Acetone Extraction and HPLC Determination of Acrylamide in Potato Chips

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A new sensitive method using high performance liquid chromatography (HPLC) and liquid extraction for the analysis of acrylamide (AA) in potato chips is reported. The method comprises extraction with acetone using ultrasonic bath and reversed phase C18-AQ (2×250 mm) column with water as eluent. Flow rate was 0.15 ml min^{-1} and the column temperature was kept constant at 40 °C. The analysis was performed using a 20 µl injection loop and a UV detector adjusted at 202 nm. In this condition, the retention time for AA was 8 min. A linear calibration curve (regression coefficient = 0.999) in the range of 20-400 ng g^{-1} was used for quantitative purposes. Limit of detection (LOD) (signal-to-noise ratio of 3:1) and limit of quantification (LOQ) (signal-to-noise ratio of 10:1) for the method was 2.46 and 3.14 ng g^{-1}, respectively. Extracted samples and standard solutions with different concentrations of AA were analyzed repeatedly in one day and different days to estimate the repeatability and reproducibility of the method. Analysis of variance on the obtained data showed no significant difference between variances in different days. Using the proposed method, different potato chips samples were analyzed in different days in another laboratory. Paired t-test showed no significant difference between the obtained results from the two laboratories.

Keywords: Acrylamide, HPLC, Determination, Potato chips, Acetone extraction

INTRODUCTION

In April 2002, researchers from the University of Stockholm and Swedish National Food Administration (NFA) reported high levels of acrylamide (AA) or 2-propenamide in a wide range of fried and oven-cooked foods [1]. AA is a known genotoxic compound and has been classified as "a probable carcinogenic to humans" by the International Agency for Research on Cancer (IARC). It causes tumors in laboratory animals [2] and nerve damage in people who have been exposed to high doses. AA is found in many different foods. Different mechanisms have been suggested for its formation [3,4].

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As it is stated by JIFSAN working group, the development and validation of sensitive and reliable analytical methods for the low level quantification of AA in different food matrices is considered essential [5]. There are a number of different methods that have been developed for the analysis of AA. The widely applicable analytical techniques for the determination of AA include liquid chromatography [6-8], gas chromatography [9,10], and for a better identification of AA, mass spectrometry coupled with gas chromatography [11-16] or liquid chromatography [17-22].

Among the variety of developed methods, good sensitivity and selectivity are reported to be achieved by chromatographic methods coupled with a mass technique. Although different methods have been developed for the analysis of AA in a number of food matrices, no "universal" extraction and

cleanup procedures have been reported to be valid for many different food matrices. Potato products, and especially potato chips, have been shown to include high levels of AA in different investigations; hence our work focuses on the determination of AA in potato chips. Having in mind that mass techniques usually require time-consuming derivatisation steps and/or exhaustive cleanup to achieve selective detection at low molecular weight, the main aim of the present work is to develop a low-cost and simple HPLC method for the analysis of AA in potato chips with a good sensitivity and reliability as is necessary for routine monitoring of AA [23]. The HPLC method has the advantages that the transfer of AA into a volatile organic solvent, or the removal of water after aqueous extraction, is not necessary as in GC techniques.

EXPERIMENTAL

Reagents and Solutions

AA (purity > 99.9%) and other chemicals (highest purity) were obtained from Merck (Darmstadt, Germany) and used without further purification.

Stock solution of AA (1000 µg ml^{-1}) was prepared by dissolving 0.1000 g of AA in deionized water and diluted to 100 ml in a volumetric flask. The successive 100 µg ml^{-1} and 2 µg ml^{-1} stock solutions of AA were prepared from this solution. Working standard solutions of AA (20, 40, 80, 160, 240, 320 and 400 ng ml^{-1}) were prepared by appropriate dilution of 2 µg ml^{-1} stock solution with water. All stock solutions and working standards were stored at 4 °C maximum for 1 month. The chips samples were purchased from local stores and were of different brands.

Apparatus

All determinations were made by a Knauer (Germany) HPLC system consisting of a K-1001 pump and a k-2501 UV detector. A Sonica (Italy) ultrasonic bath was used throughout.

Procedure

Finely ground and homogenized chips sample (4.0 g) were weighed into a closed flask, defatted twice by adding 10 ml hexane and shaking for 5 min. The mixture was dried under vacuum, after decantation.

For the extraction of AA, 20 ml of acetone and 100 µl of water were added to the defatted sample. The flask was placed in an ultrasonic bath at 40 °C for about 20 min. The acetone was filtered through a filter paper. Gently, 10 ml of the filtrate was evaporated under vacuum to dryness. Then, 2 ml of water was added and shaken thoroughly to dissolve the residue. The aqueous solution was filtered through a filter paper and injected to the column using a 20 µl injection loop. The column used was C18-AQ, 2 × 250 mm. Flow rate was 0.15 ml min^{-1} and the column temperature was kept constant at 40 °C. The analysis was performed at 202 nm with a UV detector. In this condition, the retention time for AA was 8 min.

RESULTS AND DISCUSSION

Different methods were used for the extraction of AA from potato chips. These could be divided into two main categories: extraction with water or with an organic solvent. Various solvents such as ethyl acetate, diethyl ether, acetone and dichloromethane were examined. With respect to the applied HPLC method, in all cases the extracted AA was finally transferred to an aqueous phase prior to injection.

For chloroform, ethyl acetate and dichloromethane, the extraction of AA from the matrix was not complete, phase separation (extraction of AA in water) was difficult and the final solution became diluted. The chromatograms after extraction with water, dichloromethane, 2-butanol, n-butanol and pantanol showed too many interfering peaks. Diethyl ether did not extract AA from the matrix but water and acetone showed suitable and measurable AA peaks. Therefore, acetone was chosen as the best solvent for its great range of ability to extract the AA with minimum interferences from potato chips matrix.

The spherimage-C6- 80, 250 × 4.6 mm; nucleosil-C18-100, 4 × 10 mm; Tskgel ODS-120T, 250 × 4.6 mm and C18-AQ, 2 × 250 mm columns were tested. The C18-AQ column was chosen because of its appropriateness for the separation of AA from matrix peaks.

Different eluent compositions (pure water or mixed with different portions of acetonitrile or methanol) were used. Generally, increasing organic solvents in the eluent composition decreased the retention time for AA and its peak overlapped with other peaks. More resolved peaks were obtained when pure water was used as eluent.

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The analysis at different oven temperatures ranging from 20 to 40 $^{\circ}$C showed an increase in AA retention time with a decrease in temperature.

A typical chromatogram of AA extracted from potato chips sample with and without spike is shown in Fig. 1.

Analytical Characteristics

A linear calibration curve in the range of 20-400 ng g^{-1} was obtained using the proposed procedure. The equation of the line was A = 0.0295 C-0.060 (regression coefficient = 0.9995), where C is AA concentration in ng g^{-1} and A is the AA chromatogram peak area. The AA chromatogram peaks for different concentrations are shown in Fig. 2.

Limit of detection (LOD) (signal-to-noise ratio of 3:1) and limit of quantification (LOQ) (signal-to-noise ratio of 10:1) for the HPLC method was 2.46 and 3.14 ng g^{-1}, respectively. The relative standard deviation (n = 10) for determination of 160 ng g^{-1} was 4.01%.

Validation of the Method

Recovery tests for controlling the analytical method and quantification were repeatedly performed by quantification of AA in different chips samples before and after the addition of AA (0-300 μg kg^{-1}). A blank value was determined by performing the whole analysis without addition of AA to the sample. The results are summarized in Table 1.

Extracted samples and standard solutions with different
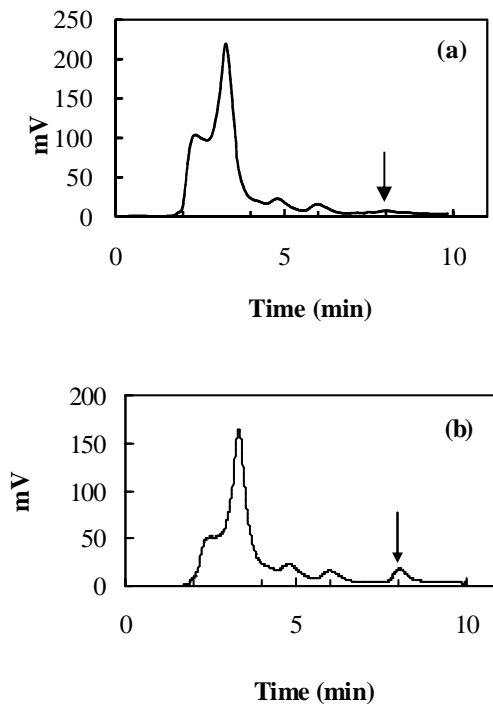
Fig. 1. Typical chromatogram of AA extracted from potato chips sample (a) without spike (b) 200 μg kg^{-1} spike.

concentrations of AA were analyzed repeatedly in one day and in different days to estimate the repeatability and reproducibility of the method (Table 2 and Table 3). The
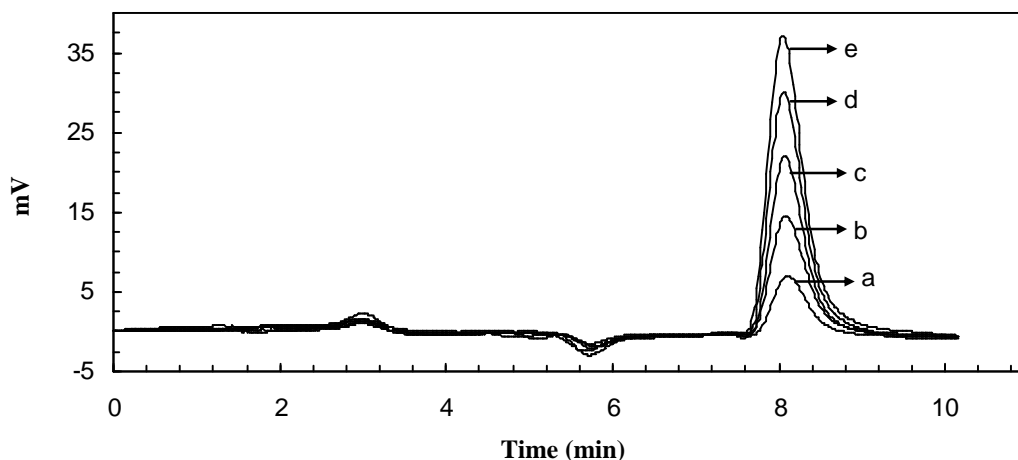
Fig. 2. Typical chromatogram of (a) 80, (b) 160, (c) 240, (d) 320 and (e) 400 ng ml^{-1} standard solutions of acrylamide.

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Table 1. Recovery Test at Different Spike Levels for Two Different Samples

Sample	No. of analysis	Spike level ($\mu g\ Kg^{-1}$)	Found value ($\mu g\ Kg^{-1}$)	Recovery (%)	Standard deviation ($\mu g\ Kg^{-1}$)	Relative standard deviation (%)
A	5	0	97.45	-	4.23	4.34
A	3	100	186.28	88.83	12.46	6.69
A	3	300	351.04	84.53	9.15	2.61
B	4	0	23.86	-	2.32	9.73
B	3	50	72.86	98.01	4.86	6.68
B	3	150	171.41	98.37	6.75	3.94
B	3	250	265.97	96.85	5.97	2.24

Table 2. Result of Four Analysis per Day of 160 and 320 ng ml^{-1} of AA Standard Solutions in Different Days

Statistic results	320 ng ml^{-1} sample			160 ng ml^{-1} sample		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mean (ng ml^{-1})	323.39	317.70	324.96	161.17	163.63	158.16
Standard deviation (ng ml^{-1})	8.55	6.90	8.42	2.18	6.56	6.54
Relative standard deviation (%)	2.64	2.17	2.59	1.35	4.01	4.10

Table 3. Result of 9 Analysis per Day of an Extracted Sample in Different Days

Statistic results	Day 1	Day 2	Day 3
Mean ($\mu g\ Kg^{-1}$)	237.47	245.39	235.82
Standard deviation ($\mu g\ Kg^{-1}$)	8.89	7.36	7.10
Relative standard deviation (%)	3.74	3.00	3.01

analysis of variance on the obtained data showed no significant difference between variances in different days ($\alpha = 0.05$, $F_{Crit.} = 5.715$, $F_{Exp.}$ for 160 $\mu g\ ml^{-1} = 0.624$; $F_{Exp.}$ for 320 $\mu g\ ml^{-1} = 0.885$).

Using the proposed method, different potato chips samples were analyzed in different days in another laboratory at 0-300 $\mu g\ kg^{-1}$ spike levels. The results are summarized in Table 4.

Paired t-test method was used to compare the systematic error between the obtained data from both laboratories which showed no significant difference (n = 21, $\alpha = 0.05$, $t_{Crit.} = 2.09$, $t_{Exp.} = 0.852$).

The proposed method was compared with another method to verify its efficiency for the extraction of AA from potato chips matrix. The other method was based on extraction with methanol and the use of Carrez solutions [15]. The obtained results showed no significant difference (n = 15, $\alpha = 0.05$, $t_{Crit.} = 2.14$, $t_{Exp.} = 1.602$).

CONCLUSIONS

The proposed method could successfully be applied for the routine analysis of AA in potato chips due to its high

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Table 4. Analysis of Different Potato Chips Samples in Different Days in another Laboratory

Sample	No. of analysis	Spike level (μg kg^{-1})	Found value (μg kg^{-1})	Recovery (%)	Standard deviation (μg kg^{-1})	Relative standard deviation (%)
C	3	0	104.97	–	4.19	3.99
C	3	100	188.06	85.39	14.50	7.71
C	3	300	383.42	93.59	5.95	1.55
D	4	0	25.36	–	3.49	13.75
D	3	50	68.03	85.35	5.04	7.41
D	3	150	176.59	100.80	7.10	4.02
D	4	200	237.01	105.82	9.34	3.94
D	3	250	252.69	90.99	19.34	7.65

Table 5. Comparison of the Presented Method with some Previously Reported Works

System	LOD (ng g^{-1})	RSD (%)	Ref.
Water extraction; HPLC column switching	10	1.5-5.2	[6]
GC-MS (bromination) or LC-MS/MS	5	5	[11]
Methanol extraction; LC-DAD Two columns	4	<5	[7]
Water extraction, GC-MS (SIM)	9	0.8- 3.9	[13]
Water extraction, GC-MS	2	<10	[16]
Acetone extraction-HPLC	2.46	4.01	This work

sensitivity and the use of fewer chemicals. Meanwhile, the present HPLC analytical method requires a relatively low-cost instrumentation compared with LC and GC-tandem MS methods. The method was fully validated using different statistical tests. Acetone was used for the extraction of AA from potato chips for the first time. The proposed method has better or comparable limit of detection and RSD values compared with some of the previously reported works (Table 5). This analytical method was successfully applied to the determination of acrylamide in potato chips without the use of any derivatization procedure with a good level of sensitivity and recovery.

ACKNOWLEDGEMENTS

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