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Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry

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Abstract

A confirmatory method for the determination of low levels of acrylamide in different food products is presented. The method entails extraction of acrylamide with water, precipitation of matrix constituents with acetonitrile, and two clean-up steps consecutively over Isolute Multimode and cation-exchange cartridges. The final extract is analyzed by liquid chromatography (LC) coupled to positive electrospray ionization tandem mass spectrometry employing [$^{13}C_3$]-acrylamide as internal standard. For the chromatographic step, a LC column based on a polymethacrylate gel is employed which shows good retention of acrylamide under isocratic flow conditions ($k' = 1.2$). Mass spectral acquisition is done by selected reaction monitoring, choosing the characteristic transitions m/z 72→55, 72→54 and 72→27. In-house validation data for breakfast cereals and crackers show good precision of the method, with intra- and interassay variation below 10%. The limits of detection for crackers and breakfast cereals, respectively are estimated at 15 and 20 μg/kg, and recoveries of fortified samples ranged between 58 and 76%. Furthermore, the method is applicable to a number of different food products, including biscuits, crisp bread, wafers, confectionery cocoa liquor, and nuts. Finally, the good results obtained in several small-scale interlaboratory tests provided additional confidence in the performance of the method.

Keywords: Food analysis; Cereals; Interlaboratory test; Validation; Isotope dilution methods; Acrylamide

1. Introduction

Recent studies in Sweden reported the presence of acrylamide (2-propenamide) in a wide range of fried and oven-cooked foods [1]. These findings have attracted considerable interest worldwide because acrylamide has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) [2]. Acrylamide is formed during the Maillard reaction, and major reactants leading to the release of acrylamide are sugars and asparagine [3,4]. The potential health risk of acrylamide in food has been considered by a number of government agencies and national authorities [5,6]. Following these deliberations, all available data on acrylamide have been reviewed at international

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level (e.g. FAO/WHO, JIFSAN Workshop) by expert Working Groups, identifying and listing a number of research gaps and priorities. Once addressed, these would allow a better assessment of the potential health risks associated with this finding [7,8].

An urgent requirement in this context is the development and validation of sensitive and robust analytical methods that can quantify acrylamide in different food matrices down to the low μg/kg level [8]. Numerous methods have in fact been developed in the past years to determine the acrylamide monomer, especially in water, biological fluids and noncooked foods (sugar, field crops, mushrooms), and the majority are classical methods based on high-performance liquid chromatography (LC) or gas chromatographic (GC) techniques [9–15]. However, these methods as such do not suffice for the analysis of acrylamide in processed/cooked foods at low levels. In particular they lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule such as acrylamide in a complex food matrix.

To date, only few analytical methods dealing with the analysis of acrylamide in cooked foods have been published in peer-reviewed journals or presented at international scientific meetings. These methods are based mainly on mass spectrometry (MS) as the determinative technique, coupled with a chromatographic step either by LC [16–21] or GC [17,21–24], the latter in most cases after derivatization of the analyte. In fact, the expert Working Group on Analytical Methods that convened during the recent JIFSAN meeting on acrylamide [8] and Clarke et al. [25] concluded that the majority of laboratories use either GC–MS or LC–MS, the advantage of the LC–MS-based methods being that acrylamide can be analyzed without prior derivatization (e.g. bromination), which considerably simplifies and expedites the analysis. Due to the low molecular mass of acrylamide (71 g/mol) and thus also its low-mass fragment ions, confirmation of the analyte can be achieved with a two-stage mass spectrometer (monitoring of more than one characteristic mass transition) [16–20]. However, acrylamide is a very polar molecule with poor retention on conventional LC reversed-phase sorbents [20], and despite the use of tandem mass spectrometry more effort may need to be placed on efficient clean-up steps to avoid interference from co-extractives.

Our laboratory has been involved in the analysis of acrylamide in many different food products since the issue was made public in April 2002, and the focus is mainly on industrially produced food. In this report, we present a robust and confirmatory LC with tandem mass spectrometry (MS–MS) that is applicable to a number of different food matrices. The method has been validated in-house and through participation in two independent small-scale interlaboratory tests for breakfast cereals and biscuits.

Finally, the report also addresses the current limitations of techniques based on LC–MS, and the difficulty of developing and applying a single “universal” clean-up approach, valid for many different food matrices and concomitantly retaining comparable method performance.

2. Experimental

2.1. Chemicals and material

Acrylamide (99%) and [$^{13}C_3$]-labeled acrylamide (isotopic purity 99%) were commercially available from Aldrich (Buchs, Switzerland) and Cambridge Isotope Labs. (Andover, MA, USA), respectively. Acetonitrile was purchased from J.T. Baker (Phillipsburg NJ, USA). Formic acid (analytical-reagent grade), LiChrosolv water and methanol were from Merck (Darmstadt, Germany). Stock solutions of acrylamide (1 mg/ml) and [$^{13}C_3$]-acrylamide (0.1 mg/ml) were prepared each by dissolving the compounds in distilled water. The stock solutions were stored at 4 °C for a maximum of 4 weeks.

The solid-phase extraction (SPE) cartridges Isolute Multimode (500 mg, 3 ml) and Accubond II SCX (100 mg, 1 ml) were obtained from IST (Hengoed, Mid-Glamorgan, UK) and Agilent Technologies (Palo Alto, CA, USA), respectively. Syringe filter units 13/0.2 RC were purchased from Schleicher and Schuell (Dassel, Germany).

2.2. Food samples

The samples of breakfast cereals (main ingredients: maize grits, wholemeal flour, sugar, and honey) and crackers (main ingredients: wheat flour, vegetable fat, condensed milk, salt, and sugar) were purchased from

retail outlets in Switzerland and stored at room temperature. The samples of the Swiss Federal Office of Public Health (BAG) and National Food Processors Association (NFPA, USA) tests were stored at 4 °C.

2.3. Sample preparation

If necessary, the samples were ground to a powder using a laboratory mill. Then, a portion of the sample (5 g) was spiked with [$^{13}C_3$]-acrylamide as internal standard to achieve a final concentration of 200 μg/kg. The sample was suspended in 50 ml of distilled water and homogenized using a dispersing tool (9500 rpm) for 1 min. The suspension (32 ml) was centrifuged at 13 800 *g* and 5 °C for 15 min. Then, the clear supernatant (8 ml) was mixed with 4 ml of acetonitrile to precipitate co-extractives. Following centrifugation (5 min, 13 800 *g*, 5 °C), the fraction of acetonitrile in the clear supernatant was evaporated under a stream of nitrogen in a heater block adjusted to 45–50 °C (reduction of the extract volume by ∼40%). For the SPE clean-up, both cartridge types were preconditioned consecutively with methanol (Isolute Multimode, 2 ml; Accubond II SCX, 1 ml) and distilled water (Isolute Multimode, 2×2 ml; Accubond II SCX, 1 ml). The residual water was removed by applying a slight vacuum. An aliquot of the sample extract (0.2 ml) was passed through the Isolute Multimode sorbent and discharged. Then, 2 ml of the extract were loaded onto the same column and collected after percolation by gravity-induced flow. An aliquot of the collected extract (1 ml) was subsequently charged onto an Accubond II SCX cartridge and the effluent collected. Prior to LC–electrospray ionization (ESI) MS–MS analysis, the effluent was filtered through a syringe filter unit and 0.1 ml of methanol was added to 0.2 ml of the final sample extract.

2.4. LC–ESI-MS–MS

Measurements were performed using an Alliance 2690 HPLC (Waters, Rupperswil, Switzerland) coupled to a Quattro LC tandem mass spectrometer (Micromass, Manchester, UK). Analytical separation was achieved with a Shodex RSpak DE-613 polymethacrylate gel column (150×6 mm I.D., Showa Denko, Tokyo, Japan). The elution mode was isocratic using a mixture of 0.01% (v/v) aqueuous formic acid–methanol (6:4, v/v) as LC solvent. The initial flow-rate was set to 0.75 ml/min that was split to 0.35 ml after the LC column using a polyether ether ketone (PEEK) T-piece. The LC eluent was directed to the MS system within the retention window set at 4–8 min using a diverter valve (LabPro PR700-100, Rheodyne, Cotati, CA, USA). The total run time was 12 min for food extracts and 10 min for standard solutions. The injection volume was 50 μl. The column and autosampler temperatures were set to 40 and 10 °C, respectively. After an overnight sequence, the LC column was flushed consecutively with 15 ml of water and 30 ml of methanol at a flow-rate of 0.5 ml/min.

The analytes were detected using electrospray ionization in the positive ion mode. Selected reaction monitoring (SRM) traces were acquired with the characteristic fragmentation transitions m/z 72→55, 72→54, and 72→27 for acrylamide and m/z 75→58 and 75→29 for [$^{13}C_3$]-acrylamide. The needle and cone voltages were set to 3.1 kV and 22 V, respectively. The collision energy was set to 20 eV except for the m/z transitions 72→55 and 75→58 (11 eV). The mass window was set to $\pm m/z$ 0.1. Nitrogen was used as nebulizer (90 l/h) and desolvation gas (680 l/h). Argon was introduced into the collision cell resulting in a cell pressure of $2.3–2.4 \cdot 10^{-3}$ mbar. The source and desolvation temperatures were set to 100 and 350 °C, respectively.

2.5. Quantitation

Acrylamide in incurred samples was quantified using a linear calibration function that was established with standard solutions of acrylamide dissolved in distilled water at the concentration levels of 0, 10, 20, 30, 40, 50, 75, 100 and 500 μg/l. Each solution contained 20 μg/l of [$^{13}C_3$]-acrylamide as internal standard. These concentration values were within the same range as encountered in the watery sample extracts. Before LC–ESI-MS–MS analysis, 0.1 ml of methanol and 0.2 ml of a standard solution were mixed as done with the sample extracts. For the calculation of the calibration parameters, the ratio of the responses in the SRM trace m/z 72→55 (acrylamide) and 75→58 ([$^{13}C_3$]-acrylamide) was plotted against the corresponding ratio of analyte amounts. In case of interference in the SRM trace m/z 72→55, the data

obtained from the trace m/z 72→27 were used for quantitation of acrylamide.

3. Results and discussion

3.1. ESI-MS–MS

Initial studies have shown that positive ESI-MS–MS is a powerful tool enabling the detection of acrylamide in different food matrices down to the low μg/kg level [16,17]. Acrylamide is a chemical of low molecular mass ($M_r = 71$) and the formation of compound-specific product ions still within the acceptable mass range of the spectrometer is not self-evident (e.g. TSQ Quantum, cut-off at 30 u). Nevertheless, the fragmentation of acrylamide reveals a prominent product ion of m/z 55, which is derived from the protonated molecule due to the loss of NH_3. This fragmentation showed a maximum response at a low collision energy of 11 eV while the formation of $[C_2H_3]^+$ (m/z 27), $[CONH_2]^+$ (m/z 44) and $[C_3H_2NH_2]^+$ (m/z 54) were observed at a higher collision energy (20 eV) as shown in Fig. 1. The intensity of the aforementioned product ions was significantly lower than that observed for m/z 55, with an ion ratio ranging from 20 to 22 for the SRM transitions m/z 72→55 and m/z 72→27 under the final acquisition conditions. The same fragmentation pattern was observed for [$^{13}C_3$]-acrylamide resulting in the corresponding product ions of m/z 58, 57, 45 and 29. Under our conditions, formic acid (0.01%, v/v) was added to the aqueous fraction of the LC solvent to improve the MS ionization efficiency, and to achieve adequate MS sensitivity. Trials with a relatively higher concentration of formic acid (e.g. 0.1%, v/v) in the LC mobile phase did not result in an improved analyte response.

The SRM transitions m/z 72→55, 72→54, and 72→27 were acquired for the detection of acrylamide (Fig. 2a). An additional trace that can be monitored for acrylamide is m/z 72→44 [16], which under our conditions provided poor MS–MS responses in food samples, probably attributable to matrix interferences. The internal standard [$^{13}C_3$]-acrylamide was recorded using m/z 75→58 and 75→29 (Fig. 2b). Acrylamide in food was confirmed if at least two positive SRM responses were obtained and matching ion ratios within an acceptable tolerance (mean ± 10–20%) versus the ratios obtained from standard solutions of acrylamide. Three SRM traces were routinely recorded although the acquisition of two SRM traces fulfils the criteria required in the Commission Decision 2002/657/EC [26]. High background levels or interfering co-extractives undergoing the same fragmentation reaction were observed especially for the SRM trace m/z 72→55 when analyzing complex food matrices such as soluble coffee or cocoa powder. However, the SRM trace m/z 72→54 turned out to be an alternative due to a higher specificity than the trace m/z 72→55. Higher injec-

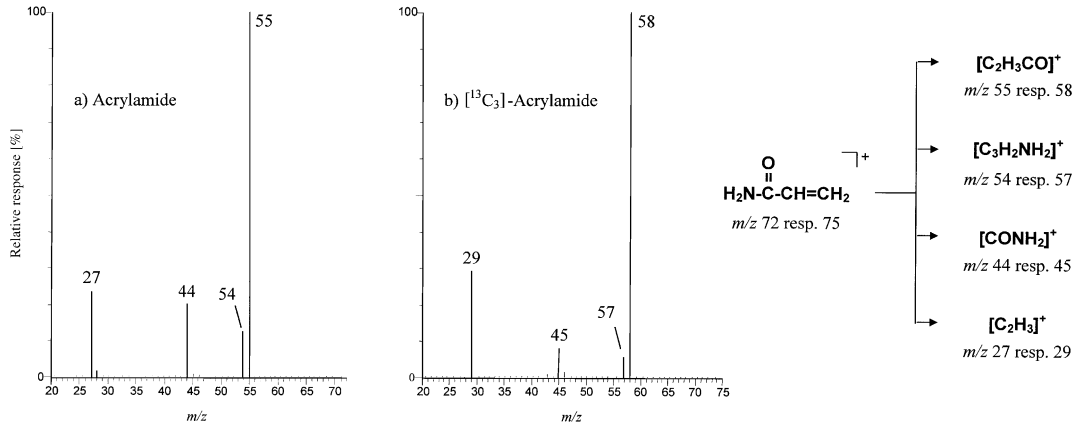

Fig. 1. Product ion spectra obtained from 3 μg/ml each of (a) acrylamide and (b) [$^{13}C_3$]-acrylamide, both dissolved in 0.1% formic acid in water–methanol (1:1, v/v). The collision cell energy was set at 20 eV.

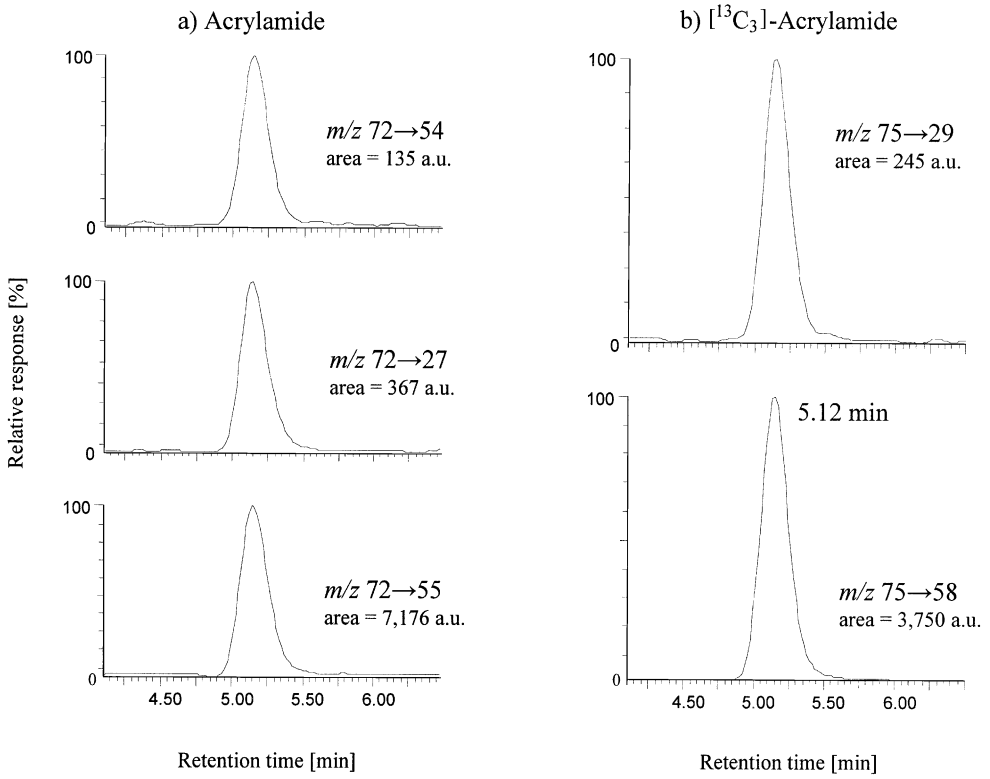

Fig. 2. SRM traces obtained from a standard solution of (a) acrylamide (40 ng/ml) and (b) [$^{13}C_3$]-acrylamide (20 ng/ml) using a Shodex RSpak DE-613 (150×6 mm I.D.) column. The LC mobile phase was composed of 0.01% formic acid in water–methanol (6:4, v/v) at flow-rate of 0.75 ml/min.

tion volume or analyte enrichment by evaporation of the final extract solvent enables to achieve adequate method sensitivity.

3.2. Liquid chromatography

Amongst the LC separation columns that were assessed during the initial stages of method development, good retention of acrylamide was achieved with the Shodex RSpak DE-613 column (polymethacrylate gel) and LC solvent composed of methanol–0.01% aqueous formic acid (1:1, v/v), using isocratic flow conditions. According to the manufacturer's specifications, the Shodex RSpak DE-613 column shows similar retention characteristics as a C_4- or C_8-silica gel column, which could explain the good retention of acrylamide even when using a mobile phase with a relatively high percentage of organic modifier. A decrease of the methanol fraction from 50 to 30% in the LC mobile phase resulted in a shift of the retention time from 5.0 to 5.8 min at ambient column temperature. Unfortunately, a concomitant decrease of the absolute MS response was observed (data not shown). Alternatively, an increase in the methanol fraction (50%, v/v) showed the best absolute MS response, unfortunately with the disadvantage in certain foods (e.g. breakfast cereals) of interfering co-extractives.

Finally, a LC mobile phase containing 40% methanol (v/v) appeared optimal with regard to analyte retention, separation efficiency, and MS response. Furthermore, an increase in column temperature from ambient to 40 °C resulted in a shorter retention time of the analyte, without loss of separation efficiency. The total analytical run-time was 10 min for standard solutions and 12 min for sample extracts, which includes 5 min to flush the LC column.

Future tests with the same LC column type but smaller inner diameter (4.6 and 2 mm) than used in this study (6 mm) may help to further simplify the current approach, with the goal of retaining equivalent analytical performance.

3.3. Sample clean-up

Food samples such as for example breakfast cereals, biscuits, crackers, and wafers were prepared according to the detailed description in Section 2.3. After addition of [$^{13}C_3$]-acrylamide as internal standard (200 μg/kg) to the ground sample portion (5 g), acrylamide was typically extracted with 50 ml of distilled water. In the case of foods that absorbed a high fraction of water (e.g. crisp bread), the sample portion was reduced to 1 or 2 g. After sample extraction, the suspension was centrifuged at a high speed and low temperature (5 °C) to achieve phase separation and to remove potential fat. Then, acetonitrile was added to the clear supernatant to precipitate proteins and other high-molecular co-extractives. After centrifugation, the organic solvent fraction in the clear supernatant was evaporated under a stream of nitrogen in a heater block, reducing the volume of the sample extract from 5 ml to approx. 3 ml. No significant improvement in sensitivity was observed in case of a further reduction of the extract volume, e.g. to 2.0 ml or 2.5 ml.

In order to remove additional interfering co-extractives, two SPE steps were performed using con-

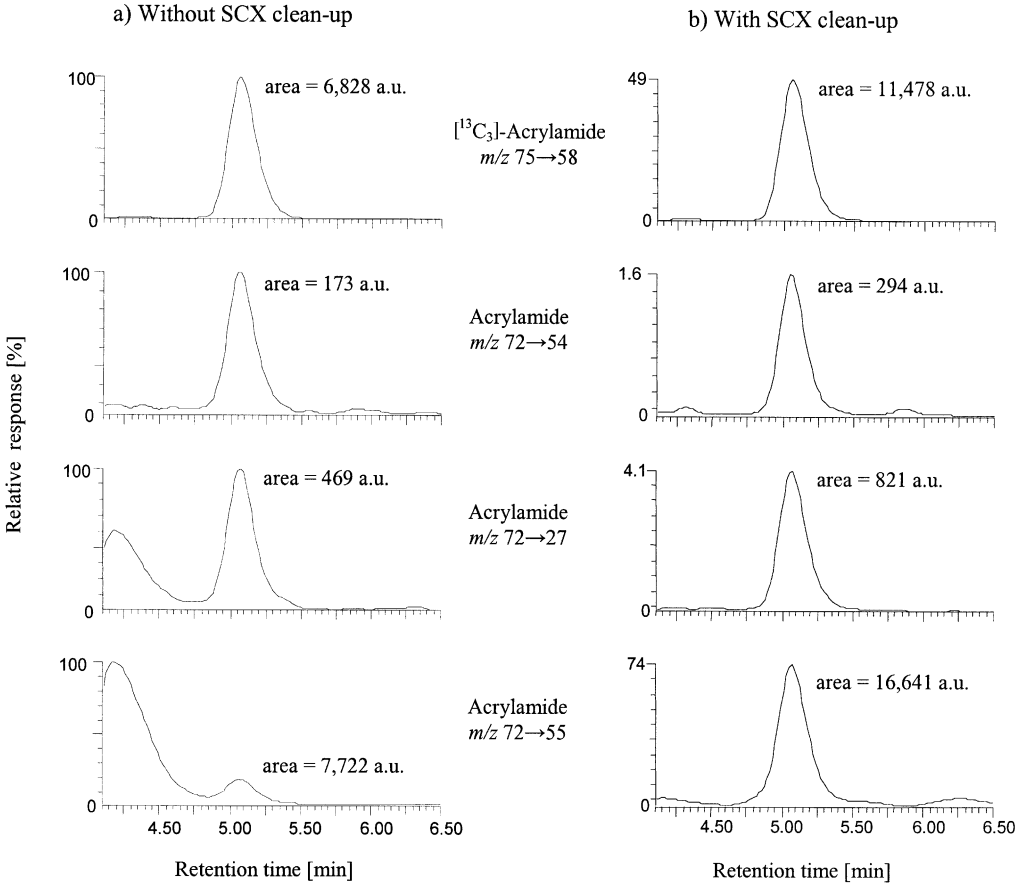

Fig. 3. SRM traces obtained from a breakfast cereal extract that was prepared according to the clean-up procedure (a) without and (b) with the Accubond II SCX SPE step. Acrylamide was determined in the sample at a level of 240 μg/kg. [$^{13}C_3$]-acrylamide was added as internal standard at 200 μg/kg.

secutively the SPE sorbents Isolute Multimode and Accubond II SCX. The characteristic features of the Multimode sorbent are hydrophobic interaction (presence of C_{18} functional groups), strong cationic (SCX), as well as anionic (SAX) exchange. The observed decrease of pH of the sample extract to 1–2 after passage through the Isolute Multimode cartridge indicated an enhanced interaction of positively charged co-extractives with the SCX functional groups.

Initially, a volume of 2–3 ml of sample extract was passed by gravity-induced flow through the Isolute Multimode sorbent and collected for LC–MS–MS analysis. However, as demonstrated for a breakfast cereal (Fig. 3a), an interfering co-extractive that eluted just before acrylamide was not sufficiently removed by the Isolute Multimode SPE clean-up. Such interferences could be avoided by including a second SPE clean-up step, i.e. passage of an aliquot of the sample extract (1 ml) over an Accubond II SCX cartridge, resulting in "cleaner" chromatograms and higher signal responses due to less ion suppression effects (Fig. 3b). Nevertheless, the efficacy of both SPE clean-up steps was strongly dependent on the matrix type and extract volume that was passed through the column. In samples with a high load of co-extractives, the column capacities clearly did not suffice. A breakthrough already after only 1 ml of the sample extract was observed, resulting in either strong interferences as described above, or suppression of ionization. On the other hand, we also observed that acrylamide may be partially retained by the SPE sorbent (Fig. 4). This behavior was demonstrated by the analysis of an eluate, which was obtained from a wash step with distilled water (0.5 ml) after passing an extract of a biscuit sample through the Isolute Multimode sorbent.

The extent of the acrylamide retention probably depends on the occurrence of co-extractives that compete with acrylamide for the active sites. Based on

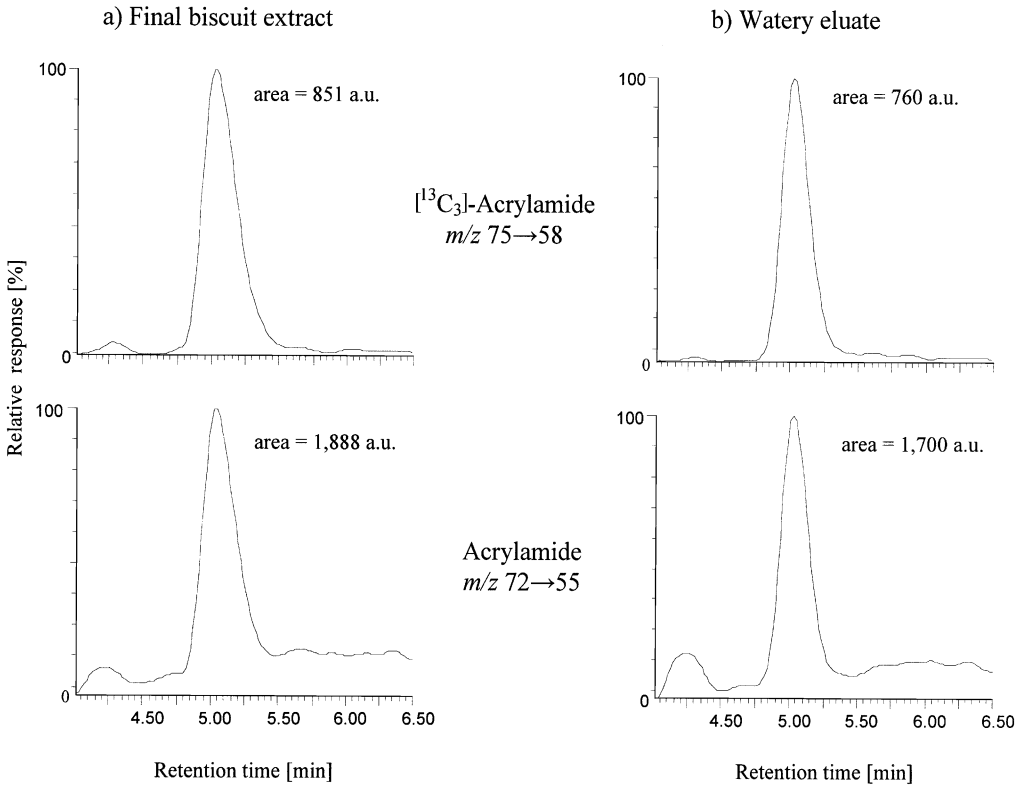

Fig. 4. SRM chromatograms recorded from (a) a biscuit extract and (b) an eluate that was collected after passing 0.5 ml of distilled water through the same Isolute Multimode cartridge.

these observations, the sample extract volume passed through both SPE cartridges should be optimized for each commodity, and acrylamide retention on the SPE sorbents should be individually tested.

The sample clean-up procedure presented in this study is suitable for a wide variety of food products such as breakfast cereals, infant cereals, pasta products, biscuits, crackers, nuts, waffles, cocoa liquor, etc. However, the method is not suited for the determination of acrylamide in cocoa powder, coffee (roast and ground and soluble), and dehydrated flavorings with a high salt content. Preliminary trials show that for such products the inclusion of a liquid-liquid extraction step (ethyl acetate) is required, and details will be communicated at a later stage.

3.4. Method performance

The analytical method was validated for breakfast cereals and crackers both containing acrylamide at 240 μg/kg. The corresponding analytical key parameters are listed in Table 1. Linearity was tested with portions of both commodities fortified to acrylamide levels of 0.39, 0.62, 0.99, 1.74, 3.94 and 7.74 mg/kg (each two independent determinations). Good coefficients of determination (r^2) of 0.999–0.9999 were achieved over the whole concentration range. A good accordance of calibration slope and intercept values calculated with data obtained from reference solutions and fortified incurred samples confirmed the compensation of matrix effects by [$^{13}C_3$]-acrylamide (data not shown).

The limit of detection (LOD) and the limit of quantitation (LOQ) for acrylamide in breakfast cereals and crackers were extrapolated from the signal-to-noise (*S/N*) ratios obtained for the responses in the SRM traces m/z 72→55 and/or m/z 72→27. The LOD ($S/N = 3$, 10 replicates) for both SRM traces ranged between 15 and 30 μg/kg (50–100 pg acrylamide on-column), with slightly elevated values for the breakfast cereal sample. Although the absolute signal response in the SRM trace m/z 72→55 was significantly higher than in the SRM trace m/z 72→27 (Fig. 2a), the LOD values of both transitions coincided. This result can be explained by the enhanced background level monitored in the SRM trace m/z 72→55 that resulted in a similar signal-to-noise ratio as observed for SRM trace m/z 72→27. For acrylamide dissolved in pure water, the on-column LOD ranged between 110 and 170 pg (average 150 pg, n=10) over time depending on the instrumental conditions. Nevertheless, the significantly different values obtained for standard solutions and the tested food commodities might be explained by the positive impact of co-extractives on the analyte ionization.

Table 1
Analytical key parameters for the determination of acrylamide in breakfast cereals and crackers using LC–ESI-MS–MS

	SRM transition m/z	Added acrylamide (μg/kg)	Breakfast cereals	Crackers
Limit of detection[a] (μg/kg)	72→55		20	15
	72→27		30	20
Limit of quantitation[a] (μg/kg)	72→55		70	45
Intra-assay RSD[b] (%)	72→55	0[c]	3.0	2.6
		300/450	3.0	2.0
Inter-assay RSD (%)		0[c,d]	4.7	4.6
		300/450[e]	3.9	5.2
Recovery[f] ± SD[g] (%)	72→55	150	62 ± 2	76 ± 2
		300	58 ± 1	74 ± 2

[a] Ten replicates.
[b] df = 7; eight replicates, four measurements.
[c] Incurred acrylamide level for the breakfast cereals and the crackers: 240 μg/kg.
[d] df = 4; five independent determinations, two replicates, three measurements.
[e] df = 3; four independent determinations, two replicates, three measurements.
[f] Six replicates, two measurements.
[g] SD, standard deviation.

The LOQ ($S/N = 10$, $n = 10$ replicates) was estimated at 45 and 70 μg/kg for the crackers and breakfast cereals, respectively (taking m/z 72→55).

The intra-assay relative standard deviations (RSDs) were first determined for both commodities without supplementation of the incurred samples with acrylamide. Since the RSD values obtained on breakfast cereals and crackers (3.0 and 2.6%, respectively) were similar, one of both commodities was fortified with either 300 or 450 μg/kg acrylamide. The same approach was used for the determination of the inter-assay RSDs that ranged from 3.9 to 5.2%.

The amount of analyte recovered after the sample clean-up was determined for both breakfast cereals and crackers based on the procedure described in EU Commission Decision 2002/657/EC [26]. Sample portions ($n = 6$) each were fortified with acrylamide (150 and 300 μg/kg) before the extraction step or after the Accubond II SCX clean-up. In order to determine the amount of incurred analyte, six sample portions were prepared without supplementation. Isotope-labeled acrylamide was added to all extracts after the Accubond II SCX clean-up step to compensate for variations due to analyte ionization efficiency or injection volume. The loss of analyte was estimated at 40 and 26% in breakfast cereals and crackers, respectively. These results confirmed the previous observation that acrylamide may be partially retained on the Isolute Multimode column or possibly on the Accubond II SCX sorbent. The recovery of acrylamide in both food commodities ranged from 95 to 103% at varying concentration levels, owing to the compensation of analyte loss by the internal standard.

The method performance was also assessed by participating in two small-scale interlaboratory tests that were organized by the Swiss Federal Office of Public Health (BAG), and independently the National Food Processors Association (NFPA). The corresponding results, which are presented in Table 2, show that our calculated concentrations are in all cases close to the mean values obtained from the results of all ring test participants.

Table 2
Acrylamide levels (mean value ± standard deviation, (μg/kg)) reported for food samples of two interlaboratory tests that were organized by the Swiss Federal Office of Public Health (BAG) and USA National Food Processors Association (NFPA)

Sample type	In-house results	Interlaboratory test mean values
BAG test, Switzerland ($n^c = 9$)		
Salt crackers, 1st round	547 ± 9[a]	588 ± 82[b]
Salt crackers, 2nd round	504 ± 42[a]	527 ± 106[b]
Breakfast cereals, 1st round	223 ± 8[a]	265 ± 98[b]
Breakfast cereals, 2nd round	158 ± 11[a]	182 ± 60[b]
NFPA test, USA ($n = 8$)		
Saltine crackers A	157 ± 4[d]	148 ± 9.4[e]
Saltine crackers B	<30[d]	N/A[e]
Saltine crackers A, spiked with 55 μg/kg	164 ± 6[d]	154 ± 14.6[e]
Potato chips	244 ± 6[d]	224 ± 15.5[e]
Laboratory water, spiked with 50 μg/kg	54 ± 1[d]	49 ± 4.6[e]

[a] Two determinations, three measurements.
[b] The analytical techniques encompassed GC–MS, GC–high-resolution MS, GC–electron capture detection, and LC–MS–MS.
[c] n, number of participants.
[d] One determination, three measurements.
[e] The values were calculated with the results obtained from all participants that performed LC–MS–MS analysis.

4. Conclusion

This work describes a confirmatory and quantitative analytical method for the determination of acrylamide in a number of different foods. The sample clean-up is based on the removal of co-extractives from the aqueous extracts, i.e. acetonitrile precipitation of proteins and two consecutive SPE clean-up steps. The sample preparation is facile and rapid in comparison to the GC–MS methods that in most cases require derivatization (bromination) of acrylamide prior to analysis. However, we clearly illustrate that the food matrix (composition and volume load) has an important impact on the efficacy of the clean-up procedure and subsequent loss of the analyte. Either suppression of ionization due to interfering co-extractives or partial retention of acrylamide on the Multimode SPE sorbent could lead to a higher LOD. Therefore, certain critical parameters such as the sample extract load onto the SPE cartridges should be individually assessed and optimized for each category of food products.

Additional advantages of the method are the use of isocratic LC conditions, and a high proportion of

methanol in the mobile phase, which also facilitates nebulization. The analyte is well retained on the LC column ($k' = 1.2$), shows no significant fluctuation in the retention time in all food products so far analyzed, and the chromatographic step takes only 12 min per measurement. The future use of a Shodex RSpak DE-213 or DE-413L column of smaller I.D. (2 mm or 4.6 mm) could simplify the laboratory handling without loss of method performance. Despite the use of a Quattro LC mass spectrometer, the method sensitivity reported here is comparable to that reported in earlier studies [16–18] using better performing instruments.

Finally, we have over the past 9 months successfully applied the method to a wide range of different food products. However, the procedure as described here cannot achieve adequate recoveries for complex matrices such as coffee, cocoa powder, or dehydrated flavors, without modification to the clean-up procedures. Further method developments are now being conducted in our laboratory on such commodities and will be communicated in due course.

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