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Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography

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Abstract

A simple and rapid method using liquid chromatography coupled to diode array detection (LC–DAD) was developed for the determination of acrylamide in potato-based foods at low levels. The method entails extraction of acrylamide with methanol, purification with Carrez I and II solutions, evaporation and solvent change to water, and cleanup with a Oasis HLB solid-phase extraction (SPE) cartridge. The final extract was analyzed by LC–DAD for quantification and by liquid chromatography coupled to mass spectrometry (LC–MS) for confirmation. The chromatographic separations were performed on a hydrophilic and a hydrophobic interaction columns having good retention of acrylamide under 100% aqueous flow conditions (k' 3.67 and 2.54, respectively). The limit of quantitation was estimated to be 4.0 μg/kg based on the signal-to-noise ratio of 3 recorded at 226 nm. Recoveries of acrylamide from potato chips samples spiked at levels of 250, 500 and 1000 ($n = 4$ for each level) μg/kg ranged between 92.8 and 96.2% with relative standard deviations of less than 5%. The results of this study revealed that a conventional LC instrument coupled to DAD can also be used accurately and precisely, as an alternative to tandem LC–MS methods for the determination of acrylamide in potato-based foods.

Keywords: Acrylamide; Potato chips; Methanol extraction; Improved retention; UV detection

1. Introduction

Detection of high concentrations of acrylamide in common heated starch-rich foodstuffs by the Swedish National Food Administration in April 2002 attained considerable public concern, since acrylamide was found to be carcinogenic in rodents and is classified as a probable human carcinogen [1,2]. Potato products, such as French fries and chips, were among the food items containing highest amounts of acrylamide [3]. Acrylamide formation was found to occur during the browning process by Maillard reaction of reducing sugars with asparagine at temperatures above 120 °C. The major reactants leading to the release of acrylamide are sugars and asparagine [4–8]. The potential health risk of acrylamide in food has been considered by a number of government agencies and national authorities. The expert working groups at international level have identified and listed a number of research gaps and priorities to allow a better assessment of health risk associated with acrylamide [9,10]. However, no maximum permitted concentration has been established for acrylamide in processed foods yet.

Numerous analytical methods have been developed in the past years to determine acrylamide monomer in water, biological fluids and non-cooked foods based on high performance liquid chromatography (LC) or gas chromatography (GC) [11–17]. However, LC-based methods were not found to be appropriate for the analysis of acrylamide in processed foods at low levels, and many researchers believe that LC must be coupled to mass spectrometry (MS) for better identification of acrylamide in processed foods. To date, the analytical methods dealing with the analysis of acrylamide in processed foods are based on MS as the detection technique coupled with a chromatographic step either by LC [18–26] or GC [27–31].

Although MS is a selective system for detection, the mass of acrylamide itself or its fragment ions are not specific due

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to presence of co-extractives that yield the same magnitude of *m*/*z* with acrylamide in the sample matrix. These interferences could not be completely avoided in these methods despite the use of tandem MS due to poor retention of polar acrylamide molecule on conventional LC reversed-phase sorbents. Therefore, the efforts were directed to modify sample preparation to develop an efficient clean-up to avoid interferences prior to tandem MS detection by some researchers [1,6,18,20–22].

When using MS detection coupled with LC, the aqueous mobile phase was used to composed of organic acids (formic, acetic, etc.) and organic modifiers (acetonitrile, methanol, etc.) to increase ionization yield and reproducibility [20–26]. According to our results, acidification had no effect on the retention of acrylamide, but, the addition of acetonitrile or methanol into aqueous mobile phase resulted in much shorter elution times during the chromatographic analysis. So, it seems very difficult to overcome the poor retention gap during LC analysis with MS detection.

This paper presents a reliable, sensitive, fast and low-cost analytical method for the determination of acrylamide in potato-based processed foods such as chips, crisps and fries. The method utilizes LC with UV detection that can be easily adopted by non-specialized analytical laboratories. The sample preparation entails the extraction of acrylamide from food by methanol, precipitation of colloids by Carrez reagents and solid-phase extraction (SPE) cleanup prior to LC analysis. Acrylamide is resolved from the co-extractives using both hydrophilic and hydrophobic interaction chromatography and detected at 226 nm. LC–MS analyses with atmospheric pressure chemical ionization (APCI) confirm the results obtained by LC–DAD analyses.

2. Experimental

2.1. Chemicals and consumables

Acrylamide (>99%) and $^{13}C_3$-labelled acrylamide (99% isotopic purity) were obtained from Sigma (Deisenhofen, Germany) and Cambridge Isotope Labs. (Andover, MA, USA), respectively. Methanol, potassium hexacyanoferrate and zinc sulfate were of analytical grade and obtained from Merck (Darmstadt, Germany). Bidistilled, deionized and 0.20 μm filtered water was used throughout the experiments. Oasis HLB (1 ml, 30 mg) SPE cartridges were supplied by Waters (Milford, MA, USA). Glass vials with septum screw caps were supplied by Agilent Technologies (Wilmington, DE, USA). Certified reference test material (FAPAS T 3007 potato crisps) were obtained from CSL (Central Science Lab., UK) to verify the accuracy of method.

2.2. Standards and reagents

Stock solution of acrylamide (1 mg/ml) and $^{13}C_3$-labelled acrylamide (0.1 mg/ml) by dissolving in distilled water. Working standards for LC–DAD analysis were prepared by diluting the stock solution of acrylamide to concentrations of 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 μg/ml with distilled water. Stock solutions and working standards were kept at 4 °C for a month. 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.

2.3. Sample preparation

Finely ground potato chips (2 g) were weighed into a 30 ml centrifuge tube. The sample was spiked with acrylamide (250, 500 and 1000 μg/kg) to determine the percentage recovery of the method at this stage. For LC–MS confirmatory tests, the sample was also spiked with $^{13}C_3$-labelled acrylamide as the internal standard. The sample was suspended in 10 ml of methanol and homogenized for 3 min with a homogenizer (Heidolph, Germany). The suspension was centrifuged at 10 000 rpm (11 180 × *g*) and 10 °C for 10 min. The clear supernatant was transferred into a centrifuge tube and treated with Carrez I and II solutions (100 μl each) to precipitate the co-extractives (*It is important to separate the solid residue from the methanolic extract prior to purification with aqueous Carrez solutions. This will exclude the transfer of water soluble co-extractives into the methanolic extract*). Following centrifugation at 10 000 rpm (11 180 × *g*) and 10 °C for 5 min, 2.5 ml of clear supernatant (0.5 g sample) was quantitatively transferred into a conical bottom glass test tube placed in a water bath at 40 °C and evaporated to dryness under a gentle stream of nitrogen (*The presence of residual water from the Carrez solutions will prevent the loss of acrylamide during evaporation by retaining it on the wall of glass tube*.). The remaining residue was immediately redissolved in 1 ml of water by mixing in a vortex mixer for 2 min. By changing the solvent, the acrylamide residue was completely transferred into water while lipids and lipid soluble co-extractives were retained on the wall of glass tube. For the SPE cleanup, Oasis HLB cartridge was preconditioned consequently with 1 ml of methanol and 1 ml of water at rate of two drops per second using a syringe. Then, 1 ml of the extract was passed through the cartridge at a rate of one drop per second using a syringe. The first seven to eight drops of the effluent were discarded to prevent any dilution of sample by replacing water held in the sorbent void fraction with the sample effluent. The forthcoming drops were collected and filtered through a 0.45 μm syringe filter. Twenty μl of the final test solution was injected onto LC column for both quantification by LC–DAD or confirmation by LC–APCI–MS.

2.4. LC–DAD analysis for quantification

The quantification of acrylamide was performed by an Agilent 1100 model HPLC system (Waldbronn, Germany) consisting of a quaternary pump with vacuum degasser, a

Table 1
Capacity factors and plate numbers calculated for the separation of acrylamide on different LC columns[a]

Column	Dimensions	t_R	k'	N
Atlantis HILIC	250 mm × 4.6 mm, 5 μm	8.9	3.67	17 373
Atlantis dC_{18}	250 mm × 4.6 mm, 5 μm	13.8	2.54	19 944
Zorbax SIL	250 mm × 4.6 mm, 5 μm	8.4	1.80	19 944
Zorbax Stable-Bond C_{18}	250 mm × 4.6 mm, 5 μm	11.2	2.39	19 250
HiChrom $5C_{18}$	300 mm × 4.6 mm, 5 μm	10.9	1.79	18 233
Luna C_{18}	250 mm × 4.6 mm, 5 μm	12.6	1.93	5 232
Synergi MAX-RP	250 mm × 4.6 mm, 4 μm	12.6	1.74	8 076

[a] Mobile phase: 0.5 ml/min at 25 °C in each case.

temperature controlled column oven and a DAD. The chromatographic separations were performed on either Atlantis dC_{18} or HILIC columns (Milford, MA, USA). During the routine analyses of food samples, the flow rate of water was adjusted to 1.0 ml/min (at 25 °C) for Atlantis dC_{18} and to 0.5 ml/min (at 5 °C) for Atlantis HILIC. Acrylamide was detected at 226 nm with continuous monitoring the peak spectra within the range of 190–350 nm for spectral confirmation and peak purity check. For comparison, Zorbax StableBond C_{18} and Zorbax SIL from Agilent Technologies, Synergi MAX-RP and Luna C_{18} from Phenomenex (Torrance, CA, USA) and $5C_{18}$ from HiChrom (UK) were also tested for the separation of acrylamide in the food matrix. Water at a flow rate of 0.5 ml/min was used as the mobile phase at room temperature for comparison analyses. Types and dimensions of columns tested for the separation of acrylamide are given in Table 1.

2.5. LC–MS analysis for confirmation

The confirmatory analyses for acrylamide were performed by an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler and a temperature controlled column oven, coupled to an Agilent 1100 MS detector equipped with atmospheric pressure chemical ionization (APCI) interface. The analytical separation was performed on a Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm) using the isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid and 0.2% acetic acid in acetonitrile (98:2, v/v) at a flow rate of 0.6 ml/min. The LC eluent was directed to the MS system after a delay time of 6.5 min using MSD software. Data acquisition was performed in selected-ion monitoring (SIM) mode using the interface parameters: drying gas (N_2) flow of 4 l/min, nebulizer pressure of 55 psig (1 psig = 6894.76 Pa above atmospheric pressure), drying gas and vaporizer temperatures of 320 °C, capillary voltage of 3 kV, corona current of 8 μA, fragmentor voltage of 55 eV. Ions monitored were m/z 72 and 55 for acrylamide and m/z 75 and 58 for $^{13}C_3$-labelled acrylamide for the quantification of acrylamide in the samples.

3. Results and discussion

3.1. Test of columns for acrylamide retention

Acrylamide is very polar molecule with poor retention ($k' < 2.0$) in conventional LC reversed phase sorbents. Therefore, LC-based methods with UV detection seem to be not suffice for the analysis of acrylamide in processed foods at low levels due to lack of selectivity. Since an additional degree of analyte certainty is required to confirm the presence of acrylamide in the complex food matrix, MS became the choice of detection step coupled to LC. However, analytical responses for compounds other than acrylamide were found to be present in the MS chromatograms (m/z 72 > 55) of certain type of food samples such as potato chips and coffee. Therefore, it was noted that more effort should be placed on efficient cleanup to avoid interferences for an accurate quantification despite the use of tandem MS [20–24].

Since the aqueous mobile phase was used to modified with organic acid and acetonitrile or methanol to increase ionization yield and repeatability, the retention of acrylamide in the analytical column cannot be improved when using MS coupled to LC. On the other hand, our results showed that the retention of acrylamide could be improved by both hydrophilic and hydrophobic interaction chromatography by avoiding organic modifiers like acetonitrile and methanol in the aqueous mobile phase.

Numbers of normal- and reversed-phase columns were tested for their ability to retain acrylamide at the beginning of this study. The capacity factors and plate numbers calculated for acrylamide on these LC columns are given in Table 1. Using water as the mobile phase at a flow rate of 0.5 ml/min, only three columns (Atlantis HILIC, Atlantis dC_{18} and Zorbax Stable Bond C_{18}) were found to have k' values exceeding 2.0 without any band broadening (N > 17 000). Among them, the aqueous normal phase (Atlantis HILIC column) designed for the retention of polar analytes exhibited the highest k' for acrylamide. However, the k' of acrylamide in the conventional normal phase (Zorbax SIL column) was almost two times lower than that obtained using the aqueous normal phase. The reversed-phase C_{18} columns greatly differed in their ability to retain acrylamide. Only Atlantis dC_{18} and Zorbax Stable-Bond C_{18} columns had k' values slightly higher than 2.0. Poor retention of polar analytes on conventional reversed-phase sorbents under highly aqueous conditions are usually associated with the reduction of void volume due to dewetting of pores.

The resolution of acrylamide from the interfering co-extractives was tested for the LC columns in potato chip matrix. Fig. 1 illustrates the chromatograms of potato chips obtained by using Atlantis HILIC, Atlantis dC_{18}, Zorbax StableBond C_{18} and Zorbax SIL columns. In the chromatograms shown in Fig. 1, acrylamide peak could be very well resolved from unidentified co-extractives in these columns under the chromatographic conditions applied, while the others (HiChrom $5C_{18}$, Luna C_{18} and Synergi Max-RP) failed to

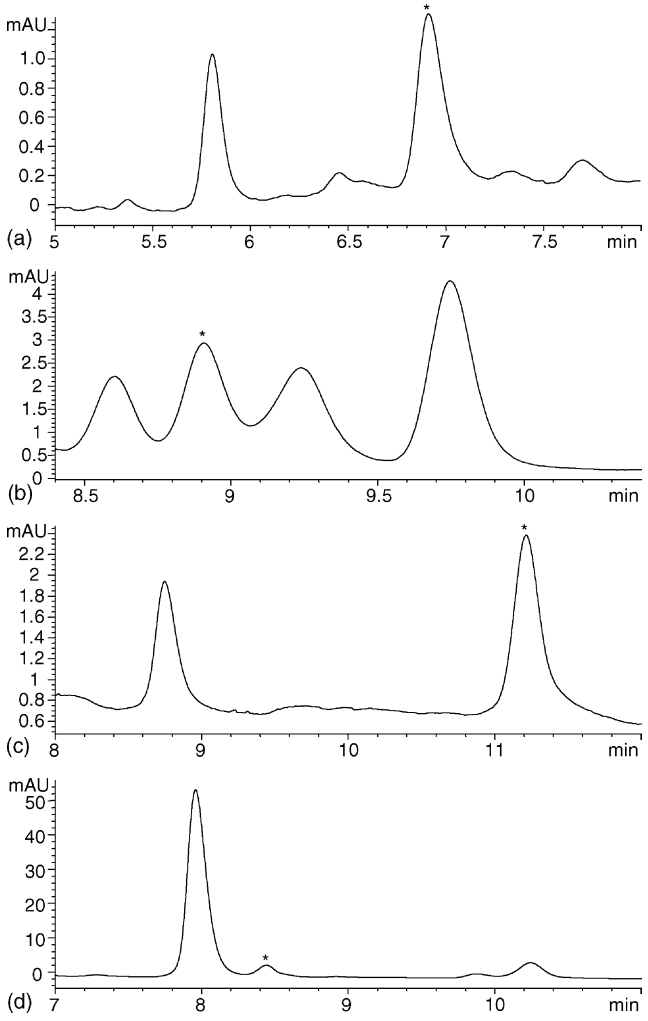

Fig. 1. Chromatograms of acrylamide in potato chips. Marks show acrylamide peak at a concentration of 1020 μg/kg. (a) Column: Atlantis dC_{18}; mobile phase: 1.0 ml/min of water at 25 °C. (b) Column: Atlantis HILIC; mobile phase: 0.5 ml/min of water at 5 °C. (c) Column: Zorbax StableBond C_{18}; mobile phase: 0.5 ml/min of water at 25 °C. (d) Column: Zorbax SIL; mobile phase: 0.5 ml/min of water at 25 °C.

resolve acrylamide from the interferences due to excessive band broadening (data not shown).

As confirmed by peak purity check using spectral analysis between the wavelength range of 190–350 nm, increasing the flow rate from 0.5 to 1.0 ml/min resulted in poorer resolution of acrylamide peak from the matrix co-extractives in the columns with an exception for the Atlantis dC_{18} column. Increasing the flow rate not only decreased the run time in Atlantis dC_{18} column, but also improved the peak shape.

Even though the best capacity factor was obtained during the separation on Atlantis HILIC column for acrylamide, the resolution of sample co-extractives from acrylamide was not good in this column at 25 °C. The interfering peak overlapped with acrylamide at 25 °C could be resolved by lowering the mobile phase temperature to 5 °C.

The analyses of acrylamide in potato chips for recovery were performed on Atlantis dC_{18} and HILIC columns.

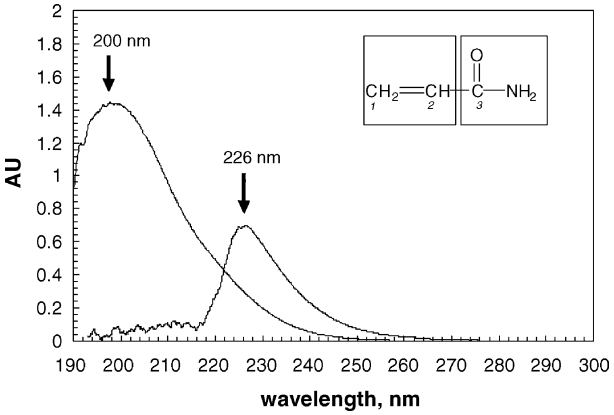

Fig. 2. UV absorbance spectra of acrylamide recorded against water blank (peak maximum at 200 nm) and 0.1 mM acetic acid solution blank (peak maximum at 226 nm).

Both columns had repeatable retention times for acrylamide over two months. The retention time of acrylamide was 6.915 ± 0.006 min on Atlantis dC_{18} column at a flow rate of 1.0 ml/min at 25 °C and 8.905 ± 0.009 min on Atlantis HILIC column at a flow rate of 0.5 ml/min at 5 °C.

3.2. Detection sensitivity of DAD

Since acrylamide is a derivative of carboxylic acid, it has a maximum absorption within the wavelength range of 195–205 nm. However, all co-extractives from the food matrix also absorb well in this wavelength range adversely affecting the detection accuracy. On the other hand, acrylamide has also a characteristic absorption at 226 nm due to double bond between C_1 and C_2 (Fig. 2). Comparing to the absorbance at 200 nm, the absorbance of acrylamide was almost two times lower at 226 nm. However, it can be analyzed at 226 nm in complex food matrices like potato chips with more precision and accuracy.

The response of DAD at 226 nm was linearly changed with the concentration of acrylamide within the range of 0.1–2.0 μg/ml ($y = 35.563x + 0.4821$, $r^2 = 0.999$). Taking into account the instrumental noise recorded at 226 nm, the limit of detection (LOD) and limit of quantification (LOQ) was calculated to be 2.0 μg/ml and 4.0 μg/kg, respectively, on the basis of a signal-to-noise ratio of 3. Additionally, acrylamide concentrations of 20 μg/kg could be successfully detected in potato chips as illustrated in Fig. 3.

3.3. Efficiency of sample preparation procedure

Because acrylamide is highly soluble in water (215.5 g/100 ml), the sample preparation was usually started by extracting the ground sample with water enough for a proper swelling in most of the methods based on LC coupled to tandem MS detection system [1,6,18,20–24]. Some researchers defatted the sample with hexane to improve swelling properties, thus extractability [25,26]. Since potato and cereal-based

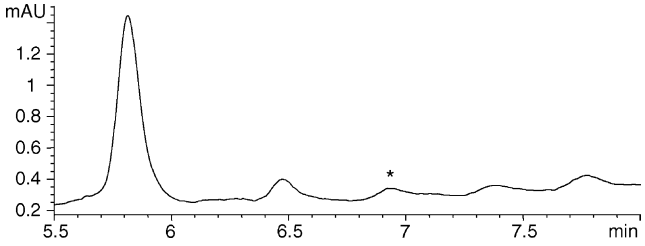

Fig. 3. Chromatogram of potato chips containing an acrylamide level of 20 μg/kg. Column: Atlantis dC_{18}, mobile phase: 1.0 ml/min of water at 25 °C.

food matrices contain high amounts of starch, water extraction forms a very viscous solution which is difficult to obtain a clear supernatant by centrifugation. Increasing the amount of water used for extraction decreases the concentration of acrylamide in the extract, and thus increases LOQ.

On the other hand, acrylamide is also highly soluble in methanol (155.0 g/100 ml) and much compatible with the food matrices containing high amounts of fat such as potato chips (30–35%) as an extracting solvent. Since methanol does not extract starch and some other polysaccharides, it yields a much more clear extract than that of water even without centrifugation. In addition, it can be easily evaporated under a gentle stream of nitrogen to improve the LOQ by concentration.

In the view of these facts, the sample preparation prior to LC–DAD analysis of acrylamide in potato chips consisted of extraction with methanol. The colloids which were soluble in methanol (like alcohol soluble proteins) were precipitated by Carrez reagents. Since their solutions were prepared in water, the addition of Carrez I and II also adds some water into the methanolic extract that can act as an extractant for water soluble molecules present in the sample. Therefore, the solid residue was separated from the methanolic extract by centrifugation prior to Carrez clarification. Carrez clarification not only purified the extract by precipitation of dissolved colloids, but also prevented the loss of acrylamide during the evaporation under a gentle stream of nitrogen. The evaporation of methanolic solution acrylamide standard to dryness under the gentle stream of nitrogen resulted in a significant loss of acrylamide (∼30%). However, 200 μl of water added into approximately 10 ml of methanolic extract in the form of aqueous Carrez I and II solutions had a significant contribution to retain acrylamide residue on the wall of glass tube during evaporation. Biedermann et al. [29] have also noted the similar problem and suggested the addition of edible oils to all samples to prevent the loss of acrylamide during evaporation.

Following evaporation to complete dryness, the residue on the wall of glass tube was redissolved by water in a vortex mixer. By changing solvent from methanol to water, lipids and highly lipophilic co-extractives present in the methanolic extract were excluded leaving them as a residue on the wall of glass tube, but acrylamide was completely transferred into water. The extract was further cleaned up by using Oasis HLB SPE cartridge. Since acrylamide does not interact with the sorbent material strongly, the pass through strategy for the SPE cleanup step was applied to retain only the matrix interferences which were hydrophobic in nature. It was determined that first seven to eight drops (∼0.4 ml) should be discarded, then collecting the remaining effluents (∼0.6 ml) during SPE cleanup using Oasis HLB (30 mg, 1 ml). It was clear from Fig. 4 that the first drops of the eluate from the cartridge had lower concentrations of acrylamide because of the dilution with water left in the cartridge following conditioning. The accuracy of method was verified by analyzing both spiked potato chips and certified reference material (FAPAS T3007 potato crisps). The recovery of acrylamide were determined by analyzing each of the spiked sample ($n = 4$) four times for levels ranging from 250 to 1000 μg/kg. Recovery samples were prepared by spiking very light colored potato chips containing an acrylamide level less than 25 μg/kg. The mean percentage recoveries exceeded 90% for all spiking levels for potato chips (Table 2). Six independent measurements of the certified reference material averaged to an acrylamide concentration of 1667.3 μg/kg (satisfactory range of 1306–2381 μg/kg) with a relative standard deviation (R.S.D.) of 3.3%. These results indicated a high accuracy and precision of the method used for the analysis of acrylamide in potato chips and crisps.

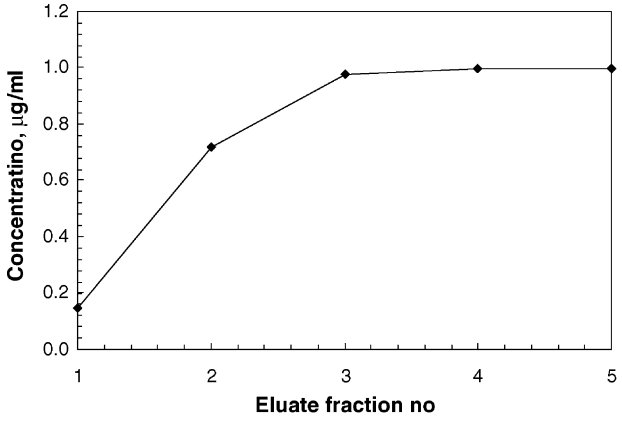

Fig. 4. The change of acrylamide concentrations in the eluate fractions collected during the elution through Oasis HLB cartridge. Sample loaded onto the cartridge was 1.0 ml of acrylamide working standard solution at a concentration of 1.0 μg/ml (the volume of each fraction was 0.2 ml).

3.4. Confirmation by LC–MS

Fig. 5 illustrates the chromatograms of potato chips spiked with both natural acrylamide and $^{13}C_3$-labelled acrylamide

Table 2
Recovery of acrylamide from potato chips

Spiking level (μg/kg)	Recovery (%)	R.S.D. (%)	*n*
250	96.2	4.1	4
500	93.6	2.3	4
1000	92.8	2.2	4

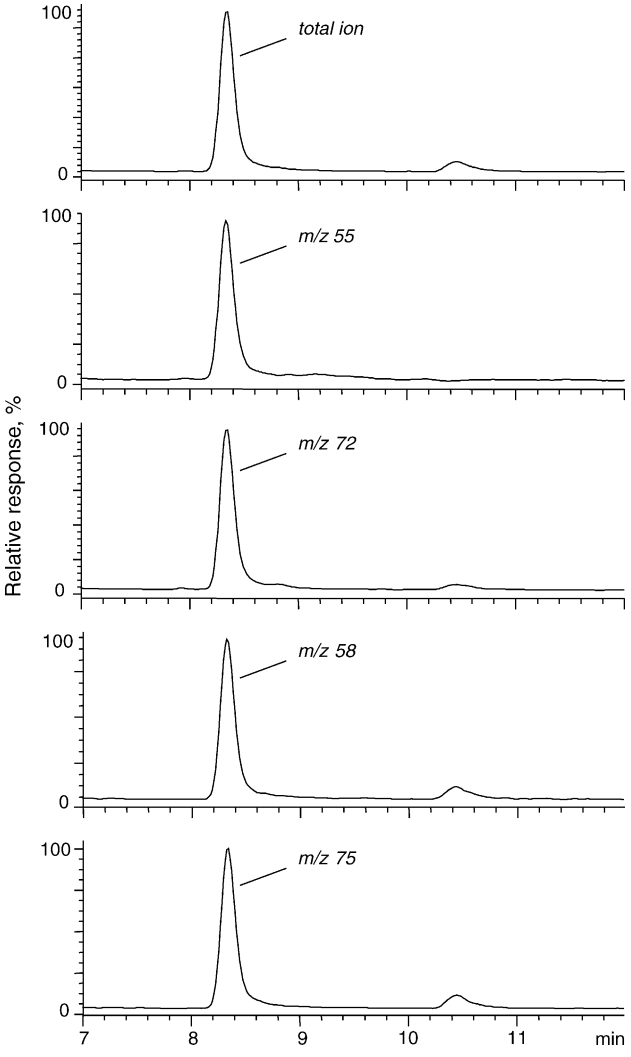

Fig. 5. LC–MS chromatogram of potato chips spiked with acrylamide and $^{13}C_3$-labelled acrylamide at a level of 1000 μg/kg.

to confirm the results obtained by LC–DAD analyses. The LC–MS chromatograms showed the presence of co-extractives giving signal in SIM mode both at *m*/*z* of 72 and 55 as reported earlier by many researchers [1,6,18,20–24]. In our study, much of these co-extractives were excluded by applying a delay time of 6.5 min during the LC–MS analyses. Calculations for LC–MS confirmation were performed using the responses of *m*/*z* 72 and 55 (acrylamide) and *m*/*z* 75 and 58 ($^{13}C_3$-labelled acrylamide).

4. Conclusion

This work describes a quantitative analytical method for the determination of acrylamide in potato-based foods. It requires a relatively low-cost instrumentation to perform when compared to tandem MS detection-based methods already published, and can be adopted by many laboratories worldwide easily. The sample preparation is simple and rapid utilizing methanol extraction, Carrez clarification, concentration and SPE cleanup steps prior to LC–DAD analysis. The improved retention in both hydrophilic and hydrophobic interaction chromatography columns using water as the mobile phase allow a successful resolution of acrylamide from the matrix co-extractives. The diode array detector set at 226 nm enables to quantify as low as 20 μg/kg of acrylamide in potato chips.

In summary, the results of a series of accuracy, precision and sensitivity tests together with the results of LC–MS analyses confirm the applicability of this method to potato-based foods. Consequently, this method should be regarded as a new, low-cost alternative for routine analysis of acrylamide in potato-based food (chips, fries, crisps, etc.). Further developments are now being conducted to expand the applicability of the method to a wide range of different food products.

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References

[1] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, J. Agric. Food Chem. 50 (2002) 4998.
[2] IARC. Acrylamide Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals, vol. 60, International Agency for Research on Cancer, Lyon, 1994, pp. 389–433.
[3] UK Food Standards Agency, Food Standards Agency study shows acrylamide in food; http://www.foodstandards.gov.uk/, 2002.
[4] D.S. Mottram, B.L. Wedzicha, A.T. Dodson, Nature 419 (2002) 448.
[5] R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.-C. Robert, S. Riediker, Nature 419 (2002) 449.
[6] A. Becalski, B.P.-Y. Lau, D. Lewis, S.W. Seaman, J. Agric. Food Chem. 51 (2003) 802.
[7] R. Weisshaar, B. Gutsche, Deut. Lebensm. Rundsch. 98 (2002) 397.
[8] D.V. Zyzak, R.A. Sanders, M. Stojanovic, D.H. Tallmadge, B.L. Eberhart, D.K. Ewald, D.C. Gruber, T.R. Morsch, M.A. Strothers, G.P. Rizzi, M.D. Villagran, J. Agric. Food Chem. 51 (2003) 4782.
[9] European Commission. Scientific Committee on Food: Opinion of the Scientific Committee on Food on New Findings Regarding the Presence of Acrylamide in Food, 3 July 2002, Brussels, 2002.
[10] FAO/WHO Consultation on the Health Implications of Acrylamide in Food. Summary Report of a Meeting held in Geneva, 25–27 June, WHO, Geneva, 2002.
[11] J. Tekel, P. Farkas, M. Kovác, Food Addit. Contam. 6 (1989) 377.
[12] L. Castle, J. Agric. Food Chem. 41 (1993) 1261.
[13] US EPS, SW 846, Method 8032A, US Environmental Protection Agency, Washington, DC, 1996.
[14] L.S. Bologna, F.F. Andrawes, F.W. Barvenik, R.D. Lentz, R.E.J. Sojka, J. Chromatogr. Sci. 37 (1999) 240.
[15] D.S. Barber, J. Hunt, R.M. LoPachin, M. Ehrich, J. Chromatogr. B 758 (2001) 289.

[16] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, Chem. Res. Toxicol. 13 (2000) 517.
[17] K. Kawata, T. Ibaraki, A. Tanabe, H. Yagoh, A. Shinoda, H. Susuki, A. Yasuhara, J. Chromatogr. A 911 (1) (2001) 75.
[18] J. Rosén, K.-E. Hellenäs, Analyst 127 (2002) 880.
[19] J.S. Ahn, L. Castle, D.B. Clarke, A.S. Lloyd, M.R. Philo, D.R. Speck, Food Addit. Contam. 19 (2002) 1116.
[20] J.A. Roach, D. Andrzejewski, M.L. Gay, D. Nortrup, S.M. Musser, J. Agric. Food Chem. 51 (2003) 7547.
[21] S. Riediker, R.H. Stadler, J. Chromatogr. A 1020 (2003) 121.
[22] M. Croft, P. Tong, D. Fuentes, T. Hambridge, Food Addit. Contam. 21 (2004) 721.
[23] D. Andrzejewski, J.A. Roach, M.L. Gay, S.M. Musser, J. Agric. Food Chem. 52 (2004) 1996.
[24] F. Calbiani, M. Careri, L. Elviri, A. Mangia, I. Zagnoni, J. AOAC Int. 87 (2004) 107.
[25] M. Murkovic, J. Biochem. Biophys. Methods (in press).
[26] K. Hoenicke, R. Gatermann, W. Harder, L. Hartig, Anal. Chim. Acta 520 (2004) 207.
[27] F. Höfler, R. Maurer, S. Cavalli, GIT Labor-Fachz. 48 (2002) 986.
[28] C. Gertz, S. Klostermann, Eur. J. Lipid Sci. Technol. 104 (2002) 762.
[29] M. Biedermann, S. Biedermann-Brem, A. Noti, K. Grob, P. Egli, H. Mändli, Mitt. Lebensm. Hyg. 93 (2002) 638.
[30] H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaka, H. Kobayashi, M. Yoshido, Food Addit. Contam. 20 (2003) 215.
[31] A. Pittet, A. Perisset, J.-M. Oberson, J. Chromatogr. A 1035 (2004) 123.