Eco-friendly ionic liquid based ultrasonic assisted selective extraction coupled with a simple liquid chromatography for the reliable determination of acrylamide in food samples

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ARTICLE INFO

Article history:
Received 23 June 2013
Received in revised form
7 October 2013
Accepted 9 October 2013
Available online 16 October 2013

Keywords:
Acrylamide
Food
Ionic liquid
Liquid chromatography
Ultrasonic extraction

ABSTRACT

Acrylamide in food has drawn worldwide attention since 2002 due to its neurotoxic and carcinogenic effects. These influences brought out the dual polar and non-polar characters of acrylamide as they enabled it to dissolve in aqueous blood medium or penetrate the non-polar plasma membrane. In the current work, a simple HPLC/UV system was used to reveal that the penetration of acrylamide in non-polar phase was stronger than its dissolution in polar phase. The presence of phosphate salts in the polar phase reduced the acrylamide interaction with the non-polar phase. Furthermore, an eco-friendly and costless coupling of the HPLC/UV with ionic liquid based ultrasonic assisted extraction (ILUAE) was developed to determine the acrylamide content in food samples. ILUAE was proposed for the efficient extraction of acrylamide from bread and potato chips samples. The extracts were obtained by soaking of potato chips and bread samples in 1.5 mol L^{-1} 1-butyl-3-methylimmidazolium bromide (BMIMBr) for 30.0 and 60.0 min, respectively and subsequent chromatographic separation within 12.0 min using Luna C18 column and 100% water mobile phase with 0.5 mL min^{-1} under 25 °C column temperature at 250 nm. The extraction and analysis of acrylamide could be achieved within 2 h. The mean extraction efficiency of acrylamide showed adequate repeatability with relative standard deviation (RSD) of 4.5%. The limit of detection and limit of quantitation were 25.0 and 80.0 ng mL^{-1}, respectively. The accuracy of the proposed method was tested by recovery in seven food samples giving values ranged between 90.6% and 109.8%. Therefore, the methodology was successfully validated by official guidelines, indicating its reliability to be applied to analysis of real samples, proven to be useful for its intended purpose. Moreover, it served as a simple, eco-friendly and costless alternative method over hitherto reported ones.

1. Introduction

At the beginning of the "Green Era", clean or eco-friendly methods were adopted by removing contaminants. Organic solvents that are usually used in both protocols of pre and within the chemical analysis are the main sources of contamination to the environment. Recently, directions toward eco-friendly solvent alternatives such as ionic liquids (ILs) were developed by substitution of hazardous organic solvents [1,2]. Furthermore, the numerous applications of ILs in analytical chemistry were significantly increasing due to their features including low vapor pressures and rarely flammable or explosive solvents [2]. Imidazolium based ILs with various anions were considered good selective solvents for the extraction of most organic compounds [3]. This could be due to the possibility of interaction between ILs and analytes by electrostatic, hydrophobic or π–π forces. Moreover, ILs were used in the effective extraction of various substances from plant samples such as alkaloids [4], lignans [5], and polyphenolic compounds [6].

In recent years, ultrasound-assisted extraction (UAE) has attracted growing interest as it is an effective eco-friendly method for the rapid and reproducible extraction of a number of compounds from food samples [7]. In particular, numerous analytical applications of UAE coupled with IL (ILUAE) were successfully established for the effective extraction of natural compounds and pollutants from food samples [8,9]. Therefore, in the current work we encouraged to use ILUAE procedure for the selective extraction of acrylamide from food samples.

Acrylamide in food has drawn worldwide attention since April 2002 [11]. It was produced when carbohydrate-rich foods are exposed to high temperatures [12]. It was estimated that the intake of acrylamide from food is 0.4 μg kg^{-1} body weight

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http://dx.doi.org/10.1016/j.talanta.2013.10.015

per day [13,14]. Acrylamide was classified by the International Agency for Research on Cancer [10] as "probably carcinogenic to humans" (Group 2A). Based on the chemical structure of acrylamide (prop-2-enamide), it exhibits both polar and non-polar characters. These characters were considered the basis of acrylamide toxicity as they enabled it to dissolve in polar aqueous blood medium or penetrate the non-polar plasma membrane. Hence, an HPLC system of polar and non-polar phases could use to investigate the distribution of acrylamide between the two phases. Furthermore, HPLC could effectively use to determine acrylamide in food which should be kept as low as possible [15,16].

Many analytical methods were developed in the past years to determine acrylamide in foods using HPLC based on UV [17] or mass spectrometer [18]. Recent reviews on the numerous methods for acrylamide detection and extraction were published [15,19]. However there was a substantial amount of previous works on the determination of acrylamide, but still there are several drawbacks regarding the complexity of cleanup steps during sample preparation, time-consuming procedures and required HPLC fractionation. As well, most of published methods used a large amount of toxic solvents or costly equipment. In order to overcome the aforementioned problems, a costless and eco-friendly analytical method should be investigated.

The objective of this work was to use a new eco-friendly and costless coupling of ionic liquid based ultrasonic assisted extraction (ILUAE) with an HPLC/UV system for the selective extraction and determination of acrylamide in food samples. Simple HPLC method of high polar single mobile phase composition and C18 non-polar stationary phase was used to find out the acrylamide interactions as a way to optimize extraction and separation. By optimizing the operating conditions, the proposed greener method was successfully validated following the requirements of selected official guidelines and applied to bread and potato chips samples.

2. Experimental

2.1. Materials

Acrylamide ($\geq$ 99% purity) was purchased from Sigma (Steinheim, Germany). Analytical reagents of sodium nitrate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Sigma (Steinheim, Germany). HPLC grade of methanol, acetonitrile and hexane were also purchased from Sigma (Steinheim, Germany). IL of 1-butyl-3-methylimidazolium bromide (BMIMBr) was synthesized as described elsewhere [20]. Purity of IL was checked with NMR and FTIR analysis [21]. FTIR spectra were recorded using BIORAD FTS-40A spectrometer (Kleve, Germany) with KBr as reference. Nuclear magnetic resonance (NMR) spectra were measured using JEOL Lambda 400 NMR spectrometer (California, USA). Water used in the preparations of solutions and mobile phases was purified by a Milli-Rx apparatus (Millipore, Milford, MA, USA). Luna C18 column (250 mm $\times$ 4.6 mm $\times$ 5 μm), Luna NH_2 column (250 mm $\times$ 4.6 mm $\times$ 5 μm) and A C18 security guard precolumn were purchased from Phenomenex (California, USA). Single randomly selected bread samples (white bread, brown bread, fino and baladi) were brought from local bakers in Jeddah, Saudi Arabia. Potato chip samples (Crispy, Lays and Doritus) were also obtained from the local store in Jeddah. Collected samples were stored at room temperature (25 °C).

2.2. Apparatus

HPLC separations were carried out with a PerkinElmer series 200, a Rheodyne injection valve (model 7725(i)) and a series 200 UV/Vis variable wavelength Detector (PerkinElmer Instruments Inc., Massachusetts, Canada). A series 200 vacuum degasser was used before pumping the mobile phase. Data were collected by TotalChrom™ Chromatography Data Handling System. The PerkinElmer 600 series link interface was used with software to acquire and buffer digital data from the instrument and to control the operating parameter of such instrument. The temperature was maintained constant by a column oven (Model 200, PerkinElmer, California, USA). The mobile phase of 100% water was pumped under constant column temperature of 25.0 °C with effluent detection at 250 nm. The stationary phase was a Luna C18 column (250 mm $\times$ 4.6 mm $\times$ 5 μm). A C18 security guard precolumn (Phenomenex) was incorporated into the system and a graphite filter was fitted between the injector and precolumn. 20 μL of the analyte solutions was usually injected. During the measurements, each sample was injected in triplicate at 0.5 mL min^{-1} flow rate.

The pH values were adjusted using pH-meter (Jenway 3510, Cambridge, UK) at 20 $\pm$ 2.0 °C. This instrument was calibrated by using standard universal buffer solutions at different pHs.

The MiniTab software package (USA statistical software) was employed to perform the statistical analysis of data.

2.3. Preparation of solutions and samples

A stock solution of acrylamide was prepared in 1.5 mol L^{-1} BMIMBr. In order to prepare BMIMBr solution, a certain mass of IL was accurately weighed in 25.0 mL water. The stock solution was stored in air dried glass bottles under the room conditions. The working solutions were daily prepared by the dilution with BMIMBr. One should be careful when dealing with acrylamide because it is toxic and readily absorbed through the skin [10]. Therefore, gloves and safety glasses should be worn at all times. Standard and sample preparations should be carried out in a fume cupboard.

Acrylamide was extracted from bread or potato chips samples by ionic liquid based ultrasonic assisted extraction (ILUAE). In this procedure, food samples were homogenized and blended into fine powder using a Braun handheld mixer (type 4169) fitted with a blender-like sample compartment (type 4297, BraunAG, Frankfurt am Main, Germany). An aliquot of approximately 4.0 g of the homogenate was transferred to a centrifuge tube and 10.0 mL of 1.5 mol L^{-1} BMIMBr was added. Potato chip and bread homogenates were soaked for 30.0 and 60.0 min, respectively. After that time, 4.0 mL of ionic liquid was again added to achieve solid:liquid ratio of 4:20 (g:mL). The resulting solid and IL mixture were sonicated for 20.0 min. The bath power was fixed at 125.0 and 250.0 W for potato chips and bread samples, respectively. Subsequently, the filtrate was extracted by centrifuge at 30,000.0 rpm for 20.0 min and an aliquot of 1.0 mL was transferred to air dried Eppendorf vials and stored at room temperature. Every extraction was conducted in parallel three times to assess repeatability. After each extraction, the corresponding extract was filtrated through a 0.45 μm filter for subsequent HPLC analysis.

2.4. Method validation procedure

The validation was performed following the requirements of official guides of US FDA Guidance for Industry and EU Regulation 2002/657/EC Decision to give more reliability of the proposed method [22].

2.4.1. Selectivity, accuracy, precision, robustness and stability

Selectivity of the proposed method was demonstrated by the use of pure reference material. The peak identity was confirmed by the spectra recorded using the variable wavelength detector.

The peak purity was estimated from the calculated peak symmetry of analyte using the TotalChrom™ software.

Accuracy of the proposed method was investigated by spiking a blank sample matrix of interest with three known concentrations of analyte (reference material). After that, recovery was obtained by calculating the concentration of analyte in the sample matrix and comparing the result to the true concentration of the pure authentic standard.

Precision tests were performed to determine both intra-day and inter-day variations in peak area. Intra-day precision test was carried out by repeating 10 runs within the same day and inter-day precision test was carried out by repeating the measurements within three consecutive days (eight runs per day).

The robustness test was conducted by varying the most effective parameters (flow rate, column temperature, IL concentration etc.) within narrow ranges around the optimized values.

The HPLC system stability was checked by injecting the concentration corresponding of the limit of quantitation (LOQ) of analyte during 48 h (short term) or 4 months (long term) and comparing the collected results with data collected from freshly prepared standard solution.

2.4.2. Linearity and method limits

The calibration graph was obtained by plotting the peak area against concentrations of acrylamide. Limit of detection (LOD) was calculated by the equation [22]: LOD = 3.3 σ/S, where σ is the standard deviation of response of blank injection and S is the slope of the calibration plot. Limit of quantification (LOQ) was calculated from LOQ = 10 σ/S [22].

3. Results and discussion

3.1. Method development

3.1.1. Reversed phase liquid chromatography (RPLC)

HPLC was chosen for the determination of acrylamide in food since it is well-known by its simplicity, efficiency and robustness in food analysis [17]. Acrylamide is a polar compound due to the presence of amide group and at the same time it has a nonpolar character due to the presence of propene chain. Acrylamide is not expected to ionize under neutral conditions based on the high predicted pK_a value due to its difficult ionization. Therefore, RPLC was chosen for the determination of acrylamide using Luna C18 stationary phase–which offers an extensive variety of selectivity, easily available and simple regeneration–in the presence of an aqueous single mobile phase composition.

The effect of relative strength of a mobile phase on both the retention factor and sensitivity of acrylamide detection was described by using a solvent polarity index (P). One advantage of using the polarity index to describe the mobile phase strength is that P changes linearly as two different solvents are mixed together. This makes this index useful in adjusting the mobile phase composition. To calculate the solvent polarity index for a mixture of two solvents, the following formula is used [23]:

$$P_{tot} = \phi_A P_A + \phi_B P_B$$

where P_{tot} is the solvent polarity index for a mixture of solvents A and B that have individual polarity indeces of P_A and P_B, respectively. P_A and P_B are constant values for each solvent [24]. ϕ_A and ϕ_B are the volume fractions of solvents A and B in the new mobile phase. A mixture of 500.0 ng mL^{-1} of acrylamide and 1000.0 ng mL^{-1} of sodium nitrate (column void time marker) was used to study the effect of mobile phase composition. Fig. 1 shows the effect of polarity index of mobile phase on the retention factor and sensitivity to acrylamide in the presence of t_0 marker. Mobile phases of 100% (v/v) water and other compositions by mixing water with methanol or acetonitrile were studied. It was found that the composition of 50% acetonitrile (solvent A) and 50% water (solvent B) with the solvent polarity index [24] of 7.92 gave the highest peak height of acrylamide (40.0 mAU) with a retention factor of 0.82. On the other hand, 100% water gave 10.2 solvent polarity index. The highest retention factor of 1.08 with a peak height of 35.0 mAU acrylamide was achieved. Therefore, there is no large difference in the response by 100% water compared with other compositions as well as a mobile phase of only water is the best eco-friendly solvent. Hence, 100% water was used as the optimal mobile phase composition for further studies.

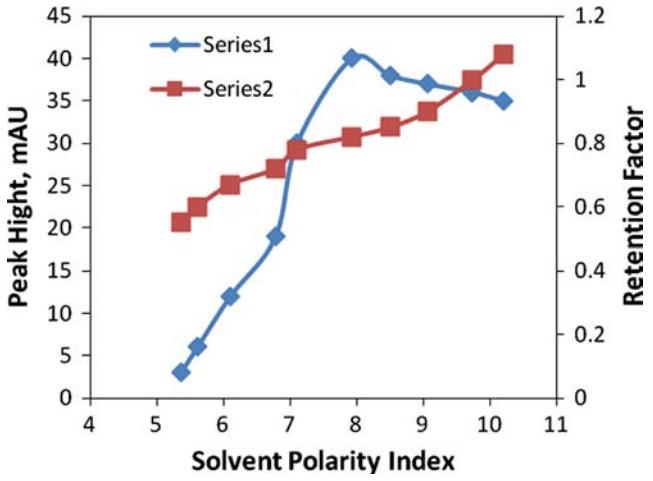

Fig. 1. Effect of solvent polarity index of a mobile phase on the peak height (series 1) and retention factor (series 2) of 500.0 (ng mL^{-1}) of acrylamide.

In order to increase the sensitivity to acrylamide, other instrumental parameters were optimized. The effect of absorption wavelength was investigated within the range of 200.0–290.0 nm. It was found that the peak height was increased 3 times at 250.0 nm in comparison with others. The effect of flow rate from 0.1 to 2.0 mL min^{-1} and column temperature from 20.0 °C to 50.0 °C was also studied. It was found that the best sensitivity of acrylamide was achieved by 0.5 mL min^{-1} flow rate under 25.0 °C of column temperature. Fig. 2 shows a representative chromatogram of acrylamide under the optimized chromatographic conditions.

3.1.1.1. Acrylamide interactions in polar and non-polar phases. From the above studies, it was observed that nonionized polar acrylamide was retained at about 10.0 min under neutral experimental conditions as indicated in Fig. 2. The suggested mechanism for the separation recognition of acrylamide could be investigated as the following.

Acrylamide under the RPLC conditions is separated based on its partitioning between water mobile phase and a nonpolar silica-based octadecyl stationary phase (Luna C18). Silica as a support material has lots of merits. One of these merits is the use of silica as a solid phase support material which permits the presence of unreacted silanol groups and hence allows the silanolphilic interactions (such as hydrogen bonding).

Acrylamide undergoes reactions typical of chemicals containing a reactive double bond and an amide group. Therefore, acrylamide exhibits both non-polar and polar characters. This dual character is considered the basis of acrylamide toxicity. As shown in Fig. 2, the distribution of acrylamide to the non-polar phase is stronger than the polar phase; this could be due to the formation

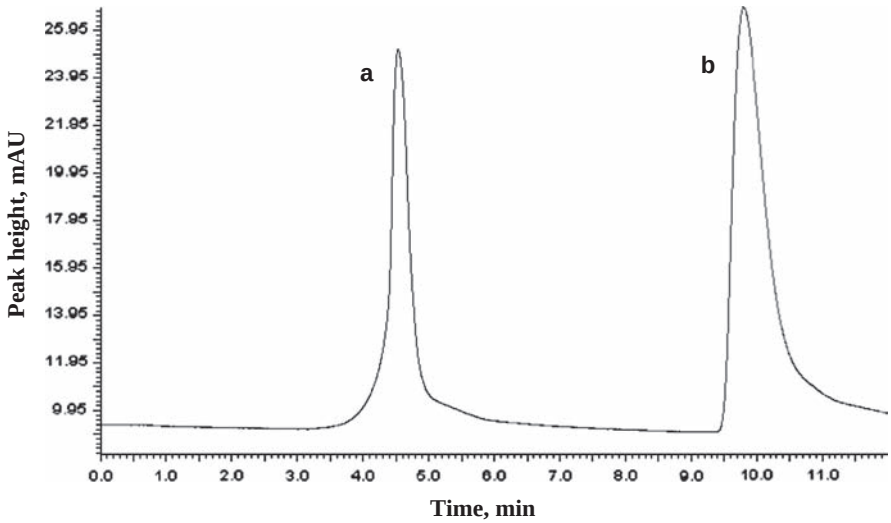

Fig. 2. A representative RPLC chromatogram of 200.0 (ng mL^{-1}) acrylamide (b) in the presence of 1300.0 (ng mL^{-1}) sodium nitrate (a) under the optimized chromatographic conditions as cited in the text.

of non-polar interaction between acrylamide and stationary phase as well as the possibility of hydrogen bond formation between $–NH_2$ group of acrylamide and –OH group of the support. We tried to use phosphate salts in the mobile phase under physiological conditions (pH 7.4) with different concentrations ranged from 10.0 to 70.0 mmol L^{-1}. It was found that the retention time of acrylamide gradually decreased with increasing salt concentration. This could be due to the formation of hydrogen bonds between $–NH_2$ polar group of acrylamide with $–PO_4$ group which reduced the retention of acrylamide by stationary phase. Therefore, the presence of salts in the polar mobile phase could enhance acrylamide flowing and could reduce its retention by non-polar stationary phase based on the salt concentration.

The possibility of these interactions was confirmed by using Luna NH_2 column instead of Luna C18 in the presence of 100% (v/v) hexane as a mobile phase. It was found that acrylamide was closely eluted with a dead time marker confirming the weak retention of acrylamide with polar $–NH_2$ stationary phase [15]. Consequently, the non-polar character of acrylamide permits preferential penetration to the non-polar phase which could be the essential reason for the toxicity of acrylamide.

3.1.2. Ionic liquid based ultrasonic assisted extraction (ILUAE)

3.1.2.1. Type and concentration of ionic liquid. ILs are usually composed of an organic cation in combination with a single or complex anion. The structure of ionic liquids has significant effects on its physicochemical properties, which might greatly affect extraction efficiency of target analyte. The effect of changing the anion and the alkyl chain length of the cation of 1-alkyl-3-methylimidazolium-type IL on the extraction efficiency was studied elsewhere [9]. It was found that 1-butyl-3-methylimmidazolium bromide (BMIMBr) gave the best results compared with others. This may be due to the ability of BMIMBr to aid expansion of cellulose in food samples facilitating the dissolution of target analyte. Therefore, BMIMBr was selected for the subsequent evaluation.

In the current work, the effect of BMIMBr concentration on the ultrasonic assisted extraction of acrylamide from food samples was studied. The extraction of acrylamide was performed on the BMIMBr-water solution of different concentrations ranged from 0.1 to 4.0 mol L^{-1}. The extraction efficiency was expressed as the observed HPLC value of acrylamide and the maximum amount was taken to be 100%. Fig. 3 A shows the effect of BMIMBr concentration on the extraction efficiency of acrylamide from seven food samples. The weight of each sample is 4.0 g. In the light of the obtained results, extraction efficiency of acrylamide significantly increased by increasing of BMIMBr concentrations over the range of 0.1–1.5 mol L^{-1}. Nevertheless the higher concentrations of 1.5–4.0 mol L^{-1} IL produced lower extraction efficiency of acrylamide. This could be due to that the high viscosity of the solvent at high IL concentrations may lead to poor infiltration of the solvent into the food tissues, resulting in decreased extraction efficiency of acrylamide [9]. Finally, 1.5 mol L^{-1} BMIMBr was selected for further studies.

3.1.2.2. Solid–liquid ratio. The solid–liquid ratio is an important parameter to increase the extraction efficiency of acrylamide. The high concentrations of solid and liquid could cause procedure complex and unnecessary waste, while lower ones would make the target extraction incomplete and lower the extraction efficiency. A series of extractions were performed with different solid–liquid ratios (4:5, 4:10, 4:15, 4:20 and 4:40 g mL^{-1}). Fig. 3B shows that the extraction efficiency rapidly increased with the increase of the BMIMBr volume before the ratio of 4:15 g mL^{-1} and then a little increase of the extraction efficiency was observed when the ratio between 4:15 and 4:20 g mL^{-1}. Further increase of the solvent amount reduced the extraction efficiency. Therefore, a solid–liquid ratio of 4:20 g mL^{-1} was used for further optimization studies.

3.1.2.3. Optimization of ultrasonic assisted extraction parameters. The effect of the parameters that might extensively affect the extraction step such as soaking time, ultrasonic power and ultrasonic time was studied. It was found that the extraction efficiency of acrylamide from bread samples was gradually increasing up to 60.0 min soaking time while the results had no obvious change when continued to prolong soaking time. In the case of potato chips samples, the optimal extraction efficiency was at 30.0 min soaking time. The increase in extraction efficiency of acrylamide after soaking with BMIMBr is probably because of the increased diffusion of solvent into the matrix and the possibility of formation of hydrogen bond between IL anion and $–NH_2$ group of acrylamide. The required soaking time for the solubilization of acrylamide from bread is longer than potato chips

due to the difference in the matrices where BMIMBr needed more time to penetrate into tightly bread bound tissues.

Further, it was observed that the extraction efficiency of acrylamide was increased dramatically when the ultrasonic power increased in the range from 100.0 to 150.0 W and 200.0 to 300.0 W for potato chips and bread samples, respectively. The complete extraction was obtained at optimal powers of 130.0 and 250.0 W for potato chips and bread samples, respectively.

To some extent, ultrasonic time played another leading function in this study. It was illustrated that the extraction efficiency of acrylamide was increased with ultrasonic time from 5.0 to 20.0 min. When the ultrasonic time reached 40.0 min, the extraction efficiency was decreased. So, 20.0 min was set for further experiments of the studied samples.

Under the above mentioned optimal parameters of ultrasonic assisted extraction (UAE), it was obvious that the extraction efficiency of acrylamide was increased three times for potato chips and two times for bread samples compared with in the absence of ultrasonic. This is because the extraction of acrylamide from the cellular structure required solvent access to the cellular compartments where acrylamide is located. An intact cell structure restricted the accessibility of the solvent to acrylamide; the ultrasound treated cells had more open structures which facilitated the efficient extraction of acrylamide.

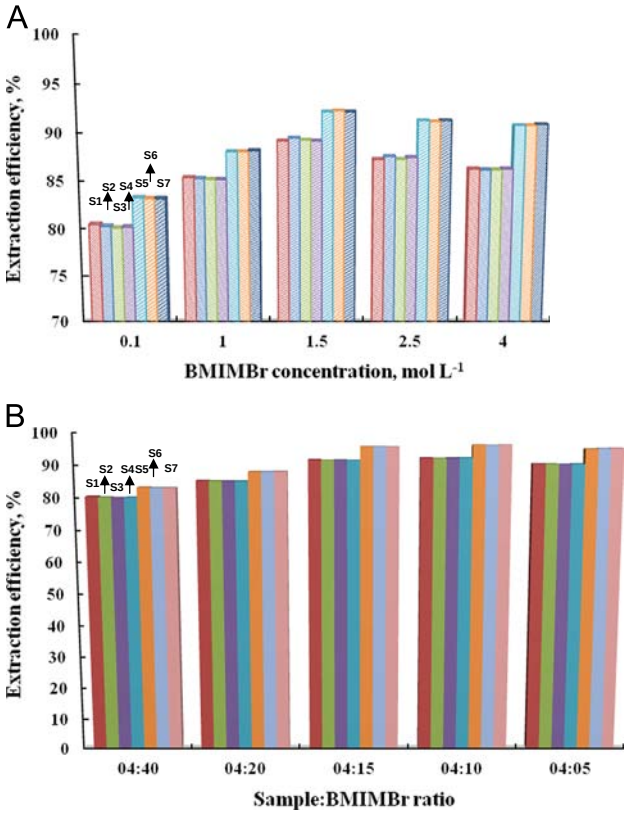

Fig. 3. A: Effect of BMIMBr concentration on the extraction efficiency of acrylamide from fino (S1), white bread (S2), brown bread (S3), Balady bread (S4), Crispy (S5), Lays (S6) and Doritus (S7) samples under experimental conditions as indicated in the text. The extraction efficiency is expressed as the observed values of acrylamide and the maximum amount in each curve was taken to be 100%. B: Effect of solid–liquid (Sample:BMIMBr) ratio on the extraction efficiency of acrylamide from fino (S1), white bread (S2), brown bread (S3), Balady bread (S4), Crispy (S5), Lays (S6) and Doritus (S7) samples under experimental conditions as indicated in the text.

3.1.2.4. Reference solvent extraction test. The reference solvent extraction tests were performed by the use of pure water and sodium chloride solution [9]. Water is the most common and inexpensive solvent, and is often selected as a co-solvent in various extraction processes. We replaced the IL by water in the optimized extraction procedure. It was observed that the extraction efficiency of acrylamide was 65.7% which is significantly lower than that by 1.5 mol L^{-1} BMIMBr (98.5%). On the other side, the extraction efficiency by 1.5 mol L^{-1} NaCl solution was 63.2%. The solvent effect of the IL was therefore more important in achieving higher extraction efficiency of acrylamide than the salt effect derived from NaCl. Hence, salt influences did not play a major role in improving the extraction efficiency of acrylamide. Therefore, ILUAE was used as the predominant extraction procedure of acrylamide in the present study. The using of BMIMBr achieved 1.5 times in the extraction efficiency of acrylamide compared with the using of water or NaCl.

3.2. Method validation

3.2.1. Selectivity, accuracy and precision

Selectivity was evaluated by comparing the retention time and spectrum of acrylamide with that obtained by analyzing real extracts of bread and potato chips samples. In addition, the peak identity of acrylamide was confirmed by spiking different concentrations of acrylamide standard solutions in the studied matrices giving linearity equations similar to the calibration equation in aqueous medium. The obtained results prove the selectivity of the proposed method beside the adequate peak purity.

The method accuracy in aqueous and real matrices was evaluated by recovery studies (each measurement replicated

Table 1
Replicate analysis and accuracy of acrylamide in bread by ILUAE/HPLC/UV method.

Food sample	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	Intra-day precision, RSD (%, $n=10$)	Inter-day precision, RSD (%, $n=24$)	Recovery (%)
Fino bread	100.0	109.8	0.8	4.2	109.8
	400.0	405.0	0.6	2.8	101.3
	800.0	801.0	0.4	2.4	100.1
White bread	100.0	108.6	0.7	3.4	108.6
	400.0	404.0	0.8	2.9	101.0
	800.0	802.0	0.3	2.3	100.3
Brown bread	100.0	90.6	0.7	3.5	90.6
	400.0	398.0	0.6	3.1	99.5
	800.0	799.0	0.2	2.9	99.9
Balady bread	100.0	91.3	0.7	3.1	91.3
	400.0	398.5	0.6	2.8	99.6
	800.0	799.0	0.1	2.3	99.9

Table 2
Replicate analysis and accuracy of acrylamide in potato chip samples by ILUAE/HPLC/UV method.

Food sample	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	Intra-day precision, RSD (%, $n=10$)	Inter-day precision, RSD (%, $n=24$)	Recovery (%)
Crispy	100.0	107.0	0.7	3.5	107.0
	400.0	403.0	0.3	2.8	100.8
	800.0	800.0	0.2	2.5	100.0
Lays	100.0	90.7	0.7	3.4	90.7
	400.0	400.0	0.6	2.9	100.0
	800.0	800.0	0.4	2.3	100.0
Doritus	100.0	106.0	0.6	3.5	106.0
	400.0	401.0	0.5	3.1	100.3
	800.0	800.0	0.2	2.8	100.0

10 times) under the optimal conditions at three different concentration levels of acrylamide covering their working concentration range. The obtained recovery varied between 91.2% and 107.0% in aqueous matrix. The accuracy of the ILUAE/HPLC/UV system was also verified in food samples. This study was performed by spiking 100.0, 400.0 and 800.0 ng mL^{-1} of acrylamide into the studied samples as described in Tables 1 and 2. The fortified food samples followed the optimal extraction procedure as described in the experimental section. The spiked amount of acrylamide was calculated by subtracting the measured values of unfortified samples from fortified samples. The obtained recoveries ranged between 90.6% and 109.8%. Therefore, the obtained results indicated that the proposed extraction and analysis procedures are accurate for the determination of acrylamide in food samples.

The method precision was tested by replicating measurements of acrylamide at concentrations ranged between 80.0 and 800.0 ng mL^{-1} in food samples within the same day (intra-day or repeatability) and three consecutive days (inter-day or reproducibility) under the optimal conditions as described in Tables 1 and 2. Relative standard deviations (RSDs) were ranged between 0.1–0.8% and 2.3–4.2% for repeatability and reproducibility, respectively. Results indicated that the null hypothesis that RSD belong to the same population is accepted; there is not a significant difference in precision at 99.0% confidence level. Therefore, our proposed HPLC method is considered precise for the determination of acrylamide in real samples.

3.2.2. Linearity and method limits

The calibration curve exhibited a good linear behavior (Peak area = 0.004 (concentration, ng mL^{-1}) + 0.094) within the range of 80.0 to 1800.0 ng mL^{-1} with a correlation coefficient (r) of 0.997. The limit of detection (LOD) and limit of quantification (LOQ) were 25.0 and 80.0 ng mL^{-1}, respectively. The obtained limits are enough to determine acrylamide in food samples.

3.2.3. Stability, robustness and completeness

The system stability was checked by injecting the LOQ concentration of acrylamide. The recoveries were ranged between 95.4% and 103.6% indicating that our system is stable for short and long-analysis time up to 3 months. The recovery of acrylamide was assumed to be indicative of the stability of the acrylamide under the extraction conditions used. The average complete recovery under the operating extraction conditions was 98.5% with no change in the retention time of acrylamide. Within the last month, the sensitivity of acrylamide was reduced to 10% of the actual value. These results suggested that acrylamide was stable in the IL solution within a reasonable time.

Table 3
Robustness study of 500.0 ng mL^{-1} of acrylamide by ILUAE/HPLC/UV method.

Parameter	Values	Peak area	Recovery (%)
Flow rate (mL min^{-1})	0.60	2.11	100.95
	0.50[a]	2.09	–
	0.40	2.07	99.04
Column temperature (°C)	27.00	2.09	100.00
	25.00[a]	2.09	–
	23.00	2.08	99.52
BMIMBr concentration (mol L^{-1})	1.70	2.13	101.91
	1.50[a]	2.09	–
	1.30	2.08	99.52
Solid:liquid ratio (g:mL)	4.5:20.5	2.20	105.26
	4.0:20.0[a]	2.09	–
	3.5:19.5	2.00	95.69
Ultrasound time (min)	22.00	2.09	100.00
	20.00[a]	2.09	–
	18.00	2.11	100.95

[a] The optimal parameter.

The robustness test was conducted by small variations of the most effective parameters around the optimized values, as indicated in Table 3. The BMIMBr concentrations were varied in the extraction procedure. Good values of RSD (0.13–1.6%) were obtained, proving that our proposed ILUAE/HPLC method is quite robust for application in real matrices.

The completeness of our proposed method was checked by applying in the following equation: $\%C = 100 \times V/n$, where $\%C$ = percent completeness, V = number of measurement judged valid and n = total number of measurements necessary to achieve at 99.0% confidence level. It was found that ILUAE/HPLC method achieved average completeness of 100.9% for acrylamide in the extracts. These methods validation studies indicate that the proposed method is credible.

3.3. Application

The proposed ILUAE coupled with HPLC/UV method was successfully applied to the determination of acrylamide in four bread samples and three potato chips samples collected in the period of October 2012 from the local stores. Fig. 4 depicts representative chromatograms for the determination of acrylamide in fino bread (A) and Doritus potato chips (B) under the optimal experimental conditions. It was found that the concentrations of acrylamide were 0.15, 0.87, 0.33, 0.36, 66.5, 70.4 and 95.0 mg Kg^{-1} in fino bread, white bread, brown bread, Balady bread, Crispy chips, Lays chips and Doritus chips, respectively. It was observed that in Fig. 4B, there is a small unknown peak which is completely separated from acrylamide peak with a resolution more than 2.5. This peak did not affect on the reliability of acrylamide determination in potato chip sample. Furthermore, there is only 0.1 min shift in the retention time of acrylamide between bread sample (Fig. 4A) and potato chip sample (Fig. 4B) due to the difference in the response based on acrylamide concentration. This difference is negligible. Besides, we confirmed the selectivity of our results following the official guidelines [22] as described above which proved that our proposed method did not need to further cleanup steps for food sample preparations. In order to validate the procedure, recovery studies have been carried out by means of comparison of the results obtained from the analysis of food samples with the results obtained from acrylamide reference solution (authentic sample) prepared in a similar way (single calibration point method). The results were not significantly different from the value obtained by the standard

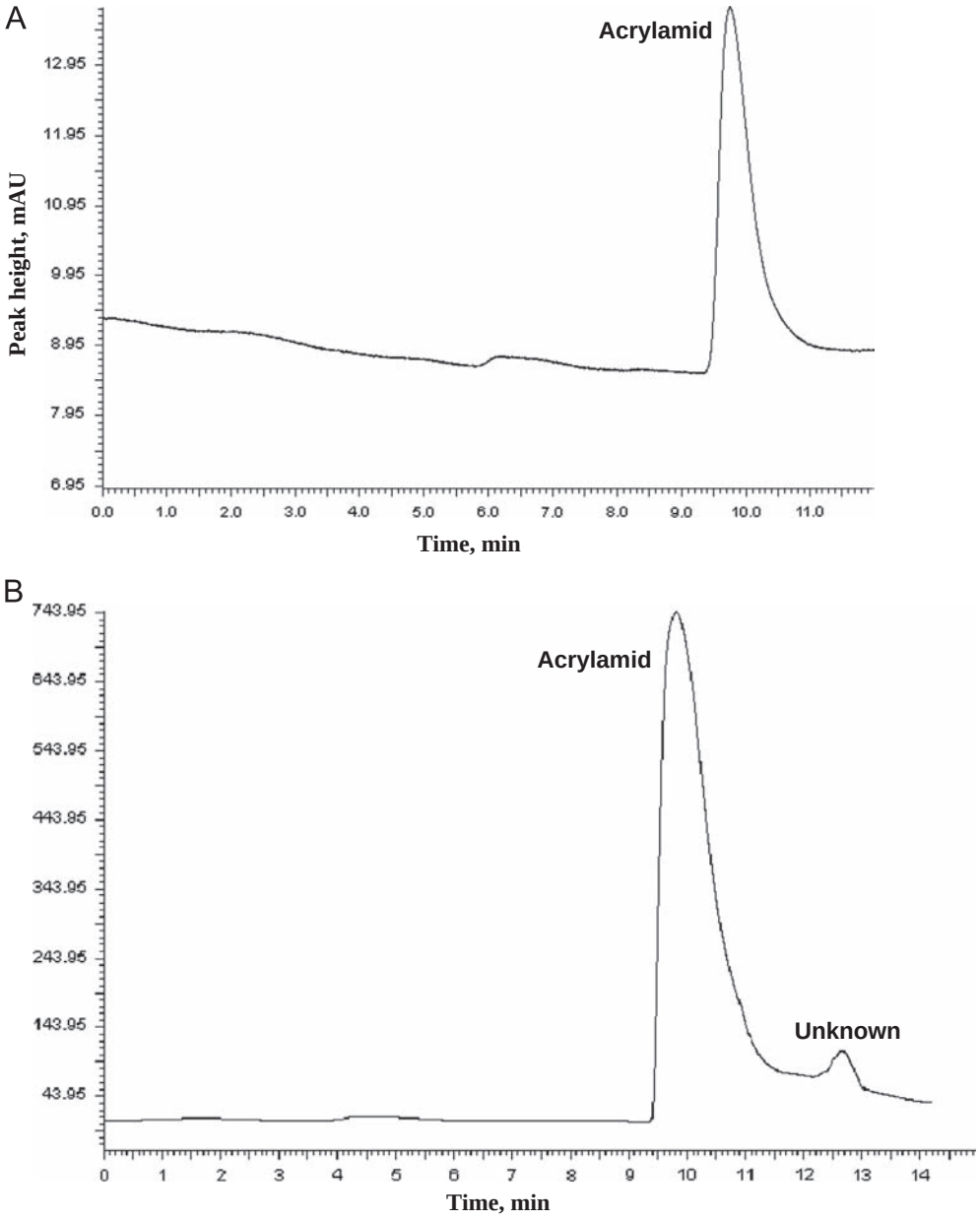

Fig. 4. HPL chromatograms for the determination of acrylamide in fino bread (A) and Doritus potato chips (B) under the optimal experimental conditions.

addition method (*F*-test and *t*-test at 95% confidence level). The obtained recoveries lay in the acceptable range of 94.0% to 108.0%.

4. Conclusion

In this study, a simple HPLC/UV system of 100% (v/v) water mobile phase and C18 non-polar stationary phase was used to find out the interaction behavior of acrylamide between the two phases. It was observed that non-polar character of acrylamide is the main part of its retention compared to its polar character. The presence of phosphate salts in polar phase reduced acrylamide penetration to the non-polar phase based on the salt concentration. In addition, a new eco-friendly and costless BMIMBr assisted ultrasonic based extraction coupled with HPLC/UV was developed for the efficient and selective extraction of acrylamide from food samples. The 1.5 times in the extraction efficiency of acrylamide was achieved by BMIMBr ionic liquid higher than the extraction efficiency by water or NaCl solution. As well, the extraction and analysis of food sample could be achieved within 2 h which also reduced the consumption of the required energy. The ILUAE/HPLC/UV system was successfully demonstrated for the determination of acrylamide in four bread samples and three potato chips. The method was correctly validated according to the requirements of official guidelines, obtaining adequate results for linearity, sensitivity, accuracy, precision and stability. Simple, costless and eco-friendly analytical method was successfully established for the routine analysis of acrylamide in food industry that is using a conventional HPLC instrument instead of procurement of high cost of other techniques.

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