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.