Method Development and Validation for the Quantification of Acrylamide in Potato Chips and Other Locally Available Food by LC-MS/MS in Bangladesh

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Abstract

A sensitive, precise, accurate and simple in house validated Liquid Chromatography and Tandem mass spectrometric (LC-MS/MS) analytical method was developed for the quantification of acrylamide in potato chips, a probable carcinogenic and neurotoxic compound in fried food items. Acrylamide was extracted and cleaned up by QuEChERS method, then analyzed by triple quadrupole mass spectrophotometer using positive electrospray-ionization mode and d3-Acrylamide internal standard. The analyte and internal standard were separated on a reversed-phase C18 column (100 × 2.0 mm, 2.5 μm) by gradient elution with water and methanol containing 0.1% formic acid as mobile phase. The analytical method was fully validated by assessment of linearity, specificity, precision, accuracy, selectivity and robustness with relative standard deviation of less than 4%. The calibration curves were found linear with correlation coefficient (r^2) of 0.9997 over the concentration range 5 - 500 μg/L. The recoveries of Acrylamide in the concentration of 250, 400, and 500 μg/L were 97% to 104%. Based on the signal to noise ratio, the lower limit of detection (LOD) and lower limit of quantification (LOQ) were achieved 2.0 μg/L and 4.0 μg/L respectively. The proposed method was also applied to other available most popular 24 types of food (265 number of sample) first time in Bangladesh. The highest level of acrylamide has been found in fried products with the range of 197.04 μg/L to 114.63 μg/L, protein-rich food lies between 79.76 to 89.14 μg/L whereas baked food products exhibited less content in the range of 35.23 to 51.17 μg/L.

Keywords

LC-MS/MS, Acrylamide, Linearity, Accuracy, Precision, LOD, LOQ

1. Introduction

Food habits are changing rapidly due to variations of lifestyles, economies and global culture. Fried food items have gained its popularity among various age people, especially young generation because of its short time of preparation and consumption. To obtain acceptable food products, it is necessary to maintain nutrients like minerals, carbohydrates, fats, proteins, vitamins, enzymes, etc. But, nutrients may be destroyed or lost when foods are processed because of their sensitivity to heat, light, oxygen, pH of the solvent or a combination of these [1]. Even some protein quality, micronutrients, vitamin and amino acid can be reduced during frying of food [2]. Furthermore, to give satisfying taste and crunch to fried foods by using hydrogenated oils may influence the risk of several diseases like obesity, high blood pressure, high cholesterol, type-2 diabetes and heart disease, etc. [3] [4]. Recently, another concern with fried food is acrylamide, a food born chemical that forms naturally when cooked at high temperatures, believed to be metabolized to glycidamide and is a potential mutagen and as well as a reproductive toxicant [5]. At the beginning of the millennium in April 2002, the Swedish National Food Authority and the Stockholm University have first reported acrylamides formation in fried foods particularly in carbohydrate-rich products during processing [6]. Many agencies like The International Agency for Research on Cancer (IARC, 1994), The U.S. National Toxicology Program's (NTP) and The U.S. Environmental Protection Agency (EPA) have reported acrylamide as "probably carcinogenic to humans (Group 2A)" [7], "reasonably anticipated to be a human carcinogen" [8] and "likely to be carcinogenic to humans" [9], respectively; while The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have warned that the unintentional contaminant acrylamide in certain foods might be of public health concern since it has been shown to cause cancer in animals [10]. Acrylamide(C_3H_5NO; 2-propenamide), is a colorless, non-volatile crystalline solid, soluble in water and has molecular weight of 71.08 kDa [11] [12] is formed in food at high temperature through Millard Reaction, where reducing sugar such as glucose, fructose and asparagine, an amino acid; are believed to be the major precursors. A salient reaction pathway has been proposed in **Figure 1** [13] [14] [15]. In absence of asparagine, acrolein and acrylic acid, that are produced by degradation of lipids (triglycerides) due to high temperature [16] [17], and ammonia play the same role in lipid-rich foods to form acrylamide [18].

Among different validated analytical methods, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) are found to be prominent for the estimation of acrylamides in various foods [19] [20] [21]. Elimination of potential matrix interferences by Solid phase extraction (SPE) cleanup step; reduction of ion suppression by APCI mode and no requirements of derivatization have given major advantage in LC-MS/MS over GC-MS [22]. Moreover, studies conducted by different countries like Sweden, China, Hong Kong, Austria and Turkey [23]-[27] in surveying
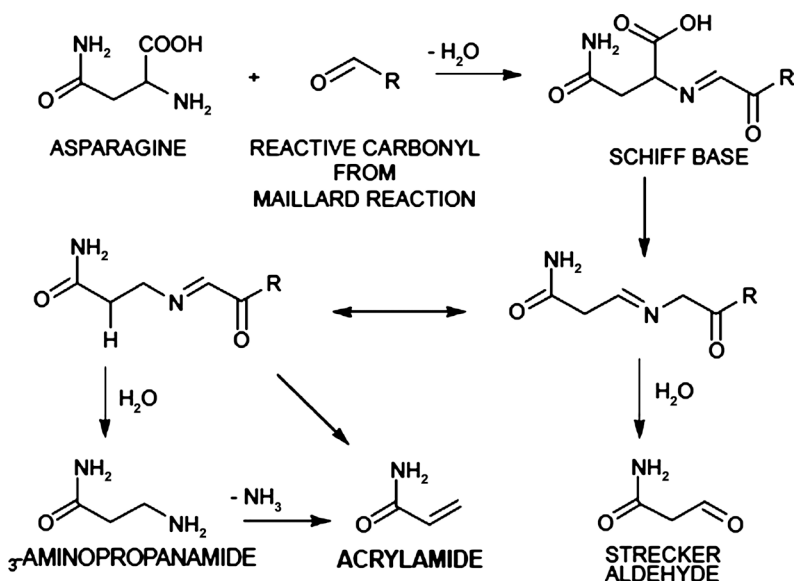
Figure 1. A salient reaction pathway for acrylamide formation in diet.

acrylamide content in various food products. However, no such study has ever been conducted in any food products in Bangladesh. Therefore, regarding the above circumstance the objective of the study was to develop a validated LC-MS/MS method for the determination of acrylamide in potato chips and other locally available foods.

2. Experimental

2.1. Standards and Chemical

Reference standard of Acrylamide (purity of 99.9%) and isotope labelled internal standard (IS) of Acrylamide d3 were purchased from Scharlu and Sigma-Aldrich, respectively. Methanol (MS Grade, purity of 99.9%), Acetonitrile (MS Grade, purity of 99.9%), Hexane (HPLC grade, purity of 97.09%) and Sodium Chloride (reagent grade, purity of 99.5%) were procured from Sigma-Aldrich. Formic acid (purity of 98%) analytical grade was purchased from BDH England, Magnesium Sulphate (purity of 99%) purchased from Merck, Primary secondary amine (PSA) procured from Agilent Technologies and highest purity demineralized water was used unless mentioned otherwise.

2.2. General Equipment

Metler Toledo analytical AL204 balance used from (China), Hwashin sonicator bath power sonic 505 and Vortex mixer 250VM (Korea), Hermle labortechnik GmbH Centrifuge Z206A (Germany), Bibby Scientific Block heater SBH130D/3 (UK), Whatman syringe filter 0.22 μm (UK) were used.

2.3. LC-MS/MS System

A quaternary liquid chromatographic system (Nexera X2 series) with a triple quadruple mass spectrometer-mass spectrometer equipped with an electrospray

interface and lab solution software (LCMS-8050, Shimadzu Corporation, Japan) were used in this study.

2.4. LC Condition

For acrylamide analysis Phenomenex C-18 (100 × 2.0 mm, 2.5 μm) column was used which maintained at 40˚C; mobile phase was mixture of two solution with gradient elution mode: B%: 1% (0 to 4.4 min)-80% (4.5 to 5.4 min)-1% (5.5 to 10 min) and delivered at flow rate of 0.2 mL/minutes; chromatographic injection volume was 10 μL and the retention time of acrylamide was about 1.8 minutes.

2.5. MS Conditions

The MS acquisition parameters were as follows: Run time: 10 minutes; Ion polarity: positive ion mode; ion source: atmospheric pressure electrospray ionisation; Capillary voltage (kV): 4.0; Block temperature: 400˚C; Dessolvation line temperature: 200˚C; CID gas: Argon (270 kPa), Nebulizing gas flow: N2, 1.5 L/min; Drying gas flow: N2, 10.0 L/min; Heating gas flow: 10 L/min; Interface temperature: 300˚C; mass spectrum scan range (m/z): 10 - 200 Da where protonated precursor ions of acrylamide and acrylamide-d_3 (IS) was m/z 72.1 and m/z 75.1 respectively. Two multiple reactions monitoring (MRM) transitions of acrylamide and acrylamide-d_3 were selected using a dwell time of 100 milli second as quantifier and confirmation ions as shown in below Table 1.

2.6. Preparation of Mobile Phase

Water and 0.1% formic acid in methanol were used as mobile phase. Both the solutions were degassed by sonication using ultra sonic bath and filtered through 0.22 μm filter membrane using the Millipore filtration unit.

2.6.1. Preparation of Stock Solution
1000 mg/L of acrylamide standard and 1.0 mg/L of IS stock solution were prepared standard in water. Both the solutions were sonicated for 10 minutes and stored at 2˚C - 8˚C for further preparation of calibration solutions.

2.6.2. Preparation of Intermediate Stock Solution
Intermediated stock solution of 10 mg/L and 1.0 mg/L of acrylamide and IS respectively were prepared by appropriate dilution of stock solutions with water.

Table 1. Multiple Reaction Monitoring (MRM) Transition of Acrylamide and IS.

Name	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Acrylamide-d_3	75.1 > 58.0*	−29	−15	−22
	75.1 > 30.1	−29	−24	−30
Acrylamide	72.1 > 55.0*	−17	−16	−25
	72.1 > 27.1	−17	−22	−30

*MRM transition as quantifier.

These were also undergone sonication for 10 minutes.

2.6.3. Preparation of Working Solution

1000 µg/L, 800 µg/L, 500 µg/L, 200 µg/L, 100 µg/L, 20 µg/L & 10 µg/L of acrylamide and 100 µg/L of IS standard were prepared by appropriate dilution of intermediated stock solutions. Prepared IS standard solution was added to each acrylamide standards in 50:50 ratio to prepare 500 µg/L, 400 µg/L, 250 µg/L, 100 µg/L, 50 µg/L, 10 µg/L and 5 µg/L acrylamide calibration solutions where 50 µg/L of IS was present in each of those. All the calibration standards were sonicated for 10 minutes in ultrasonic bath and then injected into LC-MS/MS for analysis.

2.7. Sample Preparation

A modified QuEChERS procedure was adopted in acrylamide extraction. Accurately weighed 2 grams of crushed powder sample were weighed accurately and taken into a 50 mL centrifuge tube in which 5 mL hexane, 10 mL water and 10 mL acetonitrile were introduced. Vigorously vortexed and shook for 2 minutes. The purpose of hexane was to defat the samples and to remove oils & non-polar components. Then Q-sep Q 100 Packet extraction salt (containing 4 g $MgSO_4$ and 0.5 g NaCl) and additional 4 g of $MgSO_4$ was added to absorb the water completely. Vortexed and shook again for another 5 minutes. 2 mL extract from the bottom layer was transferred into another 20 mL centrifuge tube were 100 mg primary secondary amine (PSA) was added to remove organic acids. Vortexed for 1 minute and then centrifuged at 11,000 rpm for 10 minutes. 1 mL aliquot was collected and evaporated to dryness under N2 stream. The dried sample was reconstituted with 1 ml of water and filtrated through 0.22 µm syringe filter. Then prepared sample solution and 100 ppb of IS was mixed in 50:50 ratio in a vial. This reconstituted solution and 100 µg/L IS mixed in 50:50 ratio was injected into LC-MS/MS for the determination of acrylamide in the sample.

2.8. Method Validation

The validation of LC-MS/MS method is performed using blank sample of potato chips as per guidelines of International Conference on Harmonization [28] with various parameters such as Specificity, Linearity, Precision, Accuracy, LOD, LOQ and Robustness.

2.8.1. Specificity or Selectivity

Selectivity of this method was demonstrated by making 5 µg/L and 250 µg/L acrylamide standard with acetonitrile instead of water and then analyzed by LC-MS/MS. The % RSD of retention time and area from six repeated injections at each concentration was calculated.

2.8.2. Linearity

Linearity was carried out at seven concentration levels ranging from 5 to 500 µg/L. A calibration curve was obtained by plotting the peak area vs. concentra-

tion. The % RSD from three repeated injections at each concentration and % y-intercept bias was calculated.

2.8.3. Accuracy

The accuracy of the method was evaluated at three concentration levels of acrylamide (250, 400 and 500 μg/L) by spiking into samples. The % recoveries reflect an average of six analyses at each concentration and % RSD was reported.

2.8.4. Precision

Precision was measured as intra-day precision (repeatability) and inter-day precision (reproducibility) of working standard. The intra-day and inter-day precision of the method was demonstrated by injection (in six analyses) of the standard on two consecutive days and % of RSD was calculated.

2.8.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the method were estimated on the basis of signal-to-noise ratio 3:1 and 10:1, respectively by injection series of diluted solution of known concentration. The % RSD from the six repeated injections was calculated.

2.8.6. Robustness

Robustness was studied at seven different concentration of acrylamide with change in some parameters such as column temperature, column type, injection volume and analyst. Percentage (%) RSD from six repeated injections at each concentration under change condition was calculated.

2.9. Quantification of Acrylamide in Different Types of Foods

Besides method validation, 24 types of food total 265 items of different batches represent the whole population, manufactured by different companies without revealing their names were collected in mid April 2017 from the local market randomly and prepared according to section 2.6 for quantification of acrylamide.

3. Results & Discussion

3.1. Method Development and Optimization

Chromatographic conditions like mobile phase composition, injection volume, flow rate and column oven temperature were optimized for better quantification of acrylamide in complex food matrix. Acrylamide exhibits both weakly acidic and basic properties. However, solubility varies considerably in polar and unipolar solvents whereas acrylamide is extremely high soluble in water [29] [30] and became fully protonated in the mobile phase. Method development under gradient conditions with different proportions of organic modifier was tried with change of flow rates for good separation of acrylamide. Finally, the mentioned composition of mobile phase and elution mode at flow rate 0.2 mL/min was found satisfactory. The optimized 10 μL injection volume, Phenomenex C-18 column and 40˚C column oven temperature returned the best result in separation of matrix with very little interferences and elution at about 1.8 minutes.

Atmospheric pressure electrospray ionization used as ion source for the determination of acrylamide, gave high intensity and good linearity of calibration curve. The parent and product ions were first optimized by injecting a 500 µg/L standard solution of acrylamide in both positive and negative polarity mode. Due to protonation and use of formic acid in mobile phase, the intensity was much higher in positive mode. Quantification of acrylamide was performed by using optimized MRM mode for consistent and adequate response where most abundant product ions m/z 55.0 and m/z 27.1 for the case of acrylamide precursor ion m/z 72.1 were observed by employing 16 eV and 22 eV collision energy, respectively. On the other side product ions at m/z 58.0 and m/z 30.1 was observed in the case of acrylamide-d$_3$ (IS) precursor m/z 75.1 by applying 15 eV and 24 eV collision energy, respectively. **Figure 2** and **Figure 3** represent the total ion chromatogram and mass spectra of product inos for acrylamide and acrylamide-d$_3$ (IS), respectively.

3.2. Method Performance Characteristics/Validation

3.2.1. Linearity

A seven-point calibration curve was constructed by plotting triplicate injected acrylamide concentration versus area over the range of 5 to 500 µg/L with a mean correlation coefficient (r^2) was 0.9997. The % RSD values of the repeated injection are <2% within each concentration and the Y-intercept bias is less than 2% which explains excellent linearity of the curve (**Figure 4**).

3.2.2. Precision

The precision was evaluated as the repeatability by calculating the percent relative
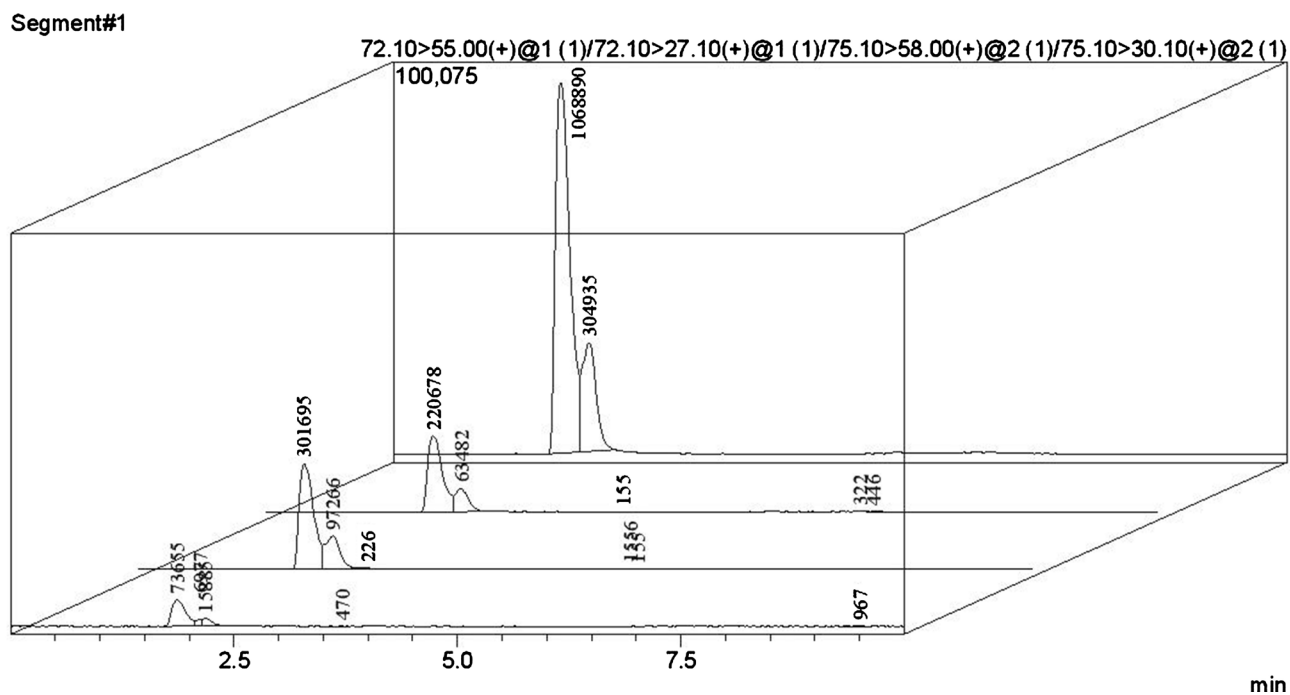

Figure 2. Breakdown of IS and Acrylamide by LC-MS/MS.

Name: IS
m/z: 75>58, 30

(a)

Mass Spectrum

Name: Acrylamide
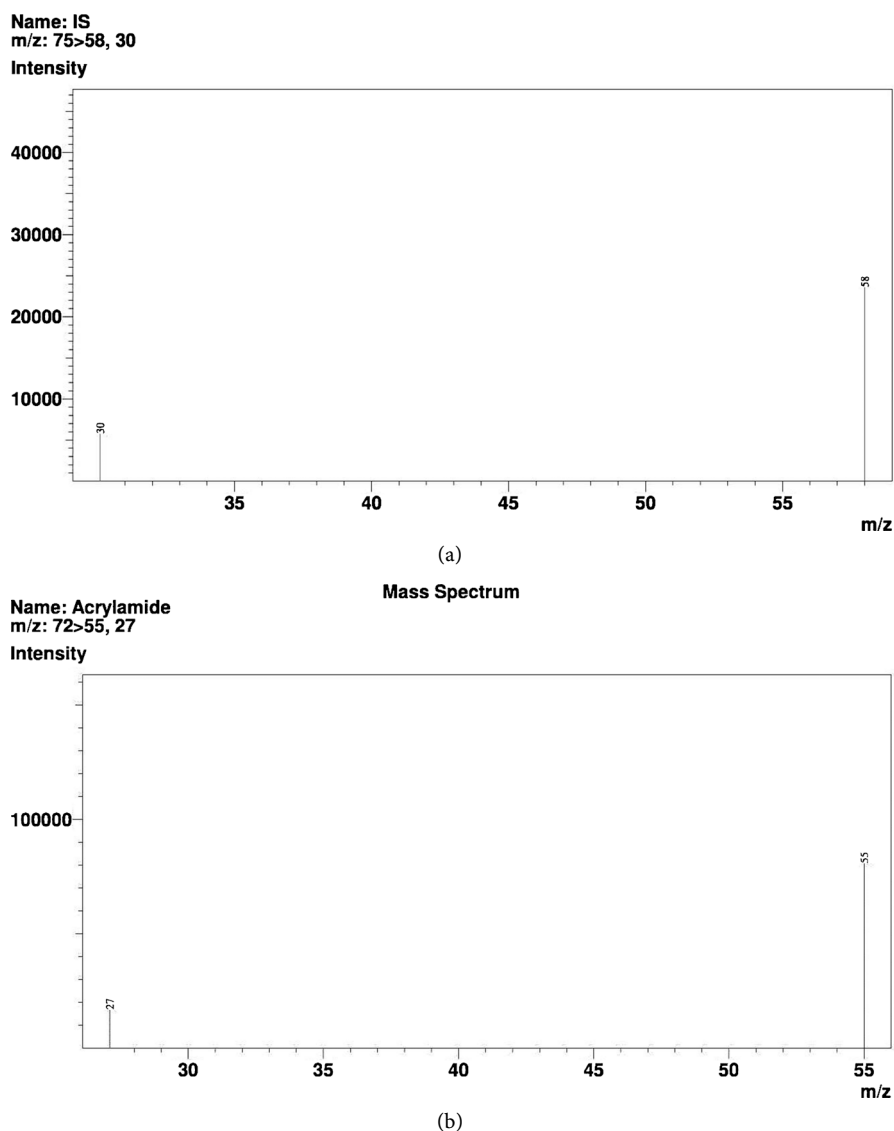**m/z: 72>55, 27**

(b)

Figure 3. Mass spectra of product ion for (a) acrylamide-d$_3$ (IS) (b) Acrylamide standard.

standard deviation (% RSD) for six determinations each for seven different solutions which were analyzed by three different analysts on the same day (Intra-day) and on the three different days (Inter-Day) as well (Table 2). Since the % RSD value is within the acceptable norms (<5) obtained for Intra-day and Inter-day at seven different concentrations, it can be said that the proposed method can be implemented for analysis.

3.2.3. Accuracy

The accuracy of the method was assessed by spiking different known concentration of acrylamide standard solution into sample. Standard solution of 250 μg/L, 400 μg/L and 500 μg/L was spiked into previously analyzed sample contained 7.05 μg/L acrylamide. The calculated and observed concentration of acrylamide was compared and recoveries of each spiked levels were determined (Table 3). It was found that the recoveries and % RSD for different spiked concentration laid

Calibration Curve

Name   : Acrylamide,   m/z   : 72.10>55.00
Quantitative Method   : Internal Standard
Function   : f(x)=0.364879*x+0.0610482
Correlation Coefficient=0.9998715
FitType   : Linear
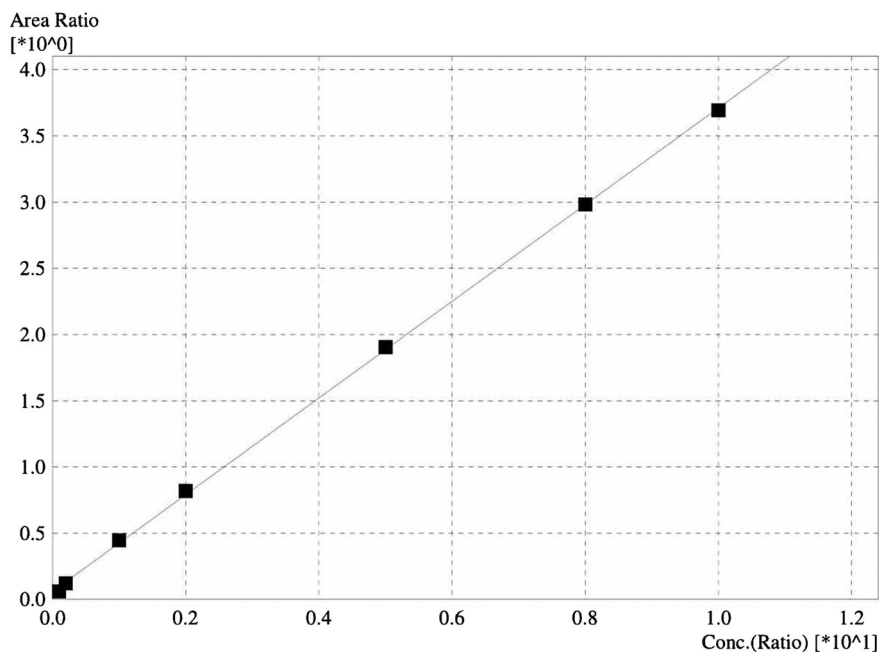ZeroThrough   : Not Through

Figure 4. Calibration curve for acrylamide from 5 to 500 μg/L.

Table 2. Precision studies for acrylamide determination.

| Standard Conc. (μg/L) | Intra-day precision | | Inter-day precision | | | | | |
	Mean observed conc. (μg/L) (n = 6)	% RSD	Days	Analyst	Mean observed conc. (μg/L) (n = 6)	Mean observed conc. (μg/L) (3 days)	% RSD	Mean % RSD
5	5	1.73	Day 1	Analyst-1	4.804	4.92	3.871	2.19
			Day 2	Analyst-2	4.95		1.977	
			Day 3	Analyst-3	5.00		0.716	
10	10.55	2.20	Day 1	Analyst-1	9.95	9.87	1.169	2.23
			Day 2	Analyst-2	9.80		2.730	
			Day 3	Analyst-3	9.85		2.779	
50	50.65	0.95	Day 1	Analyst-1	49.45	49.73	3.287	1.62
			Day 2	Analyst-2	49.7		1.643	
			Day 3	Analyst-3	50.05		1.984	
100	98.25	1.84	Day 1	Analyst-1	99.1	99.43	1.248	2.00
			Day 2	Analyst-2	99.2		1.587	
			Day 3	Analyst-3	100		3.142	

Continued

			Day 1	Analyst-1	248.8		0.368	
250	247.1	1.28	Day 2	Analyst-2	246.05	247.42	1.521	1.25
			Day 3	Analyst-3	247.4		1.833	
			Day 1	Analyst-1	393.25		2.522	
400	395.9	0.70	Day 2	Analyst-2	391.95	393.38	2.125	1.84
			Day 3	Analyst-3	394.95		0.890	
			Day 1	Analyst-1	486.45		1.532	
500	496.4	0.54	Day 2	Analyst-2	481.55	480.63	1.338	1.40
			Day 3	Analyst-3	473.9		1.338	

Table 3. Accuracy studies for acrylamide determination.

Acrylamide standard Conc.(µg/L)	Observed Conc. of Acrylamide standard (µg/L) (n = 3)	Analyte Conc. in sample (µg/L)(n = 6)	Total Conc. (Analyte Conc. in sample + Acrylamide standard Conc.) (µg/L)	Observed Conc. in sample after spiking (µg/L) (n = 6)	% Recovery	% RSD
250	265.9		272.95	261.55	104.36	0.96
400	398.45	7.05	405.5	410.7	98.73	1.08
500	489.1		496.15	511.85	96.93	0.81

between the range of 96% - 105% and 0.8 - 1.1, respectively; which is within the acceptable norms signifying the accuracy of the method.
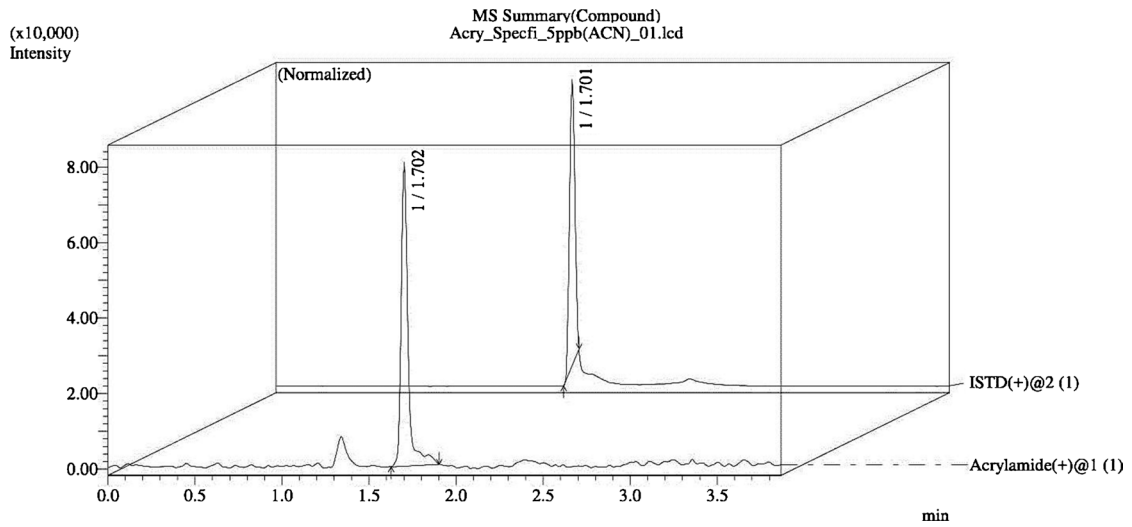
3.2.4. Selectivity

Selectivity of this method was demonstrated in the presence of acetonitrile instead of water. Six repeated injections of 5 µg/L and 250 µg/L of acrylamide standard prepared in acetonitrile along with 50 µg/L IS were analyzed by LC-MS/MS. A slight change at elution time (1.7 minutes) of acrylamide was observed with % RSD of only 0.17 to 0.23, which demonstrates that the method is selective and interferences free (**Figure 5**).

3.2.5. LOD & LOQ

LOD (Limit of Detection) is the lowest detectable but not quantifiable target analyte concentration and LOQ (Limit of Quantitation) is the lowest quantifiable concentration of target analyte with acceptable precision; was determined by considering signal to noise ratio (S/N) of the strongest mass transition with respect to background noise obtained from the blank (**Figure 6**). Signal to noise ratio (S/N) of 3:1 was taken for LOD whereas it was 6:1 for LOQ. Based on this ratio, the LOD and LOQ for acrylamide were obtained as 2.0 µg/L and 4.0 µg/L respectively with %RSD less than 5 for six replicates.

3.2.6. Robustness

By analyzing seven different concentration of acrylamide with change in some parameters such as column temperature, column type, injection volume and different analyst; the robustness of method was determined (**Table 4**). The
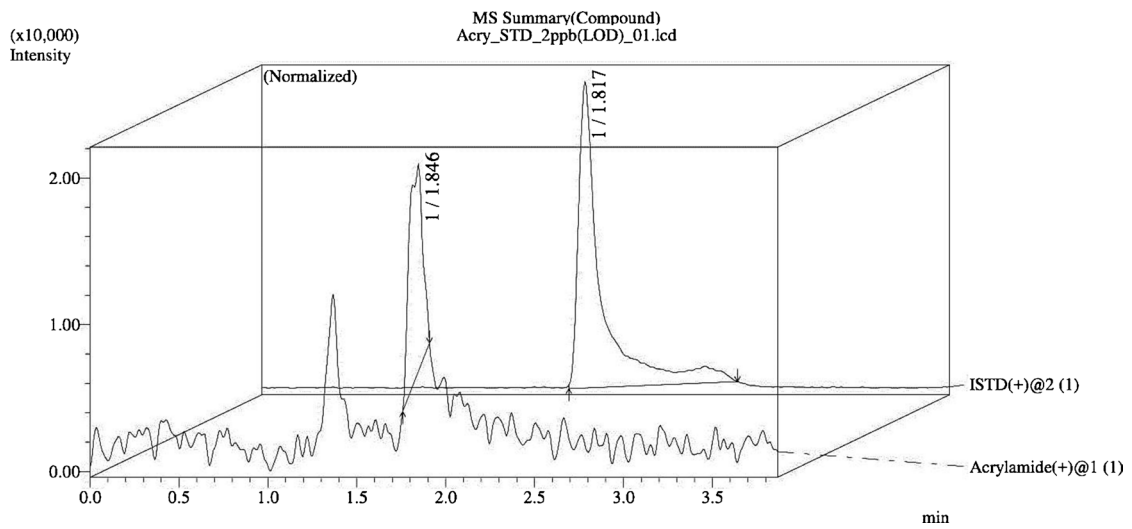
(a)
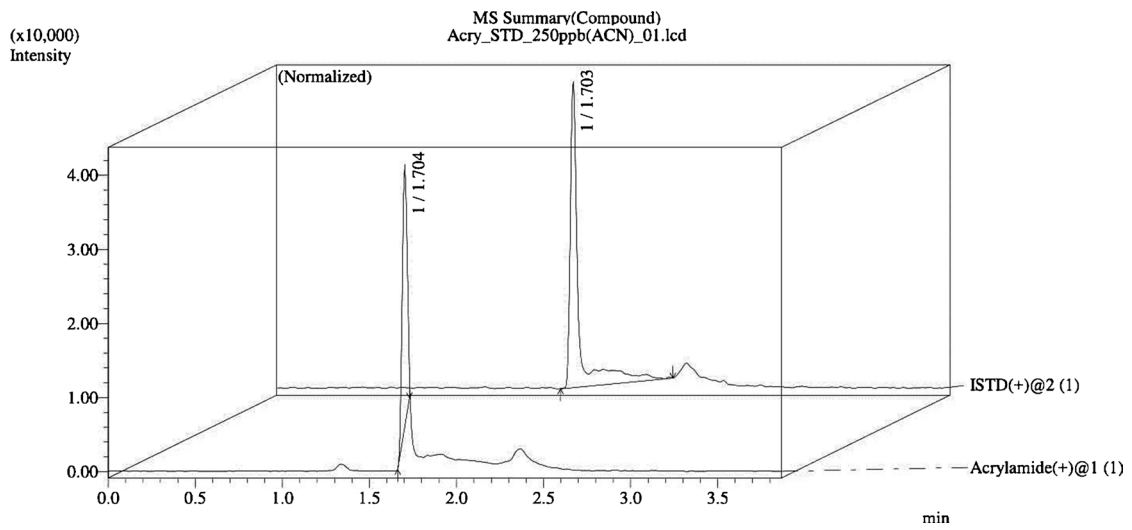
(b)

Figure 5. Total ion chromatogram of selectivity study where acetonitrile was used to analyze the effect on acrylamide standard.
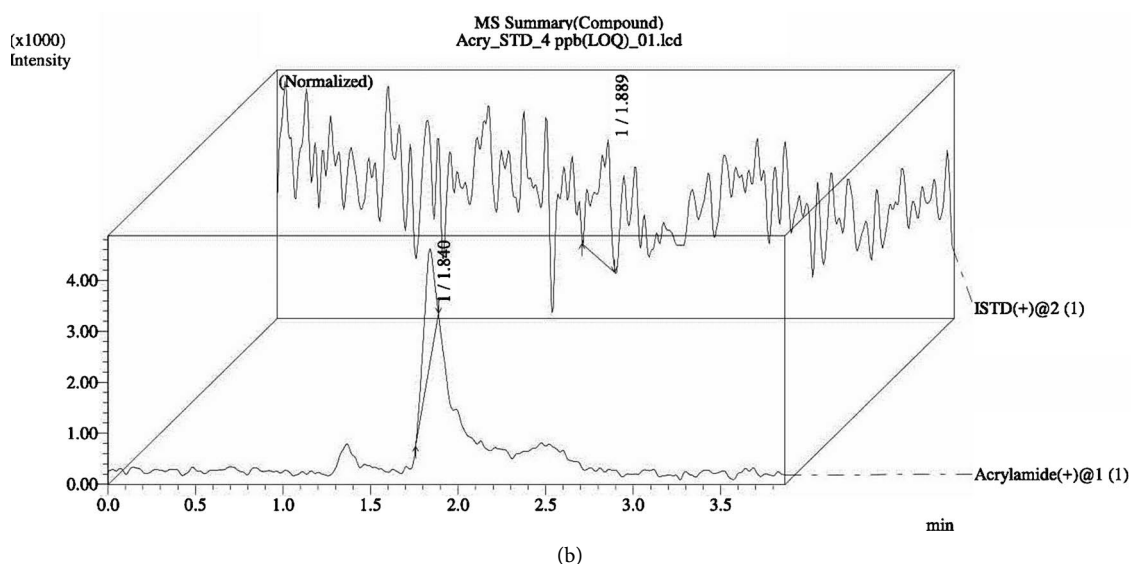

(a)

Figure 6. Chromatogram of (a) LOD and (b) LOQ for Acrylamide along with IS.

Table 4. Robustness studies for acrylamide determination under changed conditions.

Acrylamide standard concentration (μg/L)	Developed method		Change in operational method							
	Mean Conc. (μg/L) (n = 6)	RSD %	Column (Shim-pack XR-ODS II, C18, 2.0 mm × 100 mm, 2.2 μ)		Column temparature (35°C)		Injection volume (10 μl)		Analyst II	
			Mean Conc. (μg/L) (n = 3)	RSD %	Mean Conc. (μg/L) (n = 3)	RSD %	Mean Conc. (μg/L) (n = 3)	RSD %	Mean Conc. (μg/L) (n = 3)	RSD %
5	5.00	1.73	4.65	2.75	5.0	1.49	5.0	1.49	5.0	1.49
10	10.55	2.20	9.65	3.82	9.85	3.80	9.85	3.80	9.85	3.80
50	50.65	0.95	50.8	1.16	47.15	2.39	47.15	2.39	47.15	2.39
100	98.25	1.84	100.7	1.15	98.4	3.32	98.4	3.32	98.4	3.32
250	247.1	1.28	249.7	1.92	254.35	1.00	254.35	1.00	254.35	1.00
400	395.9	0.70	401.7	0.09	399.55	1.17	399.55	1.17	399.55	1.17
500	496.4	0.54	480.3	4.55	497.45	1.46	497.45	1.46	497.45	1.46

acrylamide content obtained by the developed method and that obtained under the mentioned change conditions, remained almost the same with % RSD less than 4.0 in every case.

3.3. Quantification of Acrylamide in Other Foods

The developed validate method of acrylamide quantification in potato chips by LC-MS/MS was also applied to analyze in other 265 food items like french fry, beef tikka, peyaju, chicken grill, flour chips, potato Biscuits, chanachur, cheese, puffs, coffee candy, chicken fry, puri, shik kabab, fuska, bread, brinjal fry, nut without shell, nut with shell, chicken nuggets etc. as shown in Table 5 and the frequency distribution of acrylamide content in foods is illustrated in Figure 7. 77% of the analyzed samples have returned positive levels of acrylamide ranging

Table 5. The acrylamide contents in foods using LC/MS/MS methods.

Item	Number of Sample	Samples positive	Level of Acrylamide (µg/L)			
			Min.	Max.	Mean	SD
Potato chips	52	44	44.97	252.79	131.48	62.78
French fry	43	43	162.63	244.67	197.04	22.92
Beef tikka	10	10	41.27	87.71	60.33	15.58
Peyaju	13	13	51.09	113.17	82.47	16.30
Chicken grill	11	11	142.72	169.98	156.29	9.45
Flour chips	5	5	18.12	25.17	21.19	2.82
Potato Biscuits	5	5	43.28	57.32	51.17	5.22
Chanachur	10	10	13.66	23.65	19.33	3.16
Cheese puffs	1	0	<DL			
Coffee candy	1	0	<DL			
Chicken fry	8	8	104.32	127.50	114.63	8.02
Puri	10	0	<DL			
Shik kabab	5	5	151.21	187.22	172.87	13.74
Fuska	7	7	17.77	23.29	20.43	1.92
Bread	9	0	<DL			
Brinjal fry	10	10	20.79	31.49	25.53	3.61
Nut without shell	5	0	<DL			
Nut with shell	7	0	<DL			
Chicken nuggets	5	0	<DL			
Fish fry	10	10	74.17	85.27	79.76	3.67
Egg fry	10	10	83.21	93.33	89.14	3.49
Boiled egg	5	0	<DL			
Cake	8	0	<DL			
Biscuit	15	14	10.72	57.73	35.23	16.80

from 19.33 to 197.04 µg/L, among which 54% were found to be more than 100 µg/L. Average acrylamide content in Chanachur was found to be lowest (19.33 µg/L) while in processed and cooked food like potato chips, french fries, Beef tikka, Peyaju, Chicken grill, Flour chips, Chicken fry, Shik kabab, fuska, Fish fry, Egg fry, Brinjal it was highest with average values of 20.43 - 197.04 µg/kg.

Acrylamide contents in baked products except for biscuit and other food items like cheese puffs, coffee candy, nut without shell, nut with shell, chicken nuggets and boiled egg were found to be below the detection limit. Levels of acrylamide in protein-rich food (79.76 - 89.14 µg/L) were higher than that of baked food products (35.23 - 51.17 µg/kg) indicating the occurrence of the acry-lamide contamination due to cooking procedures.

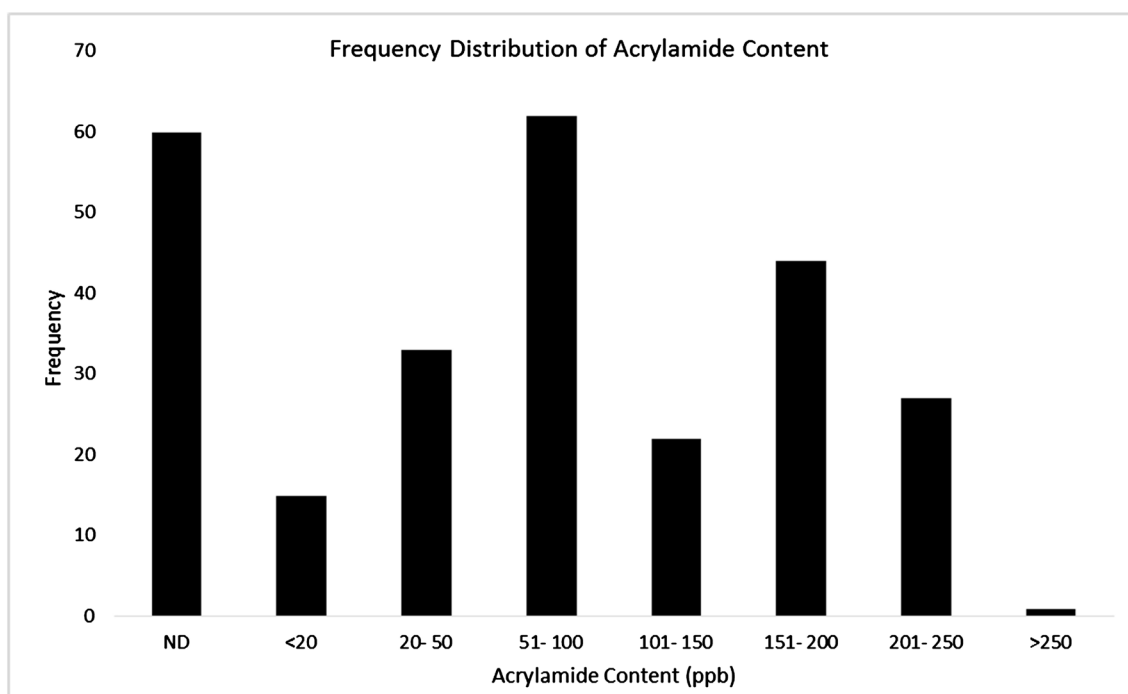Previous research also supports the above statement, that acrylamide may be

Figure 7. Frequency distribution of acrylamide content in food.

Table 6. Ranges of acrylamide in various food products [32].

Product	Acrylamide Range ($\mu g \cdot Kg^{-1}$)
Potato chips	117 - 2762
French Fries	20 - 1352
Breads & bakery products	10 - 364
Chocolate products	0 - 909
Nuts & nut butters	28 - 457
Protein foods	0 - 116
Canned fruit & vegetables	0 - 83

formed due to the differences in cooking and processing parameters, methods, type and quality of raw materials, as well as formulations [31]. The quantification of acrylamide investigated for the very first time in Dhaka, Bangladesh in different food was found to be within range and also reported by United States Food and Drug Administration (Table 6).

Though there is a declaration regarding daily allowable limit of acrylamide uptake (0.3 - 0.8 µg/kg body weight) [33], the allowable limit of acrylamide formation in food is yet to be set. It is high time to investigate current food processing practices or to develop new food processing techniques so that the acrylamide formation in food products can be minimized for the sake of public health especially for our future generations.

4. Conclusion

The developed LC-MS/MS method for the determination of acrylamide is simple, rapid, sensitive accurate and precise and can be applicable for routine analysis. The method is mainly applicable for starch-based products but can also be adopted for other sample matrices as well. Participation in Proficiency Testing might help to get a clear view about the applicability of this method. Besides, the highest acrylamide levels were found in fried products, such as potato chips, french fries, chicken grill and sheikh kabab, which indicated the general risk on public health due to toxicity of acrylamide from foods. This investigation supplied important information on acrylamide in food for public health experts, food policy makers and consumers. Further research is suggested to find out the correlation among temperature, sugar and asparagine, and also to minimize the acrylamide formation during the processing of food.

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Conflicts of Interest

All of the authors are employees of Designated Reference Institute for Chemical Measurements. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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