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Trace level determination of acrylamide in cereal-based foods by gas chromatography–mass spectrometry

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Abstract

A quantitative method has been developed for the determination of trace levels (<50 μg/kg) of acrylamide in cereal-based foods. The method is based on extraction of acrylamide with water, acidification and purification with Carrez I and II solutions, followed by bromination of the acrylamide double bond. The reaction product (2,3-dibromopropionamide) is extracted with ethyl acetate/hexane (4:1, v/v), dried over sodium sulfate, and cleaned up through a Florisil column. The derivative is then converted to 2-bromopropenamide by dehydrobromination with triethylamine and analyzed by gas chromatography coupled to mass spectrometry (GC–MS), employing ($^{13}C_3$)acrylamide as internal standard. In-house validation data for commercial and experimental cereal products showed good precision of the method, with repeatability and intermediate reproducibility relative standard deviations below 10%. The limit of detection and limit of quantitation are estimated at 2 and 5 μg/kg, respectively, and recoveries of acrylamide from samples spiked at levels of 5–500 μg/kg ranged between 93 and 104% after correction of analyte loss by the internal standard. Finally, a comparative test organized with two independent laboratories provided additional confidence in the good performance of the method, particularly at very low concentration levels.

Keywords: Cereals; Acrylamide

1. Introduction

In April 2002, researchers from the University of Stockholm and the Swedish National Food Administration (NFA) reported the presence of acrylamide (2-propenamide) in a wide range of fried and oven-cooked foods [1]. These findings have attracted considerable attention worldwide because acrylamide has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) [2]. Recent model system studies have shown that acrylamide is formed during the Maillard reaction, and that the major reactants leading to the release of acrylamide are sugars and asparagine [3,4]. The potential health risks of acrylamide in food have been evaluated by the Scientific Committee on Food (SCF) [5] and the British Food Standards Agency (FSA) [6]. In a next step, all available data on acrylamide have been reviewed at international level by expert working groups (e.g. FAO/WHO, JIFSAN Workshop), identifying and listing a number of research gaps and priorities [7,8]. Among these, the development and validation of sensitive and reliable analytical methods for the low level quantification of acrylamide in different food matrices was considered as essential [8].

Several methods have been developed in the past decade to determine acrylamide in water, biological fluids and non-cooked foods (sugar, mushrooms, and field crops such as corn, potatoes, sugar beets, and beans), 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 are not appropriate for the analysis of acrylamide in processed/cooked foods at low μg/kg levels. In particular, they lack the selectivity and additional degree of analyte certainty required to confirm the presence of a small molecule such as acrylamide in a complex food matrix. This was largely observed in our preliminary attempts to develop a GC method with electron-capture detection (ECD) for the determination of acrylamide in various food products. Recently, more selective analytical methods have been published that are based mainly on mass spectrometry (MS) as the determinative technique, coupled with a chromatographic step either by LC [16–23] or GC, the latter in most cases after derivatization of the analyte [17,20,23–26]. These methods

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have been reviewed in detail during the Joint European Commission–Swedish National Administration Workshop on "Analytical methods for acrylamide determination in food" that was held in Geel, Belgium, during 28–29 April 2003. In a recent assessment of the performance of 37 laboratories in determining acrylamide in crispbread, Clarke et al. [27] reported that the majority of laboratories use either GC–MS or LC–MS, and that there was no obvious method-dependent difference in results obtained between the two approaches.

The main advantage of LC–MS-based methods is that acrylamide can be analyzed without prior derivatization (e.g. bromination), which considerably simplifies and expedites the analysis. Due to the low molecular weight of acrylamide (71 g/mol) and thus also its low mass fragment ions, confirmation of the analyte can be achieved with tandem mass spectrometry (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, experience gathered in our laboratory has shown some limitations of this technique, like the difficulty of applying a "universal" clean-up approach valid for many different food matrices, and the difficulty of achieving a limit of quantitation lower than ~50 μg/kg in cereal products [22].

In this report, we present a robust and sensitive GC–MS method for the analysis of trace levels (<50 μg/kg) of acrylamide in cereal-based foods. This method is based on derivatization of acrylamide through bromination and is used concomitantly with our LC–MS/MS method whenever there is a need to achieve very low levels of detection. It has been validated in-house and through participation in a comparative test with two independent laboratories in Germany.

2. Materials and methods

2.1. Cereal samples

Experiments were conducted with a series of commercial products of roller dried and extruded cereal-based foods purchased from retail outlets in Brazil and stored at room temperature, as well as with some cereal bases manufactured for experimental trials in Switzerland. Nine of these samples have been sent to two private laboratories in Germany for comparative analysis.

2.2. Chemicals

Acrylamide (99%) and ($^{13}C_3$)acrylamide (isotopic purity 99%) were commercially available from Aldrich (Buchs, Switzerland) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Acetic acid (glacial) 100%, bromine, *n*-hexane, potassium bromide, potassium hexacyanoferrate(II) trihydrate, sodium thiosulfate pentahydrate, sodium sulfate anhydrous, and zinc sulfate heptahydrate were all obtained from Merck (Darmstadt, Germany) and were of analytical grade. Acetone for residue analysis and triethylamine were purchased from Fluka (Buchs, Switzerland). Florisil 60–100 mesh and hydrobromic acid 48% were obtained from Aldrich. Ethyl acetate for pesticide analysis was supplied by SDS (Peypin, France). Potassium bromide, sodium sulfate anhydrous, and Florisil were purified/activated by calcination in a crucible at 600 °C (muffle furnace) during one night and stored in tightly closed containers at room temperature.

2.3. Standards and reagents

Stock solutions of acrylamide (1 mg/ml) and ($^{13}C_3$)acrylamide (0.1 mg/ml) were prepared by dissolving the compounds in distilled water. These solutions were then appropriately diluted with water to prepare working standards at 10 and 4 μg/ml, respectively. All stock solutions and working standards were stored in a refrigerator at 4 °C for maximum 3 months. The saturated bromine–water solution (ca. 1.6%) was prepared by adding bromine (~8 ml) to 500 ml of water until precipitation became visible. Carrez I solution was prepared by dissolving 144 g of potassium hexacyanoferrate(II) trihydrate in 500 ml of water, and Carrez II solution by dissolving 288 g of zinc sulfate heptahydrate in 500 ml of water.

2.4. Safety

Acrylamide monomer is toxic and readily absorbed through the skin. It is classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer. Gloves and safety glasses should be worn at all times, and all standard and sample preparation stages should be carried out in a fume cupboard.

2.5. Extraction

Finely ground cereal samples (15 g) were weighed into a 250 ml centrifuge bottle and spiked with 250 μl of ($^{13}C_3$)acrylamide internal standard (4 μg/ml). The sample was suspended in 150 ml of distilled water and homogenized for 30 s with a Polytron homogenizer (Kinematica AG, Luzern, Switzerland). The suspension was acidified to pH 4–5 by addition of ~1 ml of glacial acetic acid, treated successively with Carrez I and II solutions (each 2 ml), and centrifuged at 16 000 × *g* (5 °C) for 15 min. Then, the clear supernatant was filtered through glass wool into a 250 ml Erlenmeyer flask.

2.6. Bromination

Calcinated potassium bromide (7.5 g) was dissolved into the filtrate with stirring, and the pH of the solution was adjusted to a value between 1 and 3 by the addition of a

few drops (~0.5 ml) of hydrobromic acid. Then, 10 ml of saturated bromine–water solution was added to the flask whilst stirring. The flask was covered with aluminum foil and transferred into an ice bath where reaction was allowed to take place for at least 1 h. After the reaction was completed, the excess bromine was decomposed by adding a few drops (~1 ml) of 1 M sodium thiosulfate solution until the yellow color disappeared. The mixture was transferred to a 250 ml separatory funnel and extracted with 50 ml of ethyl acetate/hexane (4:1, v/v) by shaking for 1 min. After phase separation, the lower aqueous layer was discarded (in case of emulsion, the mixture was centrifuged at 2600 × *g* for 10 min). The organic phase was filtered into a 100 ml round-bottom flask through glass wool covered with ca. 15 g of calcinated sodium sulfate. The separatory funnel and the filter were rinsed twice with 10 ml aliquots of ethyl acetate/hexane (4:1, v/v). Pooled fractions were evaporated to ~2 ml on a rotary evaporator (40 °C, 140 mbar), and then to dryness under a stream of nitrogen.

2.7. Florisil clean-up

The residue was transferred quantitatively onto a glass chromatography column (430 mm × 11 mm i.d.) containing 5 g of calcinated sodium sulfate and 5 g of activated Florisil, using small aliquots taken from 50 ml of hexane. The column was then eluted with the remainder of this 50 ml of hexane, and the effluent was discarded. The acrylamide derivative (2,3-dibromopropionamide) was eluted with 150 ml of acetone, at a slow steady flow rate of ~6 ml/min. The eluate was evaporated to ~2 ml on a rotary evaporator (40 °C, 200 mbar), and then to dryness under a stream of nitrogen. The residue was redissolved in 400 μl of ethyl acetate, and 40 μl of triethylamine was added to convert 2,3-dibromopropionamide to 2-bromopropenamide. The solution was finally filtered through a 0.2 μm microfilter (Spartan 13RC, Schleicher & Schuell GmbH, Dassel, Germany) into an autosampler vial for GC–MS analysis.

2.8. Bromination of calibration standards

Aliquots of 0 (blank), 5, 10, 25, 50, 100, 250, and 500 μl of acrylamide working standard solution (10 μg/ml) were transferred to a set of eight 250 ml volumetric flasks containing 100 ml of distilled water, and 250 μl of ($^{13}C_3$)acrylamide internal standard (4 μg/ml) was added to each flask. These solutions were brominated according to the procedure described earlier for sample extracts (without Florisil clean-up). Final residues were redissolved in 1000 μl of ethyl acetate, and 100 μl of triethylamine was added to each flask. The derivatized solutions were then filtered through 0.2 μm microfilters (Spartan 13RC), and aliquots (~150–175 μl) of each solution were distributed among six to seven separate autosampler vials that were stored in a freezer at −18 °C until use.

2.9. GC–MS

Brominated sample extracts and calibration standards were analyzed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph (GC) coupled to an HP 5972A benchtop mass selective detector (MSD) operated in selected ion monitoring (SIM) mode with positive electron impact (EI) ionization. The GC column was a ZB-WAX capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) connected to a deactivated fused silica guard column (1 m × 0.53 mm i.d.; Agilent Technologies, Palo Alto, CA, USA), and the carrier gas was helium at 1.6 ml/min. Following injection, the column was held at 65 °C for 1 min, then programmed at 15 °C/min to 170 °C, 5 °C/min to 200 °C, followed by 40 °C/min to 250 °C, and held for 15 min at 250 °C (total run time: 30.25 min). Injections by an HP 7683 autosampler (2 μl) were made in splitless mode (split flow 60 ml/min) with a purge activation time of 1.0 min and an injection temperature of 260 °C. The GC–MS interface transfer line was held at 280 °C. Under these conditions, the retention time of acrylamide and ($^{13}C_3$)acrylamide derivatives was 11.3 min. Ions monitored were *m/z* 70, 149, and 151 for 2-bromopropenamide, and *m/z* 110 and 154 for 2-bromo($^{13}C_3$)propenamide.

2.10. Quantification

Acrylamide in cereal samples was quantified using the ion at *m/z* 149 for 2-bromopropenamide, and the ion at *m/z* 154 for 2-bromo($^{13}C_3$)propenamide. The other ions at *m/z* 70, 110, and 151 were considered only for confirmation purposes. A calibration graph was constructed by plotting peak area ratios (149/154) against the corresponding ratios of analyte amounts, and the acrylamide contents in sample extracts were calculated from the calibration slope and intercept value. For acrylamide to be considered present in a sample extract, the following three criteria had to meet [28]: (1) the ratio of the retention time of 2-bromopropenamide to that of 2-bromo($^{13}C_3$)propenamide had to match the median ratio calculated for the seven calibration standards with a tolerance of ±0.5%; (2) at least one ratio for the ions 70/149 and 151/149 had to be within ±20% of the median ratio calculated for the seven calibration standards; (3) the signal-to-noise ratio of the GC–MS peak had to be greater than 3.

3. Results and discussion

This work describes a quantitative analytical method for the low level determination of acrylamide in cereal-based foods. The proposed method is comparable to the US EPA method for analysis of acrylamide in water [11], but improvements have been made particularly regarding sample clean-up (inclusion of a purification step with Carrez reagents before bromination, and modification of

Florisil clean-up conditions). Moreover, isotopically-labeled ($^{13}C_3$)acrylamide is added to the test portion before extraction so as to keep control on the recoveries achieved and to keep track of possible losses occurring during the whole sample pre-treatment (extraction and clean-up). Despite its low molecular weight (71 g/mol), acrylamide can be analyzed as such without derivatization [25,27], but when using GC–MS the molecule is normally brominated to form the 2,3-dibromopropionamide derivative [10,11].

3.1. Bromination

The advantage of acrylamide bromination is that a more volatile compound is produced, which leads to improved GC characteristics (less polar) and improved MS characteristics (higher mass ions and characteristic $^{79}Br/^{81}Br$ patterns). This results in an increased selectivity, which compensates for a laborious and time-consuming derivatization process. In the present method, conversion of acrylamide to 2,3-dibromopropionamide is performed according to the protocol originally described by Hashimoto [29], which involves addition of potassium bromide, hydrobromic acid, and a saturated bromine–water solution. The excess of bromine is then removed by addition of sodium thiosulfate until the solution becomes colorless. Under these conditions, the yield of 2,3-dibromopropionamide is constant and >80% when the reaction time is more than 1 h [11]. This derivative is less polar compared to the original compound and is therefore easily soluble in non-polar organic solvents like ethyl acetate and hexane. However, Andrawes et al. [30] have shown that under certain conditions, 2,3-dibromopropionamide can be converted to the more stable derivative 2-bromopropenamide on the inlet of the GC or directly on the capillary column. Because this decomposition (dehydrobromination) may yield poor repeatability and accuracy, it is preferable to deliberately convert 2,3-dibromopropionamide to the stable 2-bromopropenamide prior to GC analysis, which can be readily done by adding 10% of triethylamine to the final extract before injection. This conversion is almost instantaneous at room temperature and has been shown to be quantitative and reproducible [30].

Another bromination recipe based on the use of potassium bromide–potassium bromate ($KBrO_3$) has recently been proposed in the literature [26]. This approach offers the advantage of eliminating the handling of elemental bromine, but it has not been evaluated in the present study since low bromination yields have been reported under certain circumstances [26].

3.2. Florisil clean-up

Preliminary trials with the proposed method showed the presence of some interfering co-extractives on the GC–MS chromatograms. In order to partially eliminate this problem, it was found necessary to purify potassium bromide and anhydrous sodium sulfate salts by calcination in a crucible at 600 °C (muffle furnace) during one night, which resulted in much cleaner chromatograms for some difficult cereal matrices. The remaining impurities (present essentially in potassium bromide) were removed by a Florisil clean-up procedure, as originally proposed in US EPA Method 8032A for analysis of water [11]. However, the EPA clean-up conditions have been improved/adapted to the analysis of cereals by using hexane instead of benzene for loading sample extracts onto the column, and by using a single step elution procedure with 150 ml of acetone instead of diethyl ether/benzene (1:4) and acetone/benzene (2:1) mixtures.

3.3. GC–MS

The ions monitored for identification of the analyte, 2-bromopropenamide, were $[C_3H_4NO]^+ = 70$, $[C_3H_4{}^{79}BrNO]^+ = 149$, and $[C_3H_4{}^{81}BrNO]^+ = 151$, using *m/z* 149 for quantification. The ions monitored for identification of the internal standard, 2-bromo($^{13}C_3$)propenamide, were $[^{13}C_2H_3{}^{81}Br]^+ = 110$ and $[^{13}C_3H_4{}^{81}BrNO]^+ = 154$, using *m/z* 154 for quantification (Figs. 1 and 2). Quantification was performed by comparison with a calibration curve (0.17–17.5 ng of 2-bromopropenamide, corresponding to 5–500 μg/kg of acrylamide in the test portion), and samples with acrylamide concentrations >500 μg/kg were diluted in ethyl acetate and re-injected. Calibration curves typically produced correlation coefficients ranging between 0.997 and 1.000. The limit of detection calculated from the measurement of standard solutions is estimated at 2 μg/kg (signal-to-noise ratio of 3), and the limit of quantitation at 5 μg/kg, which corresponds to the lowest concentration of acrylamide that can be determined in a cereal matrix with an acceptable level of accuracy (repeatability) under the stated conditions. Acrylamide in cereals was confirmed if at least one ratio for the ions *m/z* 70/149 and 151/149 was matching the median ratio calculated for the seven calibration standards within an acceptable tolerance of ±20%. However, at concentration levels lower than the limit of quantitation (5 μg/kg), the two ratios were often found outside the expected tolerance range. A good estimate of the recovery rate of the analytical procedure is obtained by comparing the median of 2-bromo($^{13}C_3$)propenamide peak areas in standards with the corresponding peak area in sample extracts. Depending on the type of cereal product analyzed, the recovery rate was shown to vary between 40 and 60%. No minimum value could be determined for an acceptable recovery of the internal standard, but, as a rule, analysis was repeated if the recovery dropped below 40%.

3.4. Method performance

The method was validated by replicate analysis of two commercial cereal products under repeatability and intermediate reproducibility conditions (Table 1). Both products were first analyzed seven times in parallel so as to

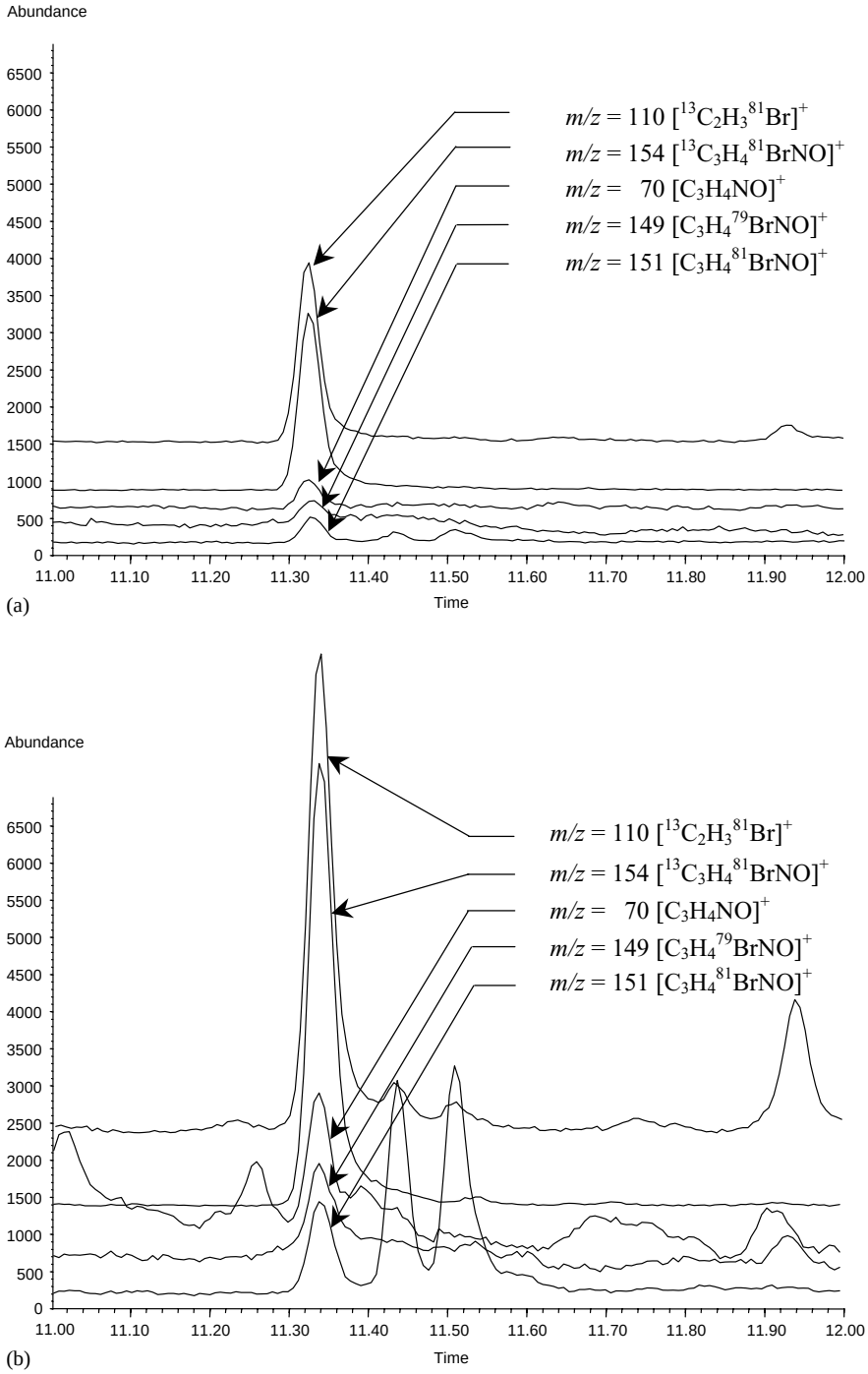

Fig. 1. GC–MS (EI) chromatograms of five selected ions from the acrylamide derivative (2-bromopropenamide) and internal standard derivative (2-bromo($^{13}C_3$)propenamide) after bromination of: (a) standard solution containing 100 ng of acrylamide; (b) rice product containing 17 μg/kg of acrylamide.

get information about within-day variation. The mean acrylamide concentrations found in these samples were 17.6 and 70.6 μg/kg, with repeatability relative standard deviations (R.S.D.(r)) of 8.6 and 5.7%, respectively. Additional precision data were obtained by duplicate analysis of the same two products on four different days, which gave practically the same concentration levels (means of 16.3 and 67.5 μg/kg) with intermediate reproducibility relative standard deviations (R.S.D.(iR)) calculated at 6.8 and 6.2%, respectively. The cereal product containing ca. 68 μg/kg of acrylamide was also analyzed by LC–MS/MS according to the other method validated in our laboratory [22], and the result was almost identical at 69 μg/kg, showing an excellent agreement between the two techniques.

The recovery rate of the method was determined by spiking a wheat semolina sample that had been shown to

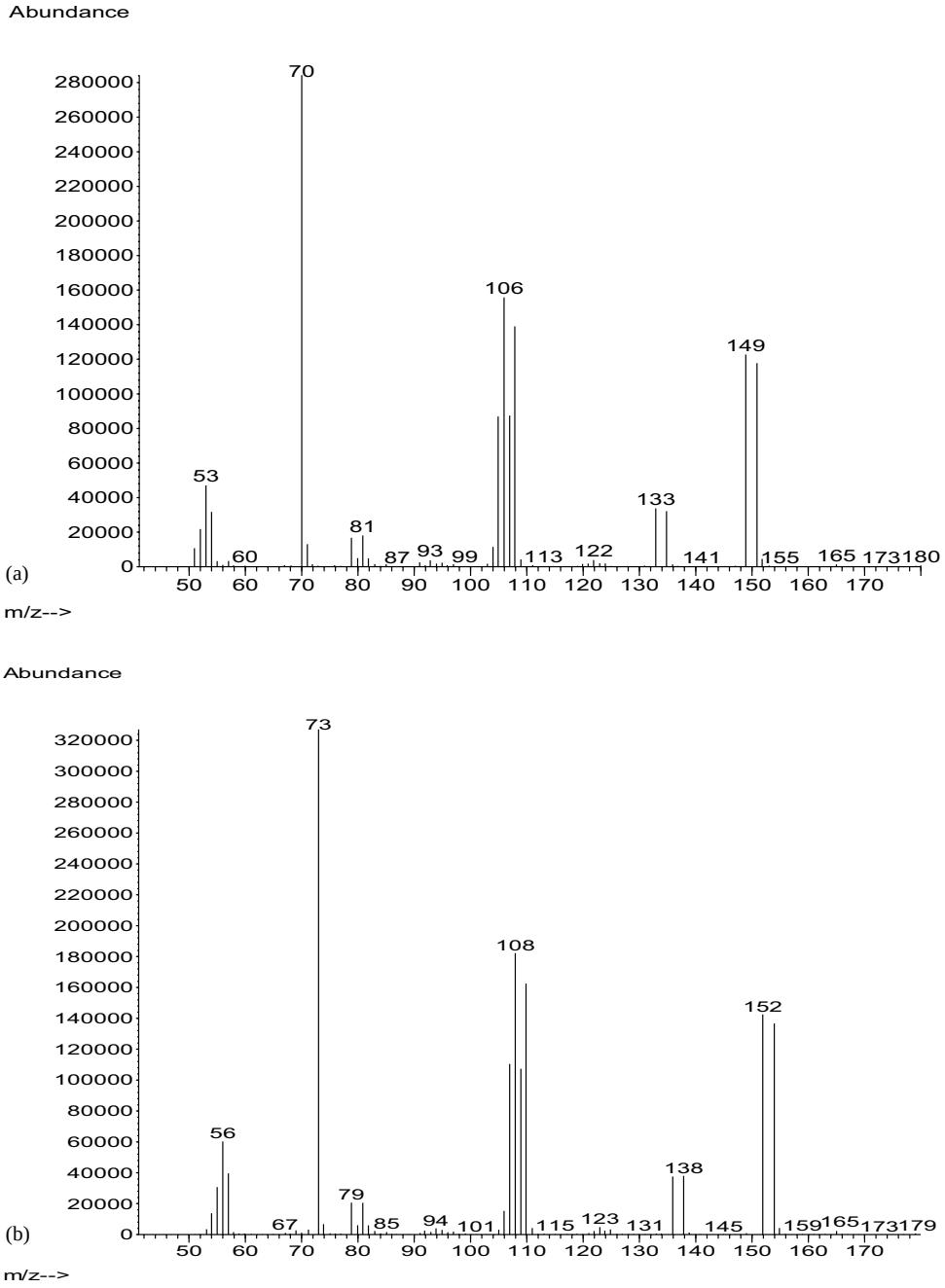

Fig. 2. Mass spectra of acrylamide derivatives: (a) 2-bromopropenamide; (b) 2-bromo($^{13}C_3$)propenamide (internal standard).

contain <5 μg/kg of incurred acrylamide ($n = 5$). Test portions were fortified with acrylamide at concentrations of 5, 10, 50, 100, 200, and 500 μg/kg before the extraction step, and at the same time isotope-labeled acrylamide was added to each flask to compensate for variations due to analyte ionization efficiency or injection volume. Taking into account the correction of analyte loss by the internal standard, the overall recoveries of acrylamide in wheat semolina ranged between 93 and 104% (Table 2). Replicate experiments at spiking levels of 5 μg/kg ($n = 8$, four different days) and 10 μg/kg ($n = 15$, five different days) gave mean recoveries of 93 and 95% with relative standard deviation of 32.6 and 11.9%, respectively. A good accordance of calibration slope and intercept values calculated with data obtained from calibration standards and fortified samples confirmed the compensation of matrix effects by ($^{13}C_3$)acrylamide (data not shown).

The accuracy of the method was further investigated through a comparative test with two independent laboratories. Test portions of commercial and experimental cereal products ($n = 9$) containing acrylamide concentrations ranging between 5 and 62 μg/kg were analyzed with the

Table 1
Acrylamide levels measured in two commercial cereal products under repeatability and intermediate reproducibility conditions

		Rice product	Three-cereal product[a]
Repeatability conditions			
Acrylamide results (μg/kg)	Day 1	17, 18, 17, 16, 16, 20, 19	65, 74, 66, 71, 74, 69, 75
Mean (μg/kg)		17.6	70.6
S.D.(r) (μg/kg)		1.5	4.0
R.S.D.(r) (%)		8.6	5.7
Intermediate reproducibility conditions			
Acrylamide results (μg/kg)	Day 2	16, 17	67, 62
	Day 3	16, 14	67, 66
	Day 4	17, 16	68, 68
	Day 5	17, 17	65, 77
Mean (μg/kg)		16.3	67.5
S.D.(iR) (μg/kg)		1.1	4.2
R.S.D.(iR) (%)		6.8	6.2

[a] The product contained wheat, oats and barley.

Table 2
Recoveries of acrylamide from a wheat semolina sample spiked at levels between 5 and 500 μg/kg

Acrylamide added (μg/kg)	*n*	Acrylamide found (μg/kg)		Mean recovery[a] (%)	R.S.D. (%)
		Range	Mean		
0	5[b]	2–5	4	–	–
5	8[c]	6–11	9	93	32.6
10	15[b]	12–16	13	95	11.9
50	1	–	54	100	–
100	1	–	108	104	–
200	1	–	191	94	–
500	1	–	505	100	–

[a] Data corrected for the amount of acrylamide found in unspiked material and for the analyte loss by the internal standard.
[b] Analyses were performed on five different days.
[c] Analyses were performed on four different days.

Table 3
Comparison of acrylamide concentrations found in nine roller dried or extruded cereal products by three different laboratories

Product description	Type	Acrylamide content (μg/kg)		
		In-house results (GC–MS)	Laboratory A (GC–MS)	Laboratory B (GC–MS/MS)
Rice (roller dried)	Commercial	18	16	25
Rice (roller dried)	Commercial	10	8	16
Maize (extruded)	Commercial	17	19	33
Wheat, oats and barley (roller dried)	Commercial	62	44	66
Wheat base (roller dried, dry)	Experimental	15	16	22
Wheat base (roller dried, wet)	Experimental	14	12	15
Rice base (extruded, agglomerated)	Experimental	9	7	10
Rice–soya base (roller dried)	Experimental	5	4	5
Wheat base (roller dried)	Experimental	5	4	5

present GC–MS method and sent to two laboratories in Germany. Laboratory A used an in-house GC–MS method, and laboratory B used an in-house GC–MS/MS method. As shown in Table 3, a good agreement was demonstrated between the three laboratories, particularly at very low concentration levels (<10 μg/kg).

4. Conclusion

Although the proposed method is similar in its principle to that recently published by Tareke et al. [17], the use of Carrez purification before the bromination step and the inclusion of an additional Florisil clean-up step after

derivatization have been shown very effective in removing interfering co-extractives identified in some difficult cereal matrices. Taken together, these two purification steps combined with bromination allow to achieve a very good sensitivity, with a limit of detection and a limit of quantitation estimated at 2 and 5 μg/kg, respectively. Moreover, the present work describes for the first time in-house validation characteristics for acrylamide in a wide range of concentrations. The use of ($^{13}C_3$)acrylamide as internal standard added to the samples at the initial step in the work-up procedure was shown to significantly improve the accuracy and precision of measurements, allowing to obtain repeatability and intermediate reproducibility relative standard deviations below 10%, even at low concentration levels (<20 μg/kg). Consequently, this method should be regarded as a new alternative for routine analysis of trace levels of acrylamide in complex cereal-based foods.

References

[1] Swedish National Food Administration, Information About Acrylamide in Food, 24 April 2002, http://www.slv.se.
[2] IARC Monographs on the Evaluation of Carcinogen Risk to Humans, vol. 60, International Agency for Research on Cancer, Lyon, 1994, p. 389.
[3] D.S. Mottram, B.L. Wedzicha, A.T. Dodson, Nature 419 (2002) 448.
[4] R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.-C. Robert, S. Riediker, Nature 419 (2002) 449.
[5] Scientific Committee on Food, Opinion of the SCF on New Findings Regarding the Presence of Acrylamide in Food, expressed on 3 July 2002, http://www.europa.eu.int.
[6] Food Standards Agency, Study on Acrylamide in Food. Background Information and Research Findings, Press briefing of 17 May 2002, http://www.food.gov.uk.
[7] Health Implications of Acrylamide in Food, Report of the Joint FAO/WHO Consultation, Geneva, Switzerland, 25–27 June 2002.
[8] Report of the Analytical Working Group, 9 November 2002, JIFSAN Acrylamide in Food Workshop, 28–30 October 2002, Chicago, USA.
[9] J. Tekel, P. Farkas, M. Kovác, Food Addit. Contam. 6 (1989) 377.
[10] L. Castle, J. Agric. Food Chem. 41 (1993) 1261.
[11] US EPA, SW 846, Method 8032A, US Environmental Protection Agency, Washington, DC, 1996.
[12] L.S. Bologna, F.F. Andrawes, F.W. Barvenik, R.D. Lentz, R.E. Sojka, J. Chromatogr. Sci. 37 (1999) 240.
[13] D.S. Barber, J. Hunt, R.M. LoPachin, M. Ehrich, J. Chromatogr. B 758 (2001) 289.
[14] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, Chem. Res. Toxicol. 13 (2000) 517.
[15] K. Kawata, T. Ibaraki, A. Tanabe, H. Yagoh, A. Shinoda, H. Suzuki, A. Yasuhara, J. Chromatogr. A 911 (2001) 75.
[16] J. Rosén, K.-E. Hellenäs, Analyst 127 (2002) 880.
[17] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, J. Agric. Food Chem. 50 (2002) 4998.
[18] A. Becalski, B.P.-Y. Lau, D. Lewis, S.W. Seaman, J. Agric. Food Chem. 51 (2003) 802.
[19] L. Hartig, Ch. Hummert, J. Buhlert, K. von Czapiewski, A. Schreiber, Poster Presentation at the 17th Symposium on Liquid Chromatography/Mass Spectrometry, Montreux, Switzerland, November 2002.
[20] J.S. Ahn, L. Castle, D.B. Clarke, A.S. Lloyd, M.R. Philo, D.R. Speck, Food Addit. Contam. 19 (2002) 1116.
[21] F. Höfler, R. Maurer, S. Cavalli, GIT Labor-Fachzeitschrift 48 (2002) 968.
[22] S. Riediker, R.H. Stadler, J. Chromatogr. A 1020 (2003) 121.
[23] H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaka, H. Kobayashi, M. Yoshida, Food Addit. Contam. 20 (2003) 215.
[24] C. Gertz, S. Klostermann, Eur. J. Lipid Sci. Technol. 104 (2002) 762.
[25] M. Biedermann, S. Biedermann-Brem, A. Noti, K. Grob, P. Egli, H. Mändli, Mitt. Lebensm. Hyg. 93 (2002) 638.
[26] S. Nemoto, S. Takatsuki, K. Sasaki, T. Maitani, J. Food Hyg. Soc. Jpn. 43 (2002) 371.
[27] D.B. Clarke, J. Kelly, L.A. Wilson, J. AOAC Int. 85 (2002) 1370.
[28] Commission Decision 2002/657/EC of 12 August 2002, Off. J. Eur. Commun. L221/8, 2002.
[29] A. Hashimoto, Analyst 101 (1976) 932.
[30] F. Andrawes, S. Greenhouse, D. Draney, J. Chromatogr. 399 (1987) 269.