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Optimization, Validation and Application of Quantitative Method for the Determination of Acrylamide in Potato Chips Samples in Yemen Using GC-NPD

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Abstract

Over the last two decades, numerous analytical methods for determining acrylamide concentration in a wide range of food products have been developed. Some studies focused on the methods of extracting acrylamide from complex food matrices. In the present study, solid Phase Extraction (SPE) and Gas Chromatography-Nitrogen Phosphorus Detector (GC-NPD) analysis were employed to develop a simple and cost-effective acrylamide determination method. Method validation and optimization steps were carried out and used to determine acrylamide concentrations in potato chips purchased from Yemeni markets. In the concentration range of 0.1-5 ppm, the validated method demonstrated good sensitivity within a 0.012 ppm limit of detection (LOD) and an excellent linearity of 0.996 correlation coefficient. Eighteen samples of potato chips purchased from local markets in Sana'a, Yemen, were analyzed using this method. Three of the 18 samples of interest contained various acrylamide concentration levels of 879.4, 795.6, and 754.7 ppb. These were higher than the EU Commission's indicative value for the acrylamide in potato chips (750 ppb). As a result, the optimized GC-NPD protocol proved to be useful for the acrylamide analysis, paving the way for the development of an acrylamide quantification prescreening tool in the food industry.

Keywords: Acrylamide, c-SPE, GC/NPD, Potato Chips

Introduction

Acrylamide (2-propenamide), is a vinylic compound with a molecular weight of 71.09 g/mol [1]. It is an unsaturated amide that has been chemically synthesized since the 1950s by hydrating acrylonitrile. It is extensively utilized in a variety of industrial processes, such as water purification, pulp, paper, and textile, in addition to the synthesis of dyes, gels, and polymer [2-7].

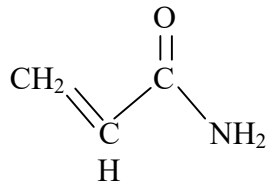

Figure 1. **Chemical Structure of the Acrylamide Molecule**

Acrylamide is considered as a hazard contaminant to human where some researches indicated neurotoxicity, harmful effects on

male reproductive, and a developmental toxicity as potential key endpoints for acrylamide conversion to glycidamide via either epoxidation reaction or conjugation with glutathione [8].

Independent studies have found that acrylamide stimulated lung and skin tumors in mice after a dose of 2 mg/kg per day administered in drinking water [9]. Furthermore, the International Agency for Research on Cancer has classified acrylamide as “probably carcinogenic to humans” because of the availability of sufficient evidence for its carcinogenicity in experimental animals and mechanistic considerations [1]. Its carcinogenicity may cause chromosomal mutations in DNA as well as nervous system damage [10,11]. Although human exposure to harmful levels of acrylamide from heat processed food sources, it wasn't until 1997 when railway workers in South-West Sweden were exposured to levels that surpassed the No Observed Adverse Effect Level (NOAEL) of acrylamide. The acrylamide concentrations in their blood ranged between 0.07-17.7 nanomol/g Hb [12]. This alarming finding stimulated scientists to carry out further investigations on the possibility of acrylamide formation in human food. In April 2002, the Swedish National Food Administration (SNFA) announced that prolonged heat treatments of some foods could result in significant amounts of acrylamide [13].

Later, several countries conducted qualitative and quantitative studies on acrylamide in various food commodities [14]. More investigations confirmed that acrylamide could be found in protein containing food. However, its primarily sources in human diet are plant-based foods rich in carbohydrates such as French fries and potato chips; cereal-grain-based foods such as cookies, crackers, breakfast cereals, toasted bread; and oil, nuts, meat, coffee, chocolate, dry milk, tea, and drinking water [10,15,16]. Experimental evidences suggested that heat treatments such as frying and baking of food items at temperature above 120 °C with low moisture levels are preferable conditions for acrylamide formation. This unfavorable by-product was due to the Maillard reaction between amino compounds and reducing sugars [17,18]. Potatoes have been used as food for centuries, but research into their nutritional value has increased their importance as a food and nutritional security alternative [19]. Due to the high content of carbohydrates in potatoes, acrylamide level could be significantly high specially in fried potatoes such as potato chips and French fries. They have been popular snack consumed by millions of people from various cultural backgrounds [10,20]. According to a recent report, potato chips and French fries account for as high as 10% and 50% dietary acrylamide intake, respectively in all age group [21].

Several studies were conducted to quantify acrylamide content in a variety of food commodities, primarily using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry /mass spectrometry (LC-MS-MS) [22-25]. The majority of the methods involved the use of solid phase extraction (SPE) as a clean-up step prior to the chromatographic analysis, as well as a derivatization reaction that may be particularly required before the GC-MS analysis, both of which were deemed costly and time-consuming [26,27]. This weakness of the GC/MS approach has given LC-MS/MS a significant advantage [28]. Furthermore, despite their dependability and great sensitivity for acrylamide detection, both GC/MS and HPLC-MS are quite expensive for laboratories with limited resources [29]. In light of the foregoing, the goal of this study was to develop a cost-effective validated method for determining acrylamide using a

SPE, which was prepared at the laboratory, and GC/NPD analytical system. This was followed by the application of the developed method for the quantification of acrylamide in potato chips collected from the Yemeni market.

Experimental

Chemicals and Material

Acrylamide, (Sigma-Aldrich) of ≥ 99% was used. GC grade solvents (n-hexane, acetone, ethyl acetate and methanol) were purchased from Scharlau (Spain). Anhydrous sodium chloride and anhydrous sodium sulphate of ≥ (99.5%) were from BDH (Dorset, UK). The water used was purified using a Direct-Q3 (Millipore, Bedford, MA, USA) water purification system. Activated charcoal (Merck-Germany) and medical syringes (China) were used to prepare SPE cartridges. A standard stock solution of acrylamide (1000 ppm) was prepared in acetone and used to prepare instrument validation solutions. For low and high concentration spiking, standard solutions of 100 and 1000 ppm of analyte were used, respectively.

GC/NPD Analysis

A Burker 450 GC gas chromatography/Nitrogen Phosphorus Detector (GC/NPD) system equipped with a CP-8410 Auto-injector and a DB-Wax capillary column 30 m x 0.2 mm I. D. Film thickness of 0.25 µm was used to analyze the samples (Agilent, Palo Alto, CA, USA). At a flow rate of 1.5 mL min^{-1}, a nitrogen of high purity (>99.99%) was used as carrier gas. In the splitless mode, samples (2 µL) were injected at 250 °C injection temperature and 280 °C detection temperature. The temperature program used in the GC-NPD was as follows: the initial temperature was 40 °C (1 min holding time), which was increased by 10 °C min^{-1} heating rate to 140 ^{o}C, and then increased to 250 °C at 30 °C min^{-1} and held for 2 min. The total run time was 16.67 min. The nitrogen content of the target compound (acrylamide) allowed the use of NPD, thereby enabling the development of a selective and sensitive assay [30].

Potato Chips Sample Preparation

Real potato chips samples were randomly collected from different local markets in Sana'a city. The samples were pulverized in a homogenizer for 10 min, sub-sampled, and stored in an acetone-precleaned glass beaker. The backers were wrapped in aluminum foil and kept at 0 °C in the dark. A portion of the sample (10 g) was spiked with standard acrylamide and then homogenized carefully.

The spiked sample was left to stand overnight before being extracted. 2g of homogenized sample was accurately weighed into a 100 mL beaker, followed by the addition of 20 mL of 0.1 v/v% formic acid/water. The sample was agitated for 60 minutes to facilitate extraction. Prior to removing the oily layer, the sample was refrigerated. After centrifuging the sample at 6000 rpm for 10 minutes, it was filtered through 4.5 µm filter paper. The extracted material was then used in the cleanup/preconcentration procedures with c-SPE.

Clean-up using Solid Phase Extraction Cartridge (c-SPE):

To make clean-up c-SPE, medical syringes were packed with various masses (0.2-0.8 g) of previously purified activated charcoal after being treated with 3 M HCl at 40 ^{o}C. In the bottom and the top of an activated charcoal layer, thin discs of pre-cleaned medical cotton were inserted. The cartridges were rinsed with acetone, and conditioned using a deionized water.

Results and Discussion

Method of Optimization

Using potato chips samples spiked with known concentrations of acrylamide, several parameters that affect clean-up/extraction efficiency were investigated to obtain the proper conditions for a good linearity, repeatability, and high extraction recovery. The optimization of the c-SPE extraction experimental procedures were achieved by comparing the GC response of the spiked sample eluted with that obtained from the standard solution.

Selection of Extraction Solvent

The extraction solvent was an important parameter because it directly affected the extraction efficiency, method cost, and toxicity. Therefore, several solvents were tested for the analyte extraction, including deionized water, acidified deionized water, methanol, ethyl acetate, and acetonitrile. Fig. 2 depicts the extraction efficiency of acrylamide (peak areas) with each solution.

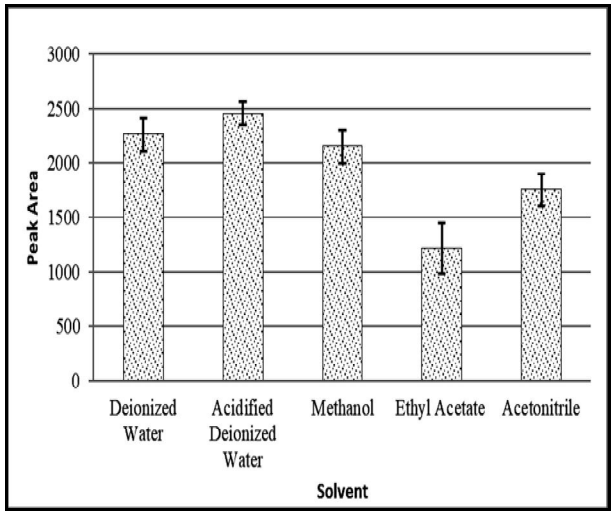

Figure 2. **Efficiency of the Extracting Solvents**

Our results clearly show that, acidified deionized water has a higher extraction capability than the other solvents, with solubilities expressed in g/100 mL of the solvent at 30 ^{o}C. This could be due to the high hydrophilic nature of the acrylamide that leads to high solubility in water, and consequently minimizes the dissolution of hydrophobic compounds in the food products. The co-extraction of other unwanted compounds in the matrix (e.g. proteins, carbohydrates), which might interfere with the detection and degenerate the chromatographic system, if they were not removed [31]. Acidified deionized water is less expensive, more stable, and has fewer side effects on the analyst and the environment. Furthermore, a simple defatting step of the extract was achieved by refrigerating at 0 °C for 15 min. As a result, acidified deionized water was chosen for further investigation.

Stirring / extraction time

The effect of the extraction time was examined. Spiked potato chips samples with 20 ppm acrylamide at room temperature were stirred for different periods of time (10-90 min.) at 1000 rpm. Fig. 3 shows that, the optimum stirring time for the acrylamide extraction from potato chips samples was 55 min, and the analytical signals remained constant at the higher periods of time, above 55 min.

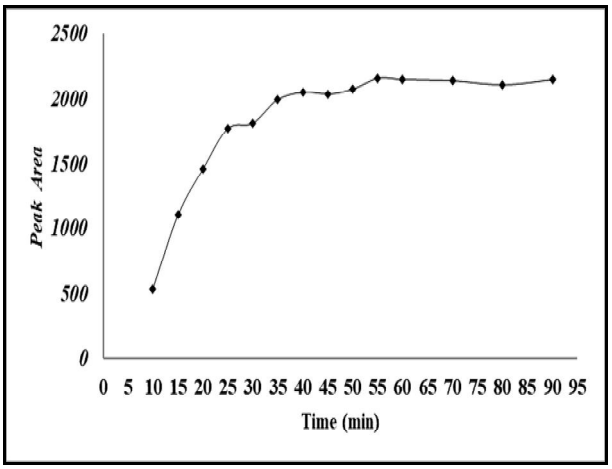

Figure 3. **Optimization of stirring/Extraction Time**

Optimization of c-SPE

Effect of solvent

The elution efficiency of the various solvents e.g. acetone, acetonitrile, methanol, and hexane on the acrylamide were examined. 5 mL cartridges were packed with 400 mg charcoal and then cleaned with acetone and conditioned with D.W. 2 mL of (20 ppm) acrylamide standard solution (in triplicate) was poured passing through each cartridge at a flow rate of 3 mL/min. The cartridges were then dried, and the trapped acrylamide in the cartridge was eluted using the various solvents. According to Fig. 4, acetone provided the best elution efficiency, with an average recovery of 97.4%. Subsequently, acetone was used for the elution procedure in all cases.

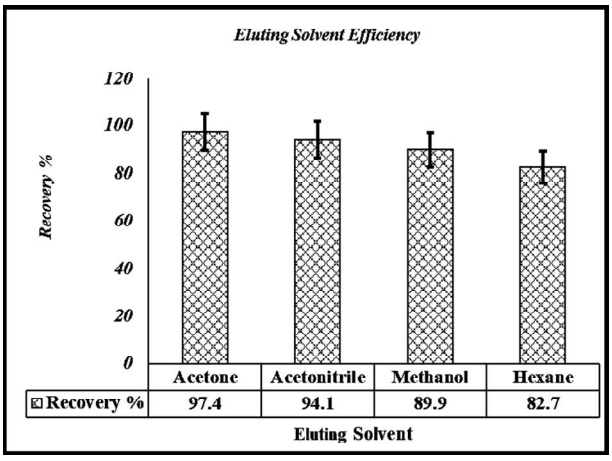

Figure 4. **Eluting solvents efficiency. (Acrylamide standard 20 ppm, activated charcoal mass = 400 mg, flow rate = 3 mL/min, 2 µL of eluent was injected to GC/NPD)**

Effect of cartridge volume

To determine the best cartridge volume/shape for recovery, three cartridges (medical syringes) with different volumes of 3, 5, and 10 mL were packed with 400 mg of charcoal. After cleaning and conditioning procedures, 2 mL of 20 ppm acrylamide standard solution was passed through each cartridge (in triplicate), the cartridges were dried, and the acrylamide trapped in the cartridge was eluted with 2 mL acetone. The obtained results show that the 3 mL cartridge volume is the most appropriate one for the used conditions, with an average recovery percentage of 98.5%, for which it has been chosen for the future work.

Effect of activated carbon mass

Various masses of activated carbon were used to investigate the effect of activated carbon mass on the acrylamide elution (200 mg, 400 mg, 600 mg and 800 mg). After conditioning procedures, 2 mL of (20 ppm) standard solution was allowed to pass through, then dried and eluted with 2 mL of acetone. The recovery percentage was 99.3% when 400 mg of the acrylamide was used; however, with 200 mg of activated carbon, the percentage recovery was 85.2%. This could be due to exceeding the breakthrough volume. The recovery percentage was found to be 93.4 and 90.6% for the larger adsorbent masses of 600 and 800 mg, respectively, but significantly improved when larger elution solvent volumes were used. However, the increased dilution factor affects the method's overall sensitivity. As a result, 400 mg of the adsorbent was used in this experiment.

Effect of eluting solvent volume

The eluting solvent volume was also tested to determine the optimal amount of the acetone for maximum elution efficiency and recovery. To accomplish this, the adsorbed acrylamide standard in the cartridge was eluted with various amounts of acetone (1, 2, 3, 4 and 5 mL). By injecting 2 µL of the eluted solvent into the GC/NPD system and performing the chromatographic analysis the recovery percentage of acrylamide was determined. Using 1 mL of acetone for the elution, although the limit of detection was the best due to a tenfold increase in preconcentration factor.

Table 1. **Influence of Eluting Solvents Volume on Method Accuracy and Precision.**

Acetone volume (mL)	Peak Area			Average Peak Area	R %	RSD %	Preconcentration Factor	LOD (ppm)
	Replicate 1	Replicate 2	Replicate 3					
1	4056.3	5161.8	4806.4	4674.8	83.4	12.1	10	0.008
2	2841.6	2786.7	2697.9	2775.4	99.1	2.6	5	0.012
3	1876.1	1793.6	1834.9	1834.9	98.3	2.2	3.33	0.018
4	1422.3	1397.6	1385.2	1401.7	100.1	1.3	2.5	0.025
5	1064.8	1146.5	1098.6	1103.3	98.5	3.7	2	0.031

Table 1 shows that the eluting solvent volume is insufficient to elute all the adsorbed acrylamide (2 mL of 20 ppm acrylamide) on activated charcoal. Higher eluting solvent volumes (i.e., 2, 3, 4, and 5 mL) were found to produce nearly complete acrylamide elution with a recovery percentage ranging from 98.3 to 100.1%. The lowest calculated LOD (0.012 ppm) was obtained with a 2 mL acetone elution volume and a preconcentration factor of fivefold. As a result, 2 mL acetone is used in the subsequent work of this study.

Analytical Characteristics

The quantitative requirements of the procedure, linearity range, precision, accuracy, and limits of determination were evaluated under optimized conditions. The method's linearity was tested using real potato chips spiked with six concentrations ranging from 0.1 to 5 ppm. The calibration curve was built by plotting the peak area of the spiked sample (after subtracting the peak area of the un-spiked sample) against the spiked concentration (Fig. 5). The results show good linearity in the studied range, with correlation coefficients of 0.9965.

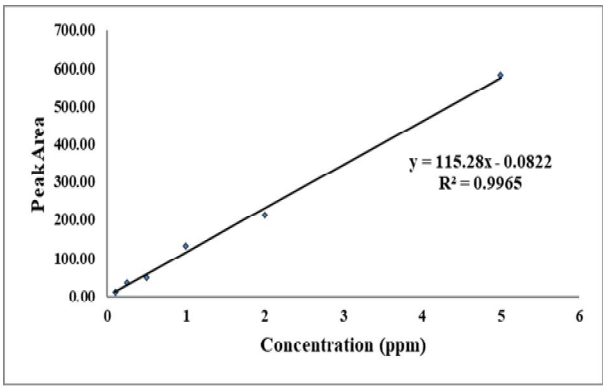

Figure 5. **Linearity of Acrylamide determination in spiked potato chips**

The three replicates of acrylamide determinations in spiked potato chips samples at all concentrations levels were used to determine reproducibility. The relative standard deviations (RSD, n = 3) varried from 4.37% to 12.58%. For the accuracy calculations, the same results were involved, and the recovery percent was calculated using the following equation [32]:

$$\% R = (A_1\text{-}A_2/A_3) \times 100$$

Where A_1 is the spiked sample's peak area, A_2 is the sample's peak area before spiking, and A_3 is the standard's peak area.

Table 2. **Spiked Potato Chips Accuracy and Precision Results.**

Conc. ppm	Standard Solution Peak Area	Spiked sample Peak Area			Average	RSD %	R %	t calculated
		Reading1	Reading 2	Reading 3				
0.1	12.85	11.05	9.94	12.75	11.25	12.58	87.52	1.96
0.25	39.65	31.68	36.52	38.49	35.56	9.85	89.69	2.02
0.5	53.77	46.10	52.30	47.30	48.57	6.77	90.33	2.74
1	143.97	139.33	131.17	125.10	131.87	5.41	91.60	2.94
2	237.82	206.40	225.80	201.50	211.23	6.08	88.82	3.58
5	626.90	607.50	579.40	556.80	581.23	4.37	92.72	3.11

Table 2 shows that recovery rates ranged between 87.52% and 92.72%. At a signal:noise ratio of 3:1, the experimental LOD was calculated [33]. The developed method's LOD was determined to be 0.021 ppm, making it fully applicable to the determination of acrylamide concentration levels expected in potato chips samples.

Method accuracy and precision

To check the test method accuracy using student t test, the following formula used:

$$t = (\overline{X} - u)\frac{\sqrt{N}}{s}$$

Where, u: is the true value, S: standard deviation, X: average measured value.

The calculated t values were shown in Table 2 for all spiked concentrations. The calculated student t values were in the range of 1.96 - 3.58, which is less than the statistical student t value at 95% confidence level for the three replicated samples. This indicates that there is no evidence for the systematic error at the 95% confidence level. To compare the method precision, F test was used to compare the standard deviation of the proposed method with three published methods for the analysis of the acrylamide using GC/NPD [30], GC/FID [33], and GC/MS [34]. The calculated values $F_{calc.}$ were 2.473, 2.813, and 3.42 respectively, which is less than the statistical F values 4.46, 19, and 19 at 95% confidence level.

Analysis of Acrylamide in Real Potato Chips Samples

The optimized method was used to analyze acrylamide in eighteen different brands of potato chips purchased from a local market in Yemen's capital, Sana'a. Triplicate analyses were performed for each sample. Fig. 6 (a,b) shows a GC/NPD chromatogram for the analysis of acrylamide in real potato chips sample. The obtained data for the real potato chips samples are summarized in Table 3. The acrylamide levels in the analyzed samples were in the 77.1 -879.4 ppb range, with RSD % of 3.4% - 12.8%.

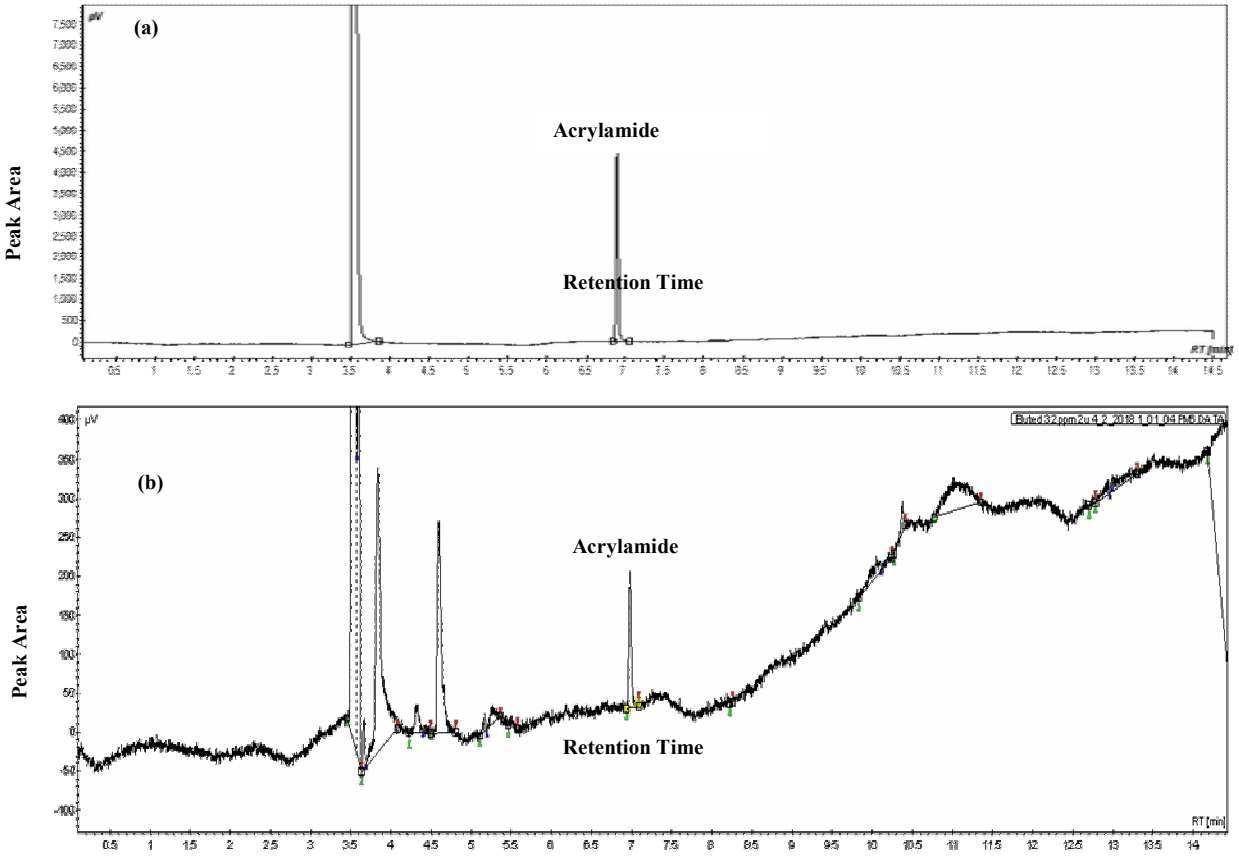

Figure 6. **(a) Standard and (b) Acrylamide Standard (5 ppm)**

Table 3. **Acrylamide in Real patato chips Samples.**

Sample Code	*Average Concentration ppb (n=3)*	*SD*	*RSD %*
PC1	77.1	6.6	8.5
PC2	121.2	4.1	3.4
PC3	139.5	4.5	11.3
PC4	243.2	11.4	4.7
PC5	319.4	12.5	3.9
PC6	198.7	15.5	7.8
PC7	879.4	51.9	5.9
PC8	115.3	9.9	8.6
PC9	795.6	11.9	6.1
PC10	345.1	23.5	6.8
PC11	417.9	15.5	3.7
PC12	223.7	10.5	4.7
PC13	89.2	4.8	9.7
PC14	165.3	8.4	12.8
PC15	113.2	7.6	6.7
PC16	754.7	30.1	4.6
PC17	297.5	11.1	3.8
PC18	417.3	33.8	8.1

In comparison to the EU Commission indicative value for the acrylamide in potato chips [35], the three (PC7 879.4 ppb, PC9 795.6 ppb and PC16 754.7 ppb) samples, out of the 18 analyzed samples were found to have higher concentration as compared to the , indicated value (750 ppb) of the EU commission. The obtained results are consistent with the 752 ppb value of the JECFA result in potato chips [36].

Conclusion

In the present study, we developed a simple, easy to use, reliable and cost-effective method for the quantitative determination of the acrylamide. The study offers highly interesting advantages, from an analytical point of view, as the final extract could be directly injected into GC/NPD without any further derivatization treatment. In comparison to the other chromatographic methods (GC/MS, GC/ECD), the results of this study showed a reasonable recovery, as a well as sufficient sensitivity and accuracy to monitor the levels of acrylamide in potato chips samples.

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Conflict of Interest

All authors declane that there in no conflict of interest

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