Pharmaceutical Sciences
December 2019, 25(4), 338-344
doi: 10.15171/PS.2019.42
https://ps.tbzmed.ac.ir/

Research Article

High-Performance Liquid Chromatography Determination of Acrylamide after Its Extraction from Potato Chips

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Article Info

Article History:
Received: 10 March 2019
Revised: 14 May 2019
Accepted: 23 May 2019
ePublished: 20 December 2019

Keywords:
-Acrylamide
-Liquid–Liquid Extraction
-Potato Chips
-Ultrasound

ABSTRACT

Background: Acrylamide is a known carcinogenic product that has been found among the substances such as potato chips which to be processed under the heat-treatment. In order to extract amounts of acrylamide from fried chips in market, an ultrasound-assisted liquid–liquid extraction (UA-LLE) technique is proposed. The UA-LLE coupled LLE and ultrasonication in a single step.

Methods: Chips samples were dissolved in an extracting organic solvent using ultrasonication to prompt transferring of acrylamide into the organic phase. As a result, the extraction time and process efficiency were significantly enhanced through increasing the collision power and mass transfer between grounded chips and organic phase.

Results: Important parameters affecting the extraction efficiency such as kind of organic solvent and its volume, re-dissolving solvent and pH were optimized. This newly proposed method has been applied to determine the trace acrylamide in potato chips samples purchased from local market.

Conclusion: UA-LLE is a handy, economic and time-saving method, with high extraction yield (over 103% average recovery) and good precision (lower than 15% relative standard deviation, RSD). Most importantly, it seems this method to be an ideal pre-treatment method for the extraction of acrylamide in food matrix in food quality control laboratories.

Introduction

Acrylamide (2-propenamide) is an odorless, crystalline solid compound with low molecular weight and high solubility in water.[1] It is formed in food as a result of cooking practices and specially in foods with high-carbohydrate content, such as potato crisp, roast potatoes, breakfast cereal, bakery products and roasted coffee.[2,3]

Maillard reaction, is the main mechanism of acrylamide formation in foods containing carbohydrates and amino acids, in particular asparagine,[4] so, it is one of the thermal process contaminants, that has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC, 1994),[4] and is known as neurotoxin announced by WHO (World Health Organisation, 2002)[2]. Acrylamide is found in various tissues of the body such as liver, kidney, brain, heart and even breast milk.[5] Determination of high concentrations of acrylamide in routine heated starch-based food products by the Swedish National Food Administration in April 2002 attracted public alarms, since acrylamide was carcinogenic for human health.[6] Potato foodstuffs, such as French fries and chips, were among the food candidates containing highest amounts of acrylamide. The estimated health risk of acrylamide in food products has been established by a number of organizations and national authorities. The expert stuffs are working in international levels have found a number of information to allow a better evaluation of health risk associated with acrylamide.[7]

European Commission started monitoring of acrylamide levels in processed foods in 2007 and eventually maximum recommended values in potato chips was around 1000 µg/kg.[8] WHO has demonstrated a safe limit of 500 ng/mL acrylamide in drinking water where higher concentrations of 100–1000 ng/g are indicated in some foods such as potato chips or French fries. Because of the significant effects that acrylamide has on human health, development of analytical methods is necessary to determine the accurate amounts of this analyte in foodstuffs.[9]

Various analytical methods such as gas chromatography (GC) or liquid chromatograph (LC) coupled with mass spectrometry (MS) have usually been selected as the first choices methods in the acrylamide determination.[3] In analysis by GC, derivatization (usually bromination) should be used to increase sensitivity and selectivity.[10] In some investigations, other chromatographic methods like HPLC-UV have been developed in the recent years.[3]

Since foodstuff matrix are complex and analytes are in combination with other compounds, different techniques have been utilized for the cleanup and extraction of acrylamide from food samples. Extraction methods are

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often used with soxhlet,[11] a liquid- liquid extraction (LLE)[12] technique, and a solid phase extraction (SPE)[13] technique. In these methods, organic solvents like hexane are used in large volumes.[14] The purpose of this study was to reduce the extraction process time and the use of solvents that are less hazardous to health and the environment.

Ultrasonic assistance (US) assisted extraction is gradually emerging as a common methodology in analytical chemistry, which utilizes this energy for speeding up the extraction rate.[15] Whether US prompts mass transfer between two immiscible phases it is reasonable if one regards the ability of this mode of power to facilitate emulsification. Because of this rational, analysts have been interested in test of US as a tool for improving extraction process.

The present work intended to use US-LLE in order to extract acrylamide from potato chips and quantified it by HPLC-UV. The developed method was assessed in terms of linearity, precision and accuracy. Finally, the applicability of method was tried on chips samples purchased from the local market.

Materials and Methods

Chemicals and solutions

Acrylamide (>99%) was obtained from Sigma (Deisenhofen, Germany). Methanol, acetic acid, acetonitrile and acetone were of analytical grade and potassium hexacyanoferrate (Carrez I) and zinc sulfate (Carrez II) were obtained from Merck (Darmstadt, Germany). Distilled, deionized and 0.20 µm filtered water was used throughout the experiments.

Stock solution of acrylamide (1 mg/mL) was prepared by dissolving in distilled water. Working standards for calibration curve were prepared by diluting the stock solution of acrylamide to concentrations of 0.5, 1.0, 3.0, 5.0 and 8.0 µg/mL with distilled water. Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 mL of water, and Carrez II solution by dissolving 30 g of zinc sulfate in 100 mL of water.

Instruments

The quantification of acrylamide was done by an Agilent 1100 model HPLC system (Waldbronn, Germany) consisting of a quaternary pump with vacuum degasser. The chromatographic separations were performed on C_{18} column (15 × 4.6 mm × 5µm) from the Agilent Company. The confirmatory analysis of blank sample was performed on the same apparatus coupled with diode array detector (DAD). During the routine analyses of food samples, the flow rate of mobile phase (acidic water) was adjusted to 1.0 mL/min. Acrylamide absorbance was recorded at 222 nm with monitoring the peak spectra within the range of 190–400 nm using spectrophotometer.

Sample Preparation

Finely smashed chips were weighted (1 g) and put into 15 mL centrifuge tube. Samples were mixed well in 10 mL acetone by the aid of US power. The samples were spiked with acrylamide at different levels in order to calculate the percentage relative recovery. The resultant suspension was centrifuged in 10 ℃ at 10000 rpm. It is important to discard the solid residues from the supernatant well and if needed the sample was centrifuged again. This will purify the acetone phase from the water-soluble co-extractive components. The upper phase was transferred into the test tube and treated with Carrez I and II solutions (100 µL each) to isolate the co-extractives. Then sample was centrifuged in 10 ℃ at 10000 rpm for 10 min and the supernatant was transferred into conical flask and was evaporated until drying in evaporator. The presence of trace water coming from the Carrez solutions will prevent the loss of acrylamide during evaporation process by retaining it on the wall of the reaction flask. The residue was dissolved in 4 mL of acidic water (pH 3) by mixing with a vortex for 2 min. The acrylamide residue was dissolved into water while lipids and lipid soluble co-extractives compounds were remained on the wall of glass flask. The solution was filtered through a 0.45 mm syringe filter. Twenty µL of the final solution was injected into LC column for quantification.

Results and Discussion

In the present work, in order to have maximum recovery in US-LLE process various parameters including kind and volume of extraction solvent, time of sonication, volume of re-dissolving agent and pH were optimized. Optimization experiments were applied on blank chips sample which was spiked by 1.0 µg/mL acrylamide and parameter were optimized using one-parameter-at-a-time method.[16-19] Optimization efficiency was evaluated by calculating the recovery.

Optimization of HPLC parameters

Since acrylamide possesses carboxylic acid functional group, therefore it shows a maximum absorption in the range of 195–230 nm. However, most of the co-existing materials coming from the chips matrix also absorb well in 195-200 nm adversely affect on the detection selectivity. In comparison with the absorbance at 200 nm, the absorbance value of acrylamide at 222 nm was lower than at 200 nm but it was more selective. However, because of the complex matrix of chips, detection at 222 nm comes with better precision and accuracy.

The mobile phase pH was tested and in order to increase ionization yield of acrylamide, acidic pH (pH 3) was selected as the optimum.

The best results in term of capacity factor and theoretical plate number (N) calculation ascribed to pure water as mobile phase. Using water as mobile phase, k' value exceeding 2.0 without any peak broadening and resulted in $N > 17\,000$.

Optimization of extraction process

In order to extract the acrylamide from spiked chips sample it is necessary to treat the chips sample with an appropriate solvent. Before going through this step, two

methods were evaluated for speeding up the rate of extraction; sonication and vortexing. Sonication using US system speeded up the rate of extraction more in comparison with vortex-assisted extraction. Therefore US-LLE set-up was designed for extraction of acrylamide from chips samples. Before starting the sonication process, it is required to ensure that during sonication each tube is exposed to same intensity, thus each tube was practiced in separate run.

In some investigations, defatted chips sample with hexane was applied to improve extractability.[20] But there is a difficulty in centrifugation of such solvent because of high content of starch and fat present in chips matrix and very bulk and swallowed media was obtained.

The solvents and solvent mixtures used in extraction process were water, acetone, methanol, acetonitrile, mixture of water and corresponding solvents i.e., acetone, acetonitrile and methanol. Acrylamide is very soluble in water and the sample treatment usually started with extracting the samples with water.[21]

Acetone did not extract the starch and fat and the obtained supernatant was much clearer than when using methanol and water. Our study showed acrylamide was highly soluble in acetone (63.1 g/100 mL)[22] and it was much more compatible with the chip's matrix than other solvents. Using the solvent mixture for extraction decreased the level of acrylamide in the final extract, and thus increased the detection limit (Figure 1a). Regarding these facts, extraction of acrylamide from chips matrix was performed with acetone. The colloids such as acetone soluble proteins were sediment by Carrez reagents. Since Carrez I and II solutions were prepared in water therefore adding these solutions into acetone extract dissolved the water-soluble compounds as well. So, it is better to discard solid residuals carefully by centrifuge before adding the Carrez solutions. Carrez solutions not only help to purify the extract from colloids but also help to prevent the acrylamide evaporation through the evaporation process. Biedermann *et al.*[23] have also seen the similar difficulty in losing the acrylamide through the evaporation process and recommended the addition of edible oils to samples to stop the loss of acrylamide.

To optimize US factors, firstly we investigated the impact of sample volume on sonication efficiency. Volume of acetone used in extraction process was tested in the range of 5, 10 and 20 mL in order to have the highest recovery of acrylamide. By increasing the extraction solvent volume up to 10 mL, recovery increased as well but by further exceeding in extraction solvent volume, the recovery of acrylamide decreased. This observation may be attributed to dilution effect. We found that sonication of samples as 10 mL aliquots in standard 15 mL polypropylene Eppendorf tubes resulted in better recovery compared to aliquots of higher volumes (Figure 1b).

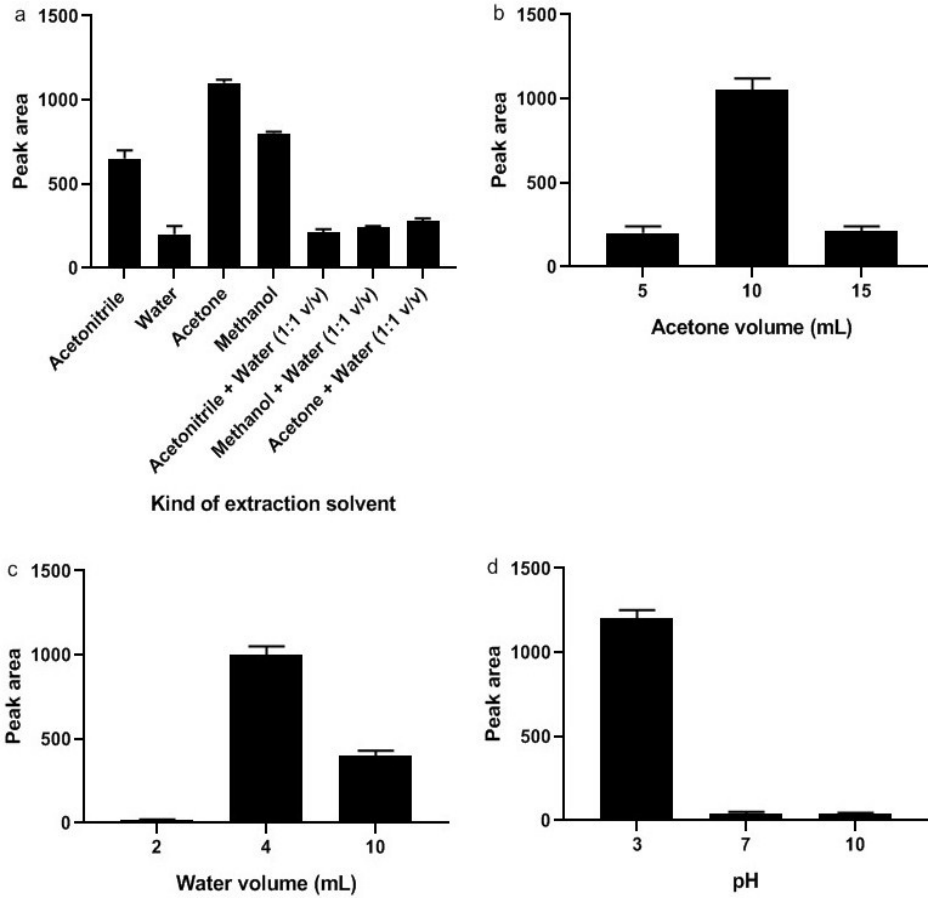

Figure 1. Optimization process of US-LLE for acrylamide extraction in potato chips.

We also assessed whether increasing the sonication time enhanced sonication efficiency. This parameter was determined empirically by different duration of sonication (5, 10 and 15 min) in the presence of a fixed concentration of acrylamide. Almost no difference was observed after 10 min and only 10 min of sonication was enough. Using US helped, acrylamide eventually reached a solid-liquid equilibrium very fast.

After solvent being evaporated by a rotary evaporator, the residual was dissolved in water (acrylamide is very soluble in water (215.5 g/100 mL)).[22] Also, we studied the effect of the water volume as shown in Figure 1c (i.e., 2, 4 and 10 mL) and 4 mL of water is enough for reconstituting the acrylamide.

Water at neutral pH is the most common pH for acrylamide extraction before chromatographic process.[24] However, it was reported that the level of acrylamide into aqueous solution increases by increasing the pH of extraction. The authors proposed that under such condition the structure of the matrix can be changed.[25] The extraction of the food samples in alcohol/water mixture in the acidic medium also were reported and proved to be adequate for the determination of acrylamide.[26]

Hence, in present work there is a need that the effect of pH was studied to verify which extraction pH reveals the maximum content of acrylamide.

Water pH effect on extraction efficiency was assessed in three occasions i.e., 3, 7 and 10.0 and results were shown in Figure 1d that at pH 3 the obtained recovery was maximum. A clear difference in recovery amount is observed for the chips samples extracted at acidic pH. It seems the results support the hypothesis that acrylamide released well at pH 3 from water-soluble components of the matrix.[25]

Method validation

To avoid matrix effects, we prefer to construct matrix-matched calibration curve. The need for matrix-matched calibration, indicates to using blank matrix in order to adequately quantify the target analyte. However, virtually every heat-treating foodstuff contains some levels of acrylamide, we used DAD to ensure that blank sample is free of acrylamide. Calibration curve was plotted by constructing peak area versus concentration of acrylamide and details are shown in Table 1. Limit of detection (LOD) and limit of quantification (LOQ), which was estimated considering the concentration value to generate a signal 3 and 10 times the noise signal, respectively. The acrylamide content in chips samples was determined by US-LLE and HPLC-UV and calculated by the calibration curve equation. In addition, precision and accuracy of the method were tried on three levels of spiked acrylamide in blank chips; intra- and inter-day as shown in Table 2.

The pre-concentration factor is around 2.1 and defined as the ratio of the calibration graph slope obtained after performing the proposed method to that of the calibration graph obtained by direct injection of the acryamide solution prepared in methanol.

Method application on real samples and efficiency of sample preparation procedure

The efficiency of developed method is practiced in real samples. Potato chips samples with different brands were purchased from the local market (Tabriz, Iran). Due to the variation between the chip's matrices being analyzed, the standard addition method was applied to ensure that matrix effect has been bypassed. Each sample was analyzed without and with spiking 1 µg/mL standard acrylamide to calculate the recovery. The extraction recoveries in different matrices were found from 88% to 118% using US-LLE and HPLC-UV. None of the sample showed the acrylamide content in the range defined for calibration curve.

Acrylamide is a polar compound which conventional LC reversed phase method was selected for its determination. As amount of acrylamide in chips samples is at low levels, so determination of acrylamide is difficult due to the lack of selectivity. Figure 2 shows that acrylamide peak was very well resolved from unknown matrix compounds and proposed US-LLE enable to clean-up the complex matrix satisfactorily.

Method comparison study

An overview of the reported methodologies for the determination of acrylamide in food samples was shown in Table 3.

Table 1. Some analytical characteristics for ultrasound assisted liquid phase extraction of acrylamide determined by HPLC-UV.

Concentration range (µg/mL) [a]	Linearity (R^2)	LOD[b] (µg/mL)	LOQ[c] (µg/mL)	RSD[d] (%)
0.5-8.0	0.99	0.12	0.31	3.0

[a]Linearity is described by the correlation coefficient for the calibration curve, [b]Limit of detection (LOD) S/N=3, [c]Limit of quantification (LOQ) S/N=10, [d] Average relative standard deviation for acrylamide levels covering the calibration curve range whereas each sample repeated three times.

Table 2. Precision and accuracy of the method.

Acrylamide concentration (µg/mL)	Intra-day (n=3)		Inter-day (n=3)	
	Precision (RSD*)	Accuracy	Precision (RSD)	Accuracy
0.50	1.01	-0.82	14.26	-0.00
0.80	4.00	-6.47	10.70	-0.15
1.00	7.30	-7.25	11.33	-0.10

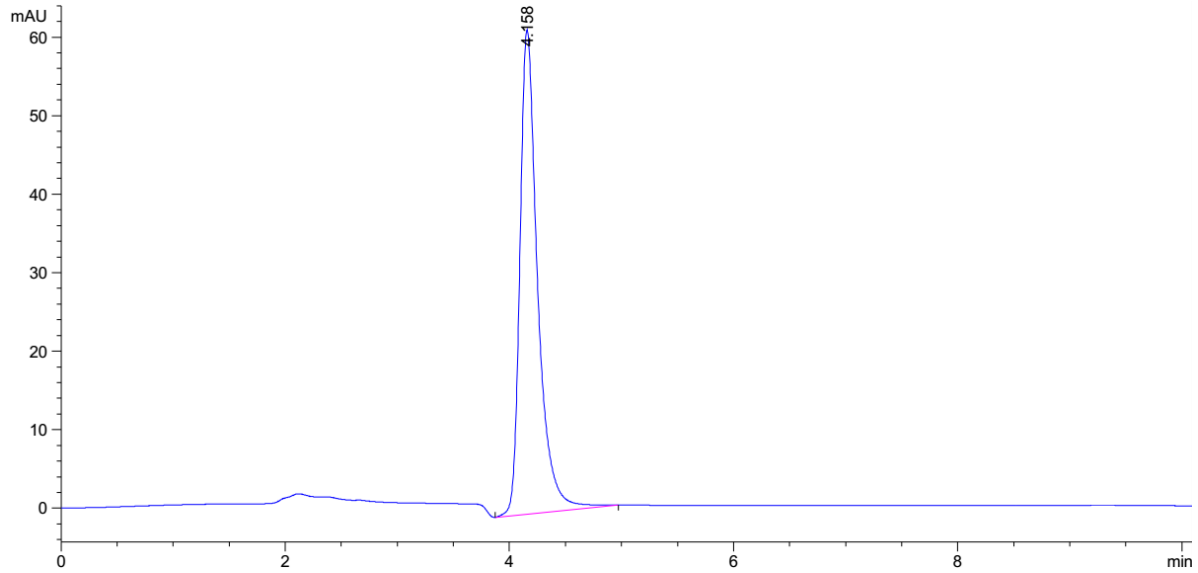

Figure 2. Chromatogram of 5 µg/mL acrylamide spiked in chips sample and analyzed using US-LLE/HPLC-UV.

Table 3. The some reported methods for the determination of acrylamide in foodstuffs.

Matrix	Sample preparation	Detection method	LOD (mg/kg)	Recovery %	Ref.
Deep-fried flour-based leavened dough	Extraction with water, filtration withPVDF syringe filter, SPE with combined Oasis HLB and Bond Elut-Accucat cartridges	HPLC-UV	6	78.0–107.0	[27]
Chips	Defatting with hexane, drying, extraction with acetone and water, filtration, evaporation to dryness, re-dissolve	HPLC-UV	0.0024	84.5–98.3	[12]
Starch-based foods	Extraction with water, deproteination with Carrez I and II solutions, defatting with hexane derivatization with 0.1 M $KBrO_3$ and KBr powder at acidic media, LLE with EtAc–hexane (4:1, v/v), Filtered the organic phase throughcalcinated Na_2SO_4, evaporate todryness, re-dissolve	HPLC-DAD	15	89.6–102.0	[28]
Potato chips and bread crust	Extraction with MeOH, SPE with activated silica gel, carbon nanotubes, magnetic chitosan, modified chitosan and C18 bonded silica gel	HPLC-UV	0.066	88.9–89.5	[29]
Potato chips	Defatting with hexane, ultrasonic-assisted extraction and dispersive liquid-liquid microextraction	GC-MS	0.00006	97.0–99.0	[30]
Heated foods	Solid-phase extraction on a reversed-phase cartridge. After the analyte was eluted from the cartridge, the eluate was cleaned up on a mixed-mode cation-exchange cartridge	LC-MS	0.1 (LOQ)	96.0–101.0	[13]
Potato chips	A defatted sample was extracted continuously with methanol, for 10 days, in a Soxhlet extractor. After about 7 days, a constant concentration of acrylamide was reached.	GC-FID and GC-MS	-	-	[11]
Potato chips	Ultrasound-assisted liquid–phase extraction	HPLC-UV	0.12 (µg/mL)	88.0-118.0	Present method

Historically, one of the first protocols published for the analysis of acrylamide was GC separation using ECD or MS in determining drinking water acrylamide after derivatization (bromination with 2,3-dibromopropionamide,[31] although this method has been practiced to complex matrices such as different food matrices.[32] This procedure seems to be very time-consuming and with confined success in regarding its reproducibility. LC-MS/MS is by far the most sensitive for acrylamide measurements and despite to GC has advantage of underivatized molecule analysis. Since molecular ion of acrylamide is very low (m/z = 72 for protonated molecule), it is not targeted for vivid spectral determination using full scan. Therefore, multiple reaction monitoring (MRM) mode of scanning with triple-quadrupole apparatus is an alternative method.

As acrylamide threshold is almost high (1000 µg/kg)[8] proposed HPLC methods with UV detection showed adequate detection limit, however, matrix effect is general limited selectivity and sensitivity of UV detectors make detection of low levels of acrylamide difficult but herein this problem bypasses by appropriate sample clean-up. In this method, acetone used for defatting and caries solutions for precipitating the proteins of the samples in the clean-up step. Using US helped to fast equilibrium between solid phase and acetone and acetone was easily

evaporated in couple of minutes and acrylamide was re-dissolved in acidic water for subsequent analysis. Present method used HPLC-UV to determine acrylamide which did not require a derivatization step. Obtained chromatogram showed that samples were free from co-existing components and the baseline was very smooth and clean. Despite from the most sample preparation methods reported in Table 3, present method did not use defatting solvent such as hazardous hexane. By optimizing the extraction conditions such as pH and extraction and re-dissolving solvents, a pure extract was obtained.

Conclusion

Unfortunately, the adverse effects of some toxic ingredients in food products come at a high price to consumer health and safety. This is why researchers have focused to find and control substances such as acrylamide that can be lurking in some chip's products. In this study, the quantification of acrylamide in chips was simply assessed by US-LLE/HPLC-UV. This set-up is reliable for routine analysis of acrylamide in chips samples in food quality control laboratories.

Acknowledgments

This work is part of a project supported by a Grant (61201) from the Food and Drug Safety Research Center, Tabriz University of Medical Science, Tabriz, Iran.

Conflict of interests

The authors claim that there is no conflict of interest.

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