Quantification of Acrylamide Content in Potato Chips and in Iraqi "Harissa"

Khalid W. S. Al-Janabi
Dept. of Chemistry/College of Education for Pure Science (Ibn Al-Haitham)/
University of Baghdad
Email: khalid.janabi@gmail.com

Received in : 26 February 2013, Accepted in : 24 July 2013

Abstract

A simple, environmentally benign, sustainable, accurate and cost effective green approach has been developed for the determination of Acrylamide (2-propenamide) in different samples of potato chips collected from the Iraqi market during the year 2012 and a traditional Iraqi meal called Harissa.

The method entails a straightforward de-fatting practice with *n*-hexane, extraction with lukewarm water, and cleanup with solid-phase extraction (SPE) cartridges containing the sorbent bed of the mixed mode ion exchangers (SiliaPrep C8/SCX-2/SAX). The final extracts are directly determined by liquid chromatography-Ultra violet (LC-UV) at a wavelength of 205nm for quantification.

The acrylamide content in the examined potato chips were in the range 339–1024 µg/kg, while for Harissa sample it was 235 µg/kg. The recoveries were in the range of 97.4 and 101.2% with relative standard deviations (RSD%) of about 4.1%. The limit of detection (LOD) and the limit of quantification (LOQ) were 20 µg/kg and 50 µg/kg in the basis of signal to noise ratios of 3:1 and 9:1, respectively.

The green goal of the proposed method was accomplished by developing a procedure that uses minimal amounts of benign reagents and the avoidance of producing toxic residues. This method can be used for routine analysis of Acrylamide in any fatty food matrices.

Key words: Acrylamide, Solid Phase Extraction, High Performance Liquid Chromatography, Harissa, Potato Chips, Iraq

Introduction

Prior to the determination of any environmental analyte, a sample preparation process must be carried out to effectively separate the analyte from its matrix quantitatively. This step is considered as the most polluting one [1]. Sample preparation typically implies a solvent extraction such as the Soxhlet technique which is slow, costly, labor intensive, and requires the use of large quantities of hazardous solvents. Extreme reduction in the use of solvents is an alternative to develop cleaner analytical procedures when the use of toxic reagents cannot be avoided [2]. Conversely the use of Solid-phase extraction (SPE) technique for sample pre-treatment complies with the general principles of green chemistry as it saves time, solvents, cost and waste with minimal labor [3].

Acrylamide, as a carcinogenic and mutagenic hazardous compound on animals and humans public health [4] [5], is consequently formed as a byproduct of certain cooking processes, such as frying of carbohydrate rich food. So it is expected to be found in potato chips [6]. The formation of acrylamide in heat-processed foods occurs via the Maillard reaction [7] [8].

The ability of acrylamide to induce chromosomal damage was observed in bone marrow cells in vivo. Frequent doses of 10-50 mg/kg body weight per day, by any route, in most laboratory animal species, causes a neuropathy affecting primarily the peripheral axons (both motor and sensory) and the visual system. While single doses exposure of 100-200 mg acrylamide/kg body weight was fatal in most animals [9].

Gas chromatography with different detectors was used for the determination of Acrylamide in foodstuff. However such methods require derivation of Acrylamide which is a time consuming step, costly, and produce waste as well as GC technique may outcome with over estimation results due to additional formation of analyte in hot GC injector [10] [11]. Similarly HPLC with different detectors is employed for the determination of Acrylamide in foodstuff [12] [13]. However, HPLC analysis does not require a tedious derivation and is a less expensive alternative to GC.

Acrylamide is a polar molecule which can be extracted efficiently by water [14].

This study has developed a green approach which is rapid, accurate, cost effective and easy to operate with minimal solvent consumption for the determination of Acrylamide in different brands of potato chips and a traditional Iraqi meal called “Harrisa” which is consisting mainly from wheat and lamb.

Experimental

Reagents and Equipment

All reagents were HPLC grade; Methanol, water, *n*-hexane and acrylamide were from Biosolve Chimie SARL (France). The mobile phase was filtered through a Whatman membrane 0.2µm pore size.

Chromatographic analyses were performed on a Shimadzu Prominence UFLC (Japan) high-performance liquid chromatograph HPLC, equipped with a quaternary pump LC-20 AD, degasser DGU-20A5, Column EC 250/4.6 Nucleodur 100-5 C18 ec, and a UV/Vis detector SPD-20A. Data collection and the performance of all components in the system were controlled by LC-Solution chromatography software of Shimadzu.

Extraction and clean-up were accomplished with (SiliaPrep C8/SCX-2/SAX, 3mL/500mg, Particle Size 40-63 µm, Pore Size 60 Å) SPE tubes from (SiliCycle inc, Canada).

Standard Solutions

Working standard solutions of acrylamide were prepared by serial dilution of a 1000mg/L stock standard solution which has been prepared by dissolving 10mg of acrylamide in 10mL of hplc grade water.

Sample preparation

Different samples of Potato Chips are locally purchased from grocery stores in Iraq in the period of May – Sep 2012. A 5 g of each sample has finely grinded with a coffee grinder (BRAUN KSM2, Germany).

Harissa sample was made in the same Iraqi traditional way. Wheat is soaked overnight in water. Then a 1 Kg of the presoaked wheat is mixed with a 200 g of lamb and boiled for 4 hr at a constant stirring. About 5 g of table salt is added during the cooking process.

Samples were de-fatted, where a 1 g mass of the homogenized and finely grinded sample is placed into a 50mL centrifuge tube, and a 20mL of n-hexane was added. The tube was shaken by vortex for 5min. Then each tube was centrifuged at 9000 rpm for 15 min, followed by filtration under vacuum and the n-hexane layer was discarded.

The de-fatted sediment is dried in the oven at 90 ºC for 15min then extracted by the addition of 5mL lukewarm water and vortex for 5min. The homogenized mixture was filtered under vacuum and the aqueous phase is collected and further cleaned-up by applying each sample to a preconditioned SPE tube. The SPE dispersive conditions removed most of the remaining fats and other interferences remaining in the aqueous aliquot.

SPE cartridges were conditioned with 3mL methanol then equilibrated with 6mL of water; the methanol and the water portions used to prepare the cartridge were discarded. A 2mL of the aqueous filtrate is directly loaded on the top of an SPE cartridge at a flow rate of 2mL/min. The extract was allowed to pass through the SPE sorbent bed followed by 0.5 mL of water. The first 0.5 mL eluted from the SPE tube is discarded while the next 1mL is collected and filtered by a 0.2µm syringe filter then introduced directly to hplc-UV analysis.

Samples are spiked appropriately with acrylamide to work out recoveries and reproducibility.

HPLC condition

Sets of experiments were conducted to adjust the best proportion of gradient mixture and to determine the optimum flow rate of the eluent capable of separating acrylamide from other interferences in the sample and the best detection. The optimum conditions of HPLC separation and detection for the acrylamide from its matrix are listed below in (Figure 1).

Results and Discussion

To average effect of Acrylamide distribution in the samples of potato chips, an exactly 1g portion of each sample was taken out of a finely grinded 5g portion. Because it is necessary to take food specimens to be analyzed from a large homogenized food sample to average effect of analyte distribution on the results [15].

In addition to time and energy consumption, enrichment of contaminants and the formation of emulsions; One serious drawback for the conventional procedures in the extraction and determination of Acrylamide in fatty food matrices is the use of relatively large amounts of organic solvents. However to comply with the principles of green chemistry and sustainability; acetonitrile, which is a common mobile phase modifier in such traditional chromatographic applications, was avoided for its expensiveness and hazardousness.

The exceptionally high water-solubility of acrylamide provides an efficient extraction of it from food samples with water effectively at room temperature. The extract is not pH adjusted since the buffer capacity of distilled and de-ionized water is extremely low. While

due to the remarkably low solubility of Acrylamide in n-hexane[16] enabled an easy de-fatting process by washing the aqueous extracts with few milliliters of n-hexane.

Clean-up has been accomplished by SPE of the sorbent bed SiliaPrep C8/SCX-2/SAX from SiliCycle. The mixed mode of this sorbent can work as a Strong Anion Exchanger (TMA Chloride, SAX) or a Strong Cation Exchanger (propyl sulfonic acid, SCX-2) (Figure 1).

During the conditioning step and the sample application, SPE cartridges never left to run dry. Dryness of the sorbent bed of the SPE cartridge may irreversibly coagulate the active sites of the SPE sorbent bed which reduces the cartridge capacity and efficiency. Elution of the conditioning solvents and the sample from the SPE tube is left to gravity and vacuum never used to speed up the elution process. SPE clean-up step removes a number of early eluting co-extractives which improved signal to noise ratios and resulting in a better precision in measurements (Figure 2).

Analytical standards were prepared freshly prior to each analysis because, especially the highly concentrated standards (> 1 mg/L) have showed a tendency for inaccurate readings after storage at room temperature [17]. A number of standard concentrations of acrylamide in water of hplc-grade are prepared and used to produce the calibration curve (Figure 3).

Each sample has been fortified with 1mg/L acrylamide standard solution to accurately identify the retention time of acrylamide in each sample and to compensate for any recovery losses during the extraction and the cleanup steps as well to ensure that results are reliable (Fig. 4).

Real samples of potato chips and one sample of Harissa were prepared, de-fatted, SPE cleaned up then LC-UV determined for acrylamide content following the pre-described conditions listed in Table 1. Extraction procedures give almost a full recovery. The samples of potato chips showed a high level of acrylamide content in the range of 339–1024 µg/kg, which is considered as a risky dosage for human health while Harissa sample exhibited less acrylamide content which is 235 µg/kg. Acrylamide content and other analytical data for samples of potato chips and Harissa are listed in (Table 2).

Conclusion

An efficient, simple and green procedure is developed for the quantification of acrylamide in potato chips and Harissa using solid phase extraction technique for sample cleanup and high-performance liquid chromatography with ultra-violet detection for quantification. It complies with the principles of green chemistry since it reduces the environmental impact in terms of reducing solvent usage, disposal, energy consumption and the avoidance of employing hazardous chemicals and reagents. This procedure can be applied for routine analysis of any other oily food stuff.

As well, this research has validated the presence of acrylamide in all brands of potato chips collected from Iraqi grocery stores for the period of May – Sep 2012 as well as in Harissa meal. All the samples exhibited acrylamide content exceeding the recommended average dosage to maintain public health.

References

1. Gonc, S. ;Pe. C.; Farré, M.; Pérez, S.; Gonçalves, C.; Alpendurada M. F.; and Barceló, D.; (2010). “Green analytical chemistry in the determination of organic pollutants in the aquatic environment;” TrAC Trends in Analytical Chemistry; vol. 29 no. 11: 1347–1362.
2. Melchert, W. R.; Infante, C. M. C. and Rocha, F. R. P. (2007). “Development and critical comparison of greener flow procedures for nitrite determination in natural waters;” Microchemical Journal; vol. 85: 209–213.

3. Anastas, P. T. and Warner, J. C. (1998). "Green Chemistry: Theory and Practice;" in Oxford University Press; New York: 30–48.
4. Kütting, B.; Schettgen, T.; Schwegler, U.; Fromme, H.; Uter, W.; Angerer, J.; and Drexler, H. (2009). "Acrylamide as environmental noxious agent: a health risk assessment for the general population based on the internal acrylamide burden.;" International journal of hygiene and environmental health; vol. 212 no. 5: 470–80.
5. Capuano, E. and Fogliano, V. (2011) "Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism; toxicity; occurrence in food and mitigation strategies;" LWT - Food Science and Technology; vol. 44 no. 4: 793–810.
6. Kaplan, O.; Kaya, G.; Ozcan, C.; Ince, M. and Yaman, M. (2009). "Acrylamide concentrations in grilled foodstuffs of Turkish kitchen by high performance liquid chromatography-mass spectrometry;" Microchemical Journal; vol. 93 no. 2: 173–179.
7. A. H. K.; Katherine, D. J. L.; Brill, A. and Jennifer, S. Li. (2005). "Detection of acrylamide in potato chips via soxhlet extraction and GC/MS analysis" The Dartmouth undergraduate journal of science; no. Winter; 29–33.
8. Praegitzer, S. M. and Smith, J. S. (2011). "Acrylamide formation and mitigation in processed potato products;" Manhattan.
9. W. H. O. United Nations Environment Programme; International Labour Organisation; (1991). "Health and safety guide no. 45;" Geneva.
10. Dunovská, L.; Cajka, T.; Hajslová, J.; and Holadová, K. (2006). "Direct determination of acrylamide in food by gas chromatography-high-resolution time-of-flight mass spectrometry.;" Analytica chimica acta; vol. 578 no. 2: 234–40.
11. Ghani, N. M.; Saim, N.; Osman, R.; and Munir, Z. A. (2007). "Accelerated solvent extraction method with one-step clean-up for hydrocarbons in soil;" The Malaysian Journal of Analytical Sciences; vol. 11 no. 1: 193–197.
12. Granda, C.; Moreira, R. G.; and Tichy, S. E. (2004). "Reduction of Acrylamide Formation in Potato Chips by Low-temperature Vacuum Frying;" Journal of Food Science; vol. 69 no. 8 405–411.
13. Gökmen, V.; Şenyuva, H. Z.; Dülek, B. and Çetin, A. E. (2007). "Computer vision-based image analysis for the estimation of acrylamide concentrations of potato chips and french fries" Food Chemistry; vol. 101 no. 2: 791–798.
14. Helmut, R. H.; Guenther, S.; Anklam, E. and Thomas, Wenzl (2007). "Acrylamide in coffee: Review of progress in analysis; formation and level reduction;" Food Additives and Contaminants; vol. 24 no. S1: 60–70.
15. Food Chemistry Section; (2002). "Determination of acrylamide in food;" Swiss Federal Office of Public Health: 1–2.
16. Habermann, C. E. Acrylamide; 4th ed. vol. 1. New York, U.S. J. Wiley & Sons (1991):251–266.
17. Brandl, F.; Demiani, S.; Ewender, J.; Franz, R.; Gmeiner, M.; Gruber, L.; Gruner, A.; Smolic, S.; Störmer, A. and Wolz, G. (2002) "A rapid and convenient procedure for the determination of acrylamide in foodstuffs;" EJEAFChe; vol. 1 no. 3: 137–144.

Table (1): HPLC conditions used for separation and determination of acrylamide

Column	EC 250/4.6 Nucleodur 100-5 C18 ec
Flow rate	0.7 mL/min
Column temperature	25 ºC
Injection volume	100 µL
Mobile phase	Isocratic elution: Methanol absolute (solvent A) and Water (solvent B) in the ratio 30% v/v A
Run time	10 min
Post time	2 min
Detection wavelength	UV at 205nm

Table (2): Acrylamide content in some samples of potato chips collected from Iraq markets in the period May – Sep 2012 and an Iraqi traditional meal "Harissa "

	Sample	**Acrylamide content (µg/Kg)**
1	Lay's/ French Cheese	437
2	Lay's/ Salt & Vinegar	398
3	Lay's/ Yogurt & herbs	342
4	Baz/ potato snacks	779
5	Pringles	1024
6	Lucky Chips	594
7	Mr Chips/ Cheese	516
8	Taycon/ Salt & Vinegar	339
9	"Harissa" meal	235

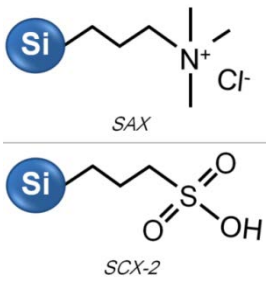


Figure (1) :Structure of the sorbents (SAX and SCX-2) used for ionic compounds characteristics

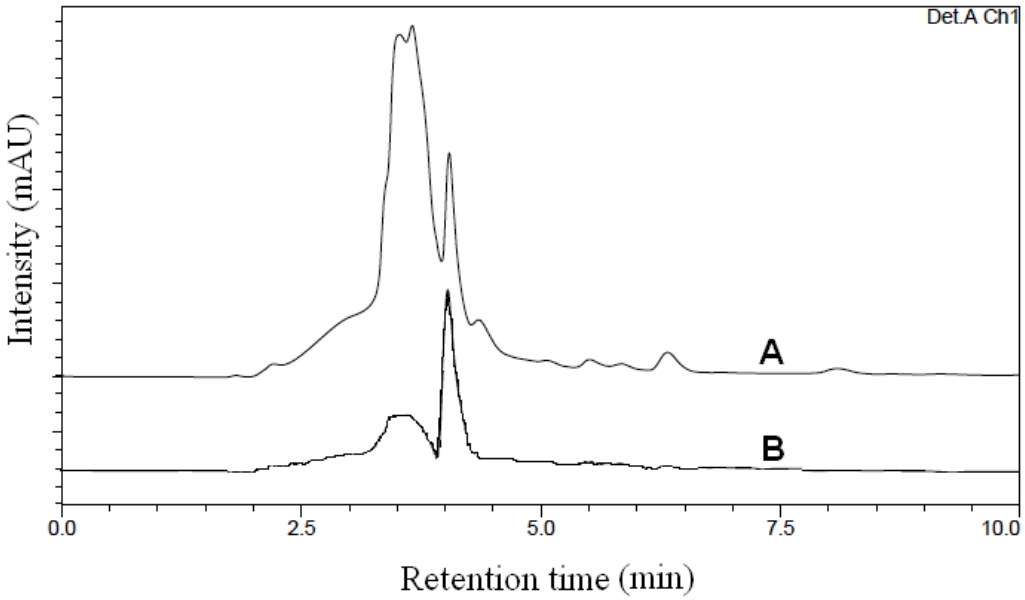


Figure (2):Comparison of two overlaid chromatograms showing the enhancement obtained after the use of SPE clean-up for Potato Chips samples; (A) before SPE clean-up (B) after SPE clean-up step.

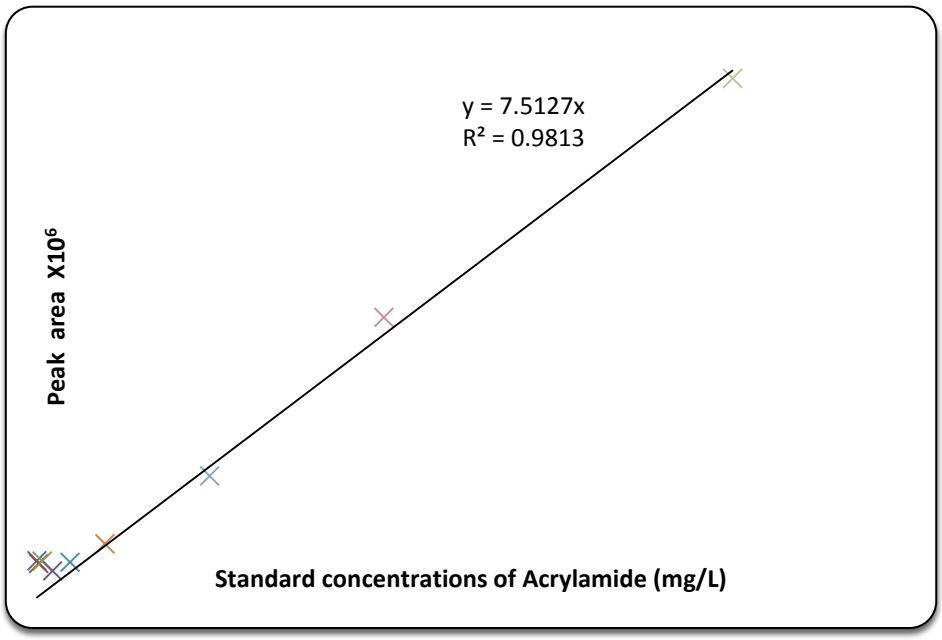
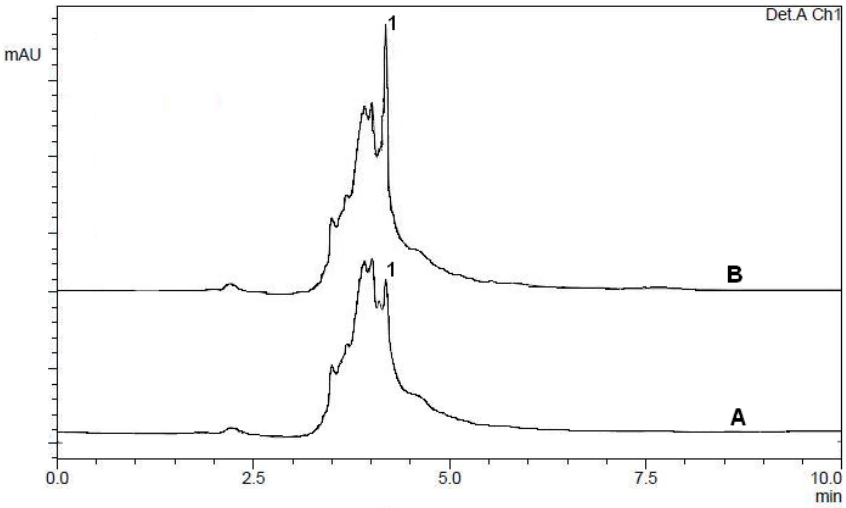


Figure (3) :Calibration curve of acrylamide in water



Figure(4) :Comparison of two overlaid graphs showing the resulting hplc chromatograms for a sample: (A) neat; (B) spiked with acrylamide. Spiking is accomplished for samples before SPE clean-up. Peak 1 is for acrylamide.

تعيين محتوى الأكريل أميد في رقائق البطاطا والهريسة العراقية

خالد وليد صالح الجنابي
قسم الكيمياء/ كلية التربية للعلوم الصرفة(إبن الهيثم)/ جامعة بغداد

استلم البحث في : 26 شباط 2013 ، قبل البحث في :24 تموز 2013

الخلاصة

طُورت طريقة خضراء بسيطة، آمنة بيئياً، مستدامة، دقيقة وذات تكلفة مناسبة لتعيين مقدار الأكريل اميد (2-بروبين أميد) في نماذج مختلفة من رقائق بطاطا جمعت من السوق العراقي للعام 2012 وفي إنموذج وجبة عراقية تقليدية تسمى "هريسة".

تضمنت الطريقة التخلص المباشر من الدسم في النماذج باستخدام الهكسان العادي ثم الاستخلاص بالماء الفاتر يتبعه التنظيف بتقنية الاستخلاص بالطور الصلب بوساطة خراطيش SPE التي تحتوي على مواد ماصة بهيئة مبادلات آيونية مختلطة نوع (SiliaPrep C8/SCX-2 / SAX). ثم يجري التقدير الكمي المباشر للمستخلص النهائي بوساطة الكروماتوغرافيا السائلة والأشعة فوق البنفسجية (LC-UV) عند الطول الموجي 205 نانومتر.

ان محتوى الأكريل اميد في نماذج رقائق البطاطا التي تم قياسها كان ضمن النطاق 339-1024 ميكروغرام/كيلوغرام، في حين كان لعينة الهريسة 235 ميكروغرام/كيلوغرام. وكانت قيم الاسترداد ضمن النطاق 97.4 الى 101.2% مع انحراف معياري نسبي RSD% يقارب 4.1%. وكان حد الكشف LOD ، حد التقدير الكمي LOQ هو 20 ميكروغرام/كيلوغرام و 50 ميكروغرام/كيلوغرام على أساس نسبة إشارة الى تشويش 3 الى 1 ، 9 الى 1 على التوالي.

أُنجز الهدف الأخضر للطريقة المقترحة من خلال تطوير إجراءات تستخدم كميات ضئيلة من الكواشف الحميدة وتتجنب إنتاج مخلفات سامة. ويمكن استخدام الطريقة في عمليات التحليل المتكرر والدوري لمادة الأكريل اميد لأي إنموذج من الأغذية الدهنية.

الكلمات المفتاحية: أكريل اميد، الاستخلاص بالطور الصلب، الكروماتوغرافيا السائلة ذات الكفاءة العالية، هريسة، رقائق بطاطا، العراق.