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Interference-free determination of acrylamide in potato and cereal-based foods by a laboratory validated liquid chromatography–mass spectrometry method

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Abstract

A simple and rapid method was developed and validated for the determination of acrylamide in potato and cereal-based foods by using a single quadrupole liquid chromatography–mass spectrometry (LC–MS) interfaced with positive atmospheric pressure chemical ionization (APCI+). Acrylamide was simply extracted with 0.01 mM acetic acid in a vortex mixer prior to LC–MS analysis. The applicability of validated method was shown for a wide range of processed foods including chips, fries, crisps, breads, biscuits and cookies. The mean recovery was found to be 99.7 with a repeatability of 1.8% in the range 100–1000 ng/g. During LC–MS analyses, the major interfering co-extractive was identified as valine which yields characteristic $[M + H]^+$ and compound specific product ions having *m/z* of 118 and 72, respectively. Valine increased the baseline signal preventing accurate and precise quantitation, and resulted in poorer sensitivity in selected ion monitoring mode. The adverse effect of valine could be limited by instrumentally adjusted delay time or by solid-phase extraction with strong cation-exchanger sorbent.

Keywords: Acrylamide; Interference; Valine; LC–MS; Delay time; Validation

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 (IARC, 1994; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Potato products, such as French fries and chips, were among the food items containing highest amounts of acrylamide (UK FSA, 2002). However, no maximum permitted concentration has been established for acrylamide in processed foods yet. Acrylamide formation was found to occur during the browning process by Maillard reaction of reducing sugars with asparagine at temperatures above 120 °C (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Weisshaar & Gutsche, 2002; Zyzak et al., 2003). 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 (EC, 2002; FAO/WHO, 2002).

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

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gas chromatography (GC) (Bologna, Andrawes, Bavenik, Lentz, & Sojka, 1999; Castle, 1993; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2000; US EPS, 1996). 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 (Ahn et al., 2002; Andrzejewski, Roach, Gay, & Musser, 2004; Croft, Tong, Fuentes, & Hambridge, 2004; Hoenicke, Gatermann, Harder, & Hartig, 2004; Murkovic, 2004; Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003; Rosén & Hellenäs, 2002; Riediker & Stadler, 2003) or GC (Biedermann, Biedermann-Brem, Noti, & Grob, 2002; Gertz & Klostermann, 2002; Hofler, Maurer, & Cavalli, 2002; Ono et al., 2003; Pittet, Perisset, & Oberson, 2004).

Although MS is a selective system for detection, the mass of acrylamide itself or its fragment ions are not specific due to presence of co-extractives that yield the same magnitude of *m*/*z* with acrylamide in the sample. 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 avoid interferences prior to tandem MS detection by some researchers. The effects of various solid-phase extraction (SPE) sorbents have been reported previously to improve MS detection (Becalski et al., 2003; Croft et al., 2004; Riediker & Stadler, 2003; Roach et al., 2003; Rosén & Hellenäs, 2002; Tareke et al., 2002). However, these reports do not contain a useful information regarding the chemical nature of these co-extractives.

This paper presents a reliable, sensitive and rapid LC–MS method for the determination of acrylamide in processed foods. The interfering co-extractives during the LC–MS analysis in selected ion monitoring (SIM) mode are successfully eliminated by instrumentally adjusting a delay time or by SPE cleanup. The study was experimentally designed: (i) to show the laboratory validation test results using potato chips; (ii) to show the effect of delay time to avoid early eluting peaks; (iii) to identify the chemical nature of interfering co-extractives; (iv) to show the applicability of method for a wide variety of foods.

2. Experimental

2.1. Chemicals and consumables

Acrylamide (99+%) and $^{13}C_3$-labelled acrylamide (99% isotopic purity) were obtained from Sigma (Diesenhofen, Germany) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Methanol, potassium hexacyanoferrate, zinc sulfate, formic acid (98%) and acetic acid (glacial) were analytical grade and obtained from Merck (Darmstadt, Germany). HPLC gradient grade acetonitrile was obtained from J.T. Baker (Deventer, Holland). Ultra pure water was used throughout the experiments (MilliQ system, Millipore, Bedford, MA, USA). Oasis MCX (30 mg) SPE cartridges were supplied by Waters (Milford, MA, USA). The analytical column (Inertsil ODS-3, 250 × 4.6 mm, 5 μm) was supplied by HiChrom (Berkshire, England). Reference test materials (FAPAS T3007 potato crisps and T3002 potato chips) were obtained from CSL (Central Science Laboratory, UK) to verify the accuracy of method.

2.2. Standards and reagents

Stock solution of acrylamide (1.0 mg/ml) and $^{13}C_3$-labelled acrylamide (0.1 mg/ml) by dissolving in distilled water. Working standards were prepared by diluting the stock solution of acrylamide to concentrations levels of 10, 25, 50, 100, 250, 500, 1000, 2000 μg/l with 0.01 mM acetic acid. Each solution contained 100 μg/l of $^{13}C_3$-labelled acrylamide. Carrez I and II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 ml of water, respectively.

2.3. Sample preparation

Dry samples like potato chips, breakfast cereal, cookies by mill and wet samples like bread were dried at 50 °C after grinding. French fries were reduced in size by using a mincer. Finely ground food sample (1 g) was weighed into a 10-ml glass centrifuge tube with cap. The sample was spiked with acrylamide to achieve the final concentrations of 100, 250, 500 and 1000 ng/g and/or with $^{13}C_3$-labelled acrylamide to achieve the final concentrations of 250 and 1000 ng/g, to determine recovery at this stage. Hundred microlitre Carrez1 and 100 μl Carrez 2 solutions were added and volume completed to 10 ml with 0.01 mM acetic acid. After mixing in a vortex mixer for 2 min, the mixture was centrifuged at 5000 rpm for 10 min at −5 °C. The clear supernatant was transferred into a vial avoiding the top fatty layer if present. The interfering co-extractives in the test sample were eliminated by (i) instrumentally adjusted delay time or (ii) SPE clean-up.

(i) *Delay time.* In order to discard early eluting peaks, the first 6.5 min of the chromatogram was diverted to waste by means of software controlled divert valve as it prevents contamination of the mass spectrometer with co-extractives.

(ii) *SPE clean-up*. The sample was passed through an Oasis MCX SPE cartridge preconditioned by passing 1 ml of methanol and 1 ml of water. After discarding the first 10 drops, the eluate was collected.

The final sample was filtered through 0.45-μm nylon syringe filter and analyzed by LC–MS analysis.

2.4. LC–MS

The LC–MS analyses for the quantitation of 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 APCI interface. The analytical separation was performed on a Inertsil ODS-3 column (250×4.6 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. Data acquisition was performed in SIM mode using the interface parameters: drying gas (N_2) flow of 4 l/min, nebulizer pressure of 55 psig, 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. Full scan analyses were performed in the mass range of 50–100 and 50–210 for the spectral identification of acrylamide and sample co-extractives, respectively.

2.5. Laboratory validation and analytical quality assurance

Three batches of potato chips each comprising eight spiked samples, two blanks and a quality control sample were analyzed at spiking levels of 100, 250, 500 and 1000 ng/g. Three batches for each of the three spiking levels were analyzed using the method described above over a period of 3 days.

Analytical quality assurance measures were employed for acrylamide which involved inclusion in each batch of eight potato chips spiked at 100, 250, 500 and 1000 ng/g and a quality control sample (FAPAS test materials, oven potato chips T3007 and potato crisps T3002).

3. Results and discussion

3.1. Laboratory validation of LC–MS method

Previous studies have shown that tandem MS–MS with positive electrospray ionization (ESI) is a powerful tool for the detection of acrylamide in foods at low levels (Riediker & Stadler, 2003; Rosén & Hellenäs, 2002; Tareke et al., 2002). Under the positive ion ESI conditions, a frequent clean of MS inlet was required due to heavy contamination with salts. This problem prevented obtaining sensitive and repeatable results using ESI in LC–MS analyses. Contrarily, LC–MS with APCI allowed us to determine acrylamide sensitively and precisely. Under the positive APCI conditions applied here, scan MS analysis of acrylamide and $^{13}C_3$-labelled acrylamide showed both $[M+H]^+$ ions with compound-specific product ions due to loss of NH_3 from the protonated molecule (Fig. 1).

Inertsil ODS-3 analytical column was used to separate acrylamide 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) as the mobile phase at a flow rate of 0.6 ml/min. Under these conditions, acrylamide was eluted at 8.33 min with good retention time repeatability (8.33 ± 0.03 min, $n = 10$). The column exhibited good performance in terms of efficiency ($N = 15{,}000$) and of asymmetry factor ($T = 0.69$) for the acrylamide peak (Fig. 2). The acrylamide response was linear over a concentration range of 1–2000 ng/ml with correlation coefficients of higher than 0.999. LOD and LOQ were determined to be 6 and 18 ng/g based on signal-to-noise ratio of 3 and 9, respectively.

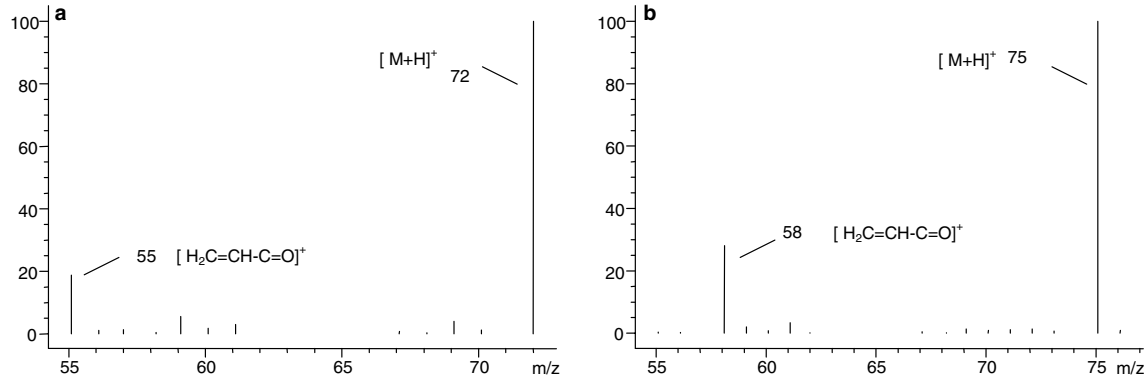

Fig. 1. Positive ion APCI mass spectra of (a) acrylamide and (b) $^{13}C_3$-labelled accrylamide at 100 ng/ml.

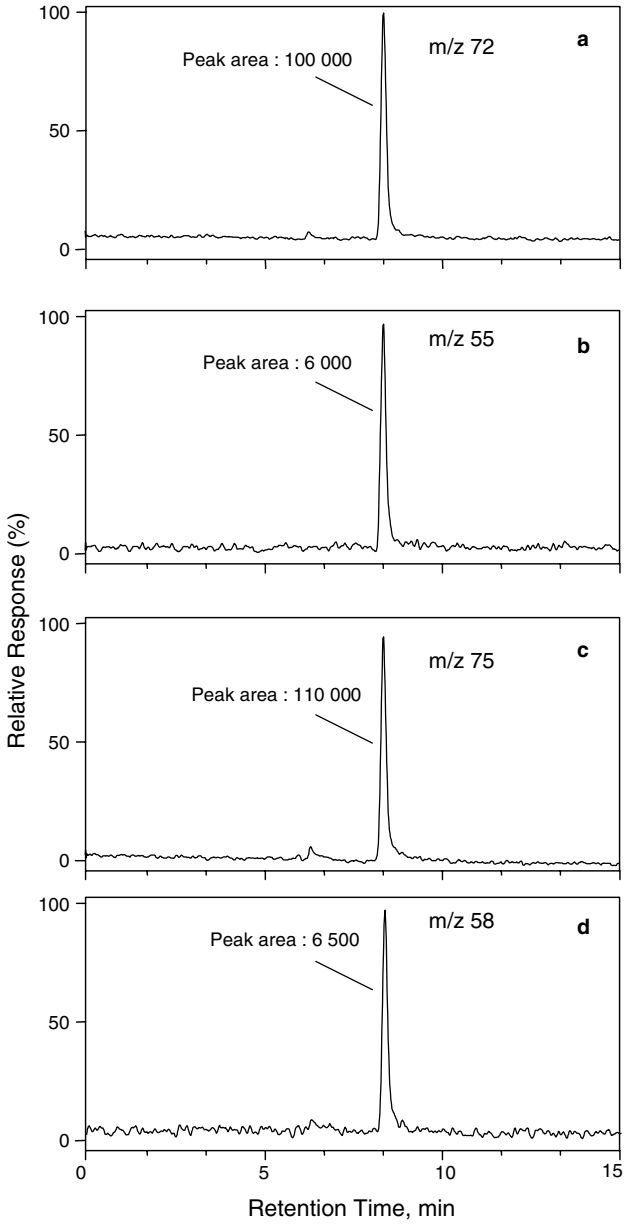

Fig. 2. LC–MS SIM chromatograms of standard solutions of (a,b) acrylamide and (c,d) $^{13}C_3$-labelled acrylamide at 100 ng/ml.

A usual approach for the extraction of acrylamide from foods entails an extraction with water followed by sample cleanup with SPE prior to LC analysis coupled to MS detection. Processed potato and cereal-based food samples are usually composed of high amounts of colloids (starch and proteins) and fat which should be separated after the extraction with water. It is well known that Carrez I and II reagents precipitates these colloids. Here, an extract free from the colloids and fat was obtained by Carrez clarification and cold centrifugation, respectively. However, small molecules soluble in water such as amino acids cannot be avoided. As demonstrated for potato chips, peak eluted at 5.6 min prevented accurate and precise quantitation, and decreased the MS detection sensitivity of acrylamide in SIM mode due to an increase of baseline signal response (Fig. 3(a)). Analysis of all potato and cereal-based processed foods showed the presence of same compound eluted at 5.6 min in varying amounts. The adverse effect of this peak was limited by adjusting a delay time of 6.5 min for APCI ionization instrumentally. Signal response increased ca. 45% and 15% for acrylamide (*m*/*z* 72) and $^{13}C_3$-labelled acrylamide (*m*/*z* 75), respectively. Instrumentally adjusted delay time had a greater effect on parent ion of acrylamide than that of $^{13}C_3$-labelled acrylamide which is important from the viewpoint of quantitation.

Laboratory validation of the method was performed by analyzing spiked potato chips. The spiking range encompasses the typical values of acrylamide found in commercial potato chips. The results from the analysis of spiked potato chip samples are presented in Table 1. Accuracy of the method was further tested analyzing reference supplied by the CSL. The assigned values of these materials were 167 ng/g acrylamide with a satisfactory range of 97–237 ng/g for FAPAS T3002, and 1843 ng/g with a satisfactory range of 1306–2381 ng/g for FAPAS T3007. The mean acrylamide concentrations of FAPAS T3002 and T3007 samples were found to be 187 and 1790 ng/g, respectively, using the validated method.

The simplified sample preparation together with LC–MS analysis was found to be applicable for a wide variety of foods. Table 2 reports the mean recoveries for foods collected from the local markets in Ankara. The preliminary results about the presence of acrylamide in these foods should not be generalized about acrylamide levels in particular food types, because these samples are not statistically representative of specific foods.

3.2. Identification of co-extractives

As illustrated in Figs. 3(a) and 4(b), analytical responses for compounds other than acrylamide were present in the MS chromatograms (*m*/*z* 72) of food samples. The extract was examined in detail to determine the chemical structures of these compounds. Taking the molecular weights of amino acids into account, the extract was analyzed in scan mode using a *m*/*z* range of 50–210. Mass spectral examination of the peak eluted at ca. 5.6 min showed the presence of characteristic $[M + H]^+$ and compound specific product ions having respective *m*/*z* values of 118 and 72 similar to that of amino acid valine. The presence of valine in the extract was further confirmed by analyzing a pure standard solution of valine and comparing its retention and mass spectral data to that of the peak eluted at ca. 5.6 min. Similarly, some other amino acids were also identified in the extract, which were asparagine and aspartic acid (4.18 min), lysine (4.22 min), glutamine (4.24 min),

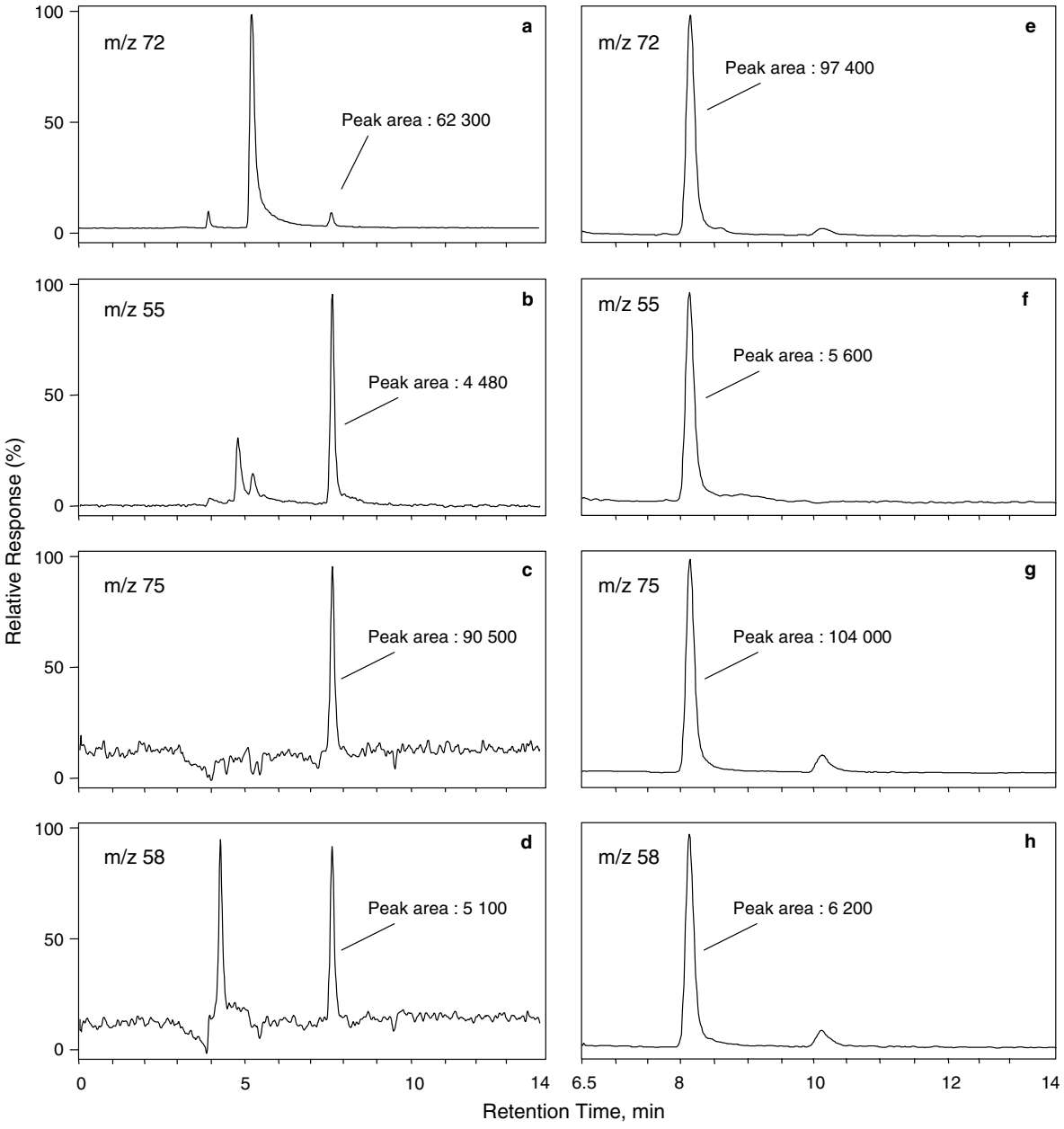

Fig. 3. LC–MS chromatograms of potato chips containing 1020 ng/g of acrylamide and 1000 ng/g $^{13}C_3$-labelled acrylamide (a–d) without delay time (e–h) with delay time of 6.5 min.

glutamic acid (4.35 min), proline (4.83 min), methionine (6.92 min), isoleucine (9.04 min), tyrosine (9.66 min), leucine (9.87 min), arginine (9.96 min), phenylalanine (20.26 min) and tryptophan (21.25 min) by matching both mass spectral and retention data of pure amino acid standards.

Table 1
The results of laboratory validation study of acrylamide in potato chips

Spiking level (ng/g)	Acrylamide					$^{13}d_3$-Acrylamide		
	Recovery (%)	Within batch		Between batch		Recovery (%)	Within batch	
		RSD (%)	*n*	RSD (%)	*n*		RSD (%)	*n*
100	98.5	1.5	8	1.9	3			
250	99.4	0.5	8	0.7	3	100.3	2.3	8
500	100.4	2.2	8	2.2	2			
1000	99.2	2.7	8	2.6	2	95.0	4.7	8

Table 2
Mean recoveries for a range of matrices as determined by standard addition of $^{13}C_3$-labelled acrylamide at level of 500 ng/g

Matrix	Acrylamide (ng/g)[a]	Recovery (%)[b]
Infant biscuits	148–152	96.8 (95.5–98.1)
Breakfast cereal	28–31	94.0 (90.6–97.6)
French fries	198–200	98.2 (97.7–98.7)
Diet biscuits	344–350	96.0 (95.1–96.9)
Biscuits with dietary fiber	467–486	96.0 (95.1–97.0)
Simit	82–86	81.6 (79.2–84.0)
Turkish breads	36–42	81.5 (73.8–89.2)
Cookies	248–261	95.8 (93.1–98.5)
Roasted chick pea	64–71	84.0 (79.8–89.2)

[a] Incurred mean acrylamide levels in foods ($n = 3$).
[b] Mean recoveries obtained 500 ng/g ($n = 2$).

After appropriately identifying the chemical nature of co-extractives, it could be possible to design a further cleanup treatment to eliminate them. It is very well known that amino acids can be either positively or negatively charged according to pH of environment because of their amphoteric property. Since the extraction was performed by acidified water in this study, the most of amino acids present in the extract were positively charged. These amino acids, therefore, can be easily retained by passing through a SPE cartridge packed with a strong cation-exchanger sorbent. This hypothesis was tested using both a standard mixture of valine and acrylamide prepared in 0.01 mM acetic acid, and a food extract prepared from potato crisps. One millilitre of the standard mixture of valine and acrylamide (5 μg/ml each), or the sample extract was passed through an Oasis MCX SPE cartridge preconditioned by 1 ml of methanol and water. After discarding the first 10 drops, the eluate was collected and analyzed by LC–MS. As shown in Fig. 4, valine present in the mixture was completely retained by the cartridge while acrylamide passed through without a loss. Clean-up of extract by using Oasis MCX SPE cartridge, the signal response for acrylamide parent ion increased ca. 25% for a potato crisp sample as shown in Fig 4(b).

As stated before, the effects of various SPE sorbents have been reported by previous researchers to improve MS detection. However, the most of these reports seem like simple observations using various cartridges, and did not deal with the identification of co-extractives. Riediker and Stadler (2003) have noted a decrease of pH of the sample extract to 1–2 after passage through the Isolute Multimode cartridge featuring strong hydrophobic interaction; strong cationic (SCX) as well as anionic (SAX) exchange characteristics. They have concluded the decrease of pH as the presence of positively charged co-extractives strongly interacting with the SCX functional groups.

4. Conclusion

The results of this study emphasize the identification of major interfering compound as valine during the analysis of acrylamide in potato and cereal-based processed foods. Instrumentally adjusted delay time and SPE with a strong cation exchanger based sorbent can

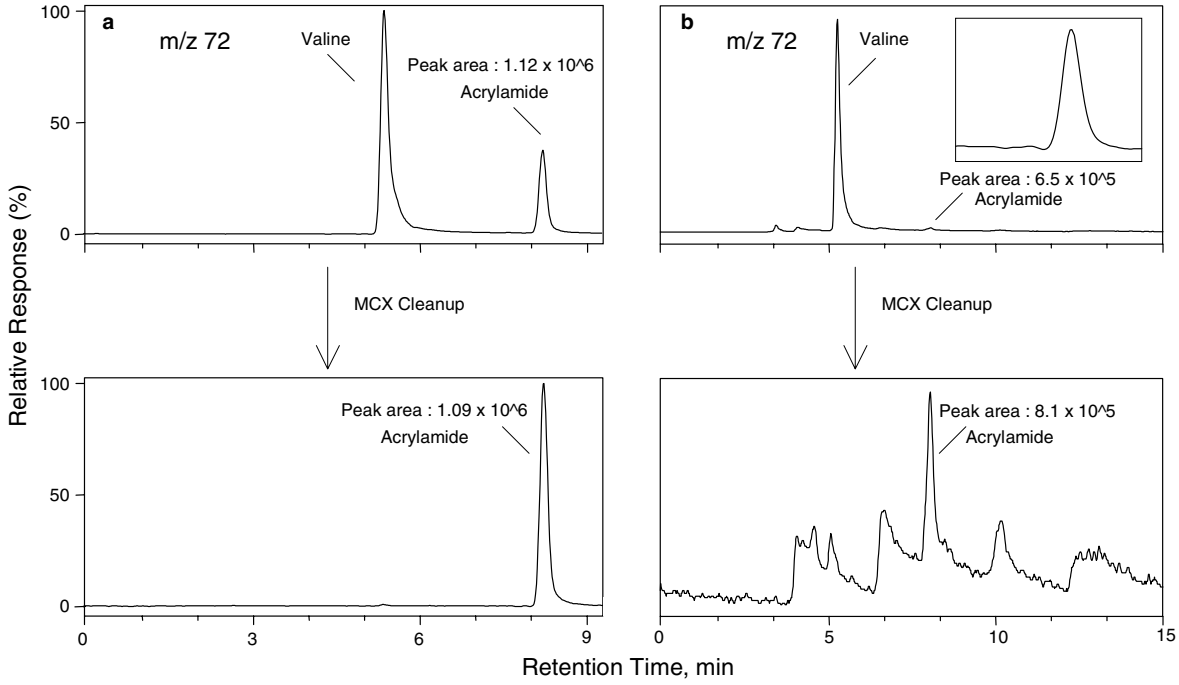

Fig. 4. LC–MS chromatograms of (a) a standard mixture containing 5 μg/ml of valine and acrylamide and (b) potato crisp sample (FAPAS T3007) before and after Oasis MCX cleanup.

successfully avoid the adverse effect of valine to improve accuracy, precision and sensitivity. The analytical procedure described here passed a series of laboratory validation tests.

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