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Short communication

Development of a quantitative method for determination of acrylamide in infant powdered milk and baby foods in jars using isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry

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Abstract

An improved method has been developed for the determination of acrylamide in infant powdered milk and baby foods in jars, a particular class of foodstuffs which represent an important source of nutrition for young infants and babies. This method uses isotope dilution liquid chromatography coupled to a tandem mass spectrometer with electrospray ionization and is significantly more sensitive than previous published methods with a limit of quantification estimated at 1 $\mu g\,kg^{-1}$. The new method offers effective sample preparation procedures including defatting with petroleum ether, extraction with aqueous solution of sodium chloride, further liquid–liquid extraction with ethyl acetate and clean-up by solid-phase extraction (SPE) with HLB 200 mg cartridges. The analytical method was well validated and good results were obtained with respect to repeatability (RSD < 5%) and recovery (86–97%) which fulfilled the requirements defined by European Union (EU) legislation. The acrylamide level in infant powdered milk and baby foods in jars were 3.01–9.06 $\mu g\,kg^{-1}$ and 6.80–124.93 $\mu g\,kg^{-1}$, respectively. Especially, this new method is successfully applied to the trace quantification of acrylamide in infant/baby foods, the content of which is less than 10 $\mu g\,kg^{-1}$.

Keywords: Acrylamide; Infant powdered milk; Baby foods in jars; LC–MS/MS

1. Introduction

In April 2002, researchers in Swedish National Food Administration (SNFA) and Stockholm University announced that carbohydrate-rich foods that are fried or baked at high temperature contain relatively high levels of acrylamide [1]. It has been shown that the likely reactants which produce significant levels of acrylamide in foods are asparagine and reducing sugars [2]. Recently in 2005, World Health Organization (WHO) and Food and Agriculture Organization (FAO) together announced that certain foods processed or cooked at high temperature especially Western-style snacks contain considerable levels of acrylamide and may harm human health to a certain extent [3]. Under such situation, researches on the analytical method and risk assessment of acrylamide in different food matrixes have once again become a hotspot since the discovery of acrylamide in 2002.

Infant powdered milk and baby foods in jars belong to a particular class of foodstuffs which represent an important source of nutrition for young infants and babies. However, the high consumption of these foods by infants or babies in the whole world may be a significant source of daily exposure to acrylamide. Recently, several research groups were involved in rapidly developing methods to reliably quantify acrylamide in a large variety of different foodstuffs by LC–MS/MS, such as cereal-based foods [4,5], coffee [6–8] and olives [9]. However, few publications focused on the acrylamide level in infant/baby foods and the development of trace analytical methods for the determination of acrylamide [10]. The aims of this study were (i) to investigate acrylamide that probably exists in infant powdered milk and baby foods in jars and (ii) to develop a trace method that works well for the detection and quantification of acrylamide in infant/baby foods by LC–MS/MS, especially the acrylamide level of which is less than 10 $\mu g\,kg^{-1}$.

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2. Experimental

2.1. Samples

Several representative infant/baby foods including powdered milk and foods in jars were collected from supermarkets in Hangzhou (Zhejiang Province, China) in the winter of 2004. The analytical survey comprised a series of commercial products such as starter and infant formula powdered milk and baby foods in jars including chicken/*lentinusedodes* mash, pork liver/medlar mash, tunny mash and pork/kelp/carrot mash.

2.2. Chemicals

Acrylamide (99%) and $^{13}C_3$-labelled acrylamide (isotopic purity 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Formic acid (96%) and ethyl acetate (HPLC-grade) were obtained from Tedia (Fairfield, OH, USA) while methanol (HPLC-grade) was supplied by Merck (Whitehouse Station, NJ, USA), respectively. All of other solvents and chemicals used for the analysis of acrylamide were of analytical grade. Water was purified with a Milli-Q system (Millipore, Bedford, USA). All solutions prepared for LC were passed through a 0.45 μm nylon filter before use as a dilute medium.

2.3. Analytical instruments and conditions

The quantification of acrylamide in infant/baby foods was performed on LC–MS/MS with electrospray positive ionization (ESI+) using a Micromass Quattro Ultima coupled to Waters 2695 HPLC (2695 quaternary pump, 2695 microvacuum degasser, 2695 thermostated autosampler, 2695 thermostated column compartment) with an Atlantis dC_{18} column (210 mm × 1.5 mm, 5 μm; Waters, Milford, MA, USA) together with a precolumn Hypercarb Guard (10 mm × 2 mm; Thermo Electron, San Jose, CA, USA).

Standards and sample portion extracts (10 μl) were analyzed on a Waters 2695 HPLC system coupled to a mass spectrometer. The temperature of both precolumn and column were maintained at 25 °C. The mobile phase was 10% methanol/0.1% formic acid in water with a flow speed of 0.2 ml min^{-1}. On the other hand, the conditions of MS/MS detection and quantification were as follows: capillary voltage, 3.5 kV; cone voltage, 50 V; source temperature, 100 °C; desolvation gas temperature, 350 °C; desolvation gas flow, 400 l h^{-1} nitrogen; cone gas flow, 45 l h^{-1} nitrogen; and argon collision gas pressure to 6×10^{-3} mbar for MS/MS. The LC–MS/MS run time was 10 min per sample. The collision energy for each monitored transition was optimized as 6 eV for both acrylamide and $^{13}C_3$-acrylamide in MRM mode. MS conditions were optimized by observing responses for loop injections of 100 pg of acrylamide on-column.

2.4. Calibration procedure

The calibration was achieved by the internal standard method. A plot was made of the ratio A_{aa}/A_{is} against C_{aa}/C_{is} (A_{aa}/A_{is}, the peak–area ratio of acrylamide and $^{13}C_3$-acrylamide; C_{aa}/C_{is}, the concentration ratio of acrylamide and $^{13}C_3$-acrylamide). By adding the same concentration of internal standard (1 ng ml^{-1}) to the sample and fixing the aliquot portion, concentration can be used as the abscissa of plot. Meanwhile, concentrations of acrylamide ranged from 0.1 to 5 ng ml^{-1} were studied.

2.5. Sample extraction and clean-up

The baby foods in jars were homogenized in HL-2070 multifunction food processor (Shanghai Herine Electric Appliance Co., Ltd., Shanghai, China) prior to sampling while powdered milk products were sampled directly. Aliquots (1.50 g) of abovementioned samples were weighted into 50 ml centrifuge tubes, and 500 μl of $^{13}C_3$-labelled acrylamide solution (1 μg ml^{-1}) was homogeneously added. To make a defatting process, 20 ml of redistilled petroleum ether was added and each tube was then clamped and shaken in an ultrasonic shaker to mix the tube contents for 10 min. The supernatant petroleum ether was removed and the defatting step was then performed again. Seven milliliters of aqueous solution of sodium chloride was added into the residue of each tube, which was capped and shaken in an ultrasonic shaker to extract the analyte for 20 min. The tubes were centrifuged at 15,000 rpm for 15 min with an Allegra 21 Beckman Coulter centrifuge (Beckman Coulter Inc., Fullerton, CA, USA). The clarified aqueous layer was promptly removed by pipet. The residues were extracted again by 8 ml of aqueous solution of sodium chloride and the extraction step was performed as described above. The supernatant fluids during two extraction steps were removed and merged into corresponding separatory funnels by pipet for further extraction. Then, the aqueous solution was extracted by 15 ml of ethyl acetate for three times. The organic phase was removed from separatory funnel, concentrated by rotatory evaporator and dried by nitrogen gas both at 50 ± 1 °C. The residue was re-dissolved by water and removed for clean-up. Oasis HLB SPE cartridges (6 cm^3, 200 mg) purchased from Waters were conditioned with 3.5 ml of methanol followed by 3.5 ml of water; the methanol and water portions were discarded. Each cartridge was loaded with 1.5 ml of re-dissolved extract. The extract was allowed to pass through the sorbent material and discarded. Then the cartridge was eluted with 3 ml of water and the eluant was collected. Aliquots of the eluant were transferred into amber glass autosampler vials for LC–MS/MS analysis.

3. Results and discussion

3.1. Optimization of chromatographic conditions

In order to optimize the LC conditions, the retention and peak shape on elution of acrylamide using different concentration ratios of methanol as mobile phase were studied. Finally, the LC mobile phase containing 10% methanol (v/v) appeared optimal with regard to analyte retention, separation efficiency, and MS response. Furthermore, an increase in column temperature from ambient to higher level resulted in a shorter retention time of the analyte, with loss of good peak shape and

Table 1
Examples of recovery tests of LC–MS/MS method[a]

	No. I Infant powdered milk[b]	No. II Chicken/*lentinusedodes* mash[b]	No. III Pork liver/medlar mash[b]
Before addition ($\mu g\,kg^{-1}$)	4.61 ± 0.04	33.78 ± 1.68	127.26 ± 4.97
Added amount ($\mu g\,kg^{-1}$)	5	50	200
After addition ($\mu g\,kg^{-1}$)	9.00 ± 0.20	81.62 ± 2.04	323.58 ± 2.07
Recovery (%)	86.8 ± 5.1	93.6 ± 2.7	97.2 ± 2.2

[a] The concentration data of acrylamide are shown as mean ± SD ($n = 3$). Three representative samples spiked at 5, 50 and 200 $\mu g\,kg^{-1}$ of acrylamide standard solution were regarded as low, intermediate and high level spiked food matrix, respectively.
[b] Sample.

separation efficiency so that room temperature was set during LC separation. The total analytical run-time was 10 min both for standard solutions and 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 (5 μm) may help to further simplify the current approach, with the goal of retaining equivalent analytical performance.

3.2. Optimization of sample treatment

Another challenge in this study was therefore to improve the recovery of acrylamide throughout the sample pretreatment and concomitantly reduce the amount of co-extractives in difficult matrixes. Some different commercially available SPE cartridges such as non-polar stationary phase (Varian Bond Elut-C_{18}, 1 cm^3/100 mg or 3 cm^3/500 mg) or hydrophilic–lipophilic balanced copolymer (Oasis HLB, 3 cm^3/60 mg or 6 cm^3/200 mg) were tested. Acrylamide was not completely adsorbed by cartridges with small size such as Varian Bond Elut-C_{18} (1 cm^3, 100 mg) and Oasis HLB (3 cm^3, 60 mg). Good adsorbability and SPE recovery of acrylamide were found when using Oasis HLB (6 cm^3, 200 mg) as SPE cartridges, i.e. $90.3 \pm 2.8\%$, $97.5 \pm 0.4\%$ and $98.1 \pm 5.2\%$ of SPE recovery ($n = 5$) under the acrylamide concentration of 0.5, 25 and 100 ng ml^{-1}, respectively. On the other hand, efforts on ameliorating the recovery of acrylamide were focused on a combination of liquid–liquid plus SPE. Due to the hydrophilic nature of acrylamide, the analyte was extracted three times with ethyl acetate (volumetric ratio ethyl acetate/water = 1:1), which achieved a satisfactory extraction yield.

3.3. Calibration and method performances

For qualitative purposes, the method was evaluated by taking into account the precision of retention time, the interference of co-elution and peak purity of the analyte. A high repeatability of the retention time was obtained with RSD values lower than 1% for both standards (non-labelled and labelled acrylamide) and different food matrixes. In no case were impurities or co-elutions were observed (match factor ≥ 95%). For quantitative purposes, the method was validated by defining the linearity, LOD and LOQ, repeatability, precision and recovery. The calibration curve for the determination of acrylamide in infant powdered milk and baby foods in jars ($A_{aa}/A_{is} = 0.843C_{aa}/C_{is} + 0.001$) was linear over the range of 0.1–5 ng ml^{-1} with a coefficient of determination (r^2) of 0.9992 ($n = 5$). Some extracts of baby foods in jars containing relatively high levels of acrylamide were diluted before injection in order to match the linear range of calibration. The limit of quantification (LOQ) evaluated by the statistical software of MassLynx v4.0 (Micromass, Manchester, Lancashire, UK) was calculated as 1 $\mu g\,kg^{-1}$, which adequately satisfied the trace quantification of acrylamide in infant/baby foods. Good repeatability was obtained for three kinds of powdered milk with infant formula (3.59, 6.85 and 9.06 $\mu g\,kg^{-1}$) and two kinds of baby foods in jars (7.86 $\mu g\,kg^{-1}$ for pork/kelp/carrot

Table 2
Quantitative analysis of acrylamide level in infant powdered milk and baby foods in jars

Sample	Acrylamide level ($\mu g\,kg^{-1}$)						Mean ($\mu g\,kg^{-1}$)	RSD (%)
Infant powdered milk with different brands								
Powdered milk 1	3.36	3.07	3.12	3.33	3.31	3.09	3.21	4.1
Powdered milk 2	3.70	3.53	3.30	3.73	3.64	3.62	3.59	4.3
Powdered milk 3	6.93	7.84	6.84	7.10	8.56	7.65	7.49	8.8
Powdered milk with starter formula	8.92	9.08	9.26	9.42	8.44	9.21	9.06	3.8
Powdered milk with infant formula 1	6.42	6.92	7.07	6.73	7.02	6.94	6.85	3.5
Powdered milk with infant formula 2	4.69	4.65	4.75	4.18	4.60	4.57	4.57	4.5
Baby foods in jars								
Chicken/*lentinusedodes* mash	34.16	31.49	30.46	34.48	34.87	34.01	33.24	5.4
Pork liver/medlar mash	128.20	127.60	126.86	118.21	121.21	127.48	124.93	3.3
Tunny mash	6.45	6.67	6.78	6.48	7.33	7.08	6.80	5.1
Pork/kelp/carrot mash	7.52	7.72	8.31	7.75	7.91	7.98	7.86	3.4

mash and 124.93 μg kg^{-1} for pork liver/medlar mash, respectively) with the RSD range of 3.3–4.4%. The within-day precision (RSD) of two representative samples, powdered milk (3.59 μg kg^{-1}) and pork liver/medlar mash (125.21 μg kg^{-1}), were 4.2% and 2.9%, respectively ($n = 6$). The day-to-day precision (RSD) of them were 4.9% and 3.7%, respectively ($n = 6$). Recovery of the method was demonstrated in three tests employing the standard spiking method. The average recovery was >86% for all the three samples (see the details in Table 1). Considering all of the above data for method performance, this LC–MS/MS and sample pretreatment method employed in the present work can be regarded as selective, precise and robust.

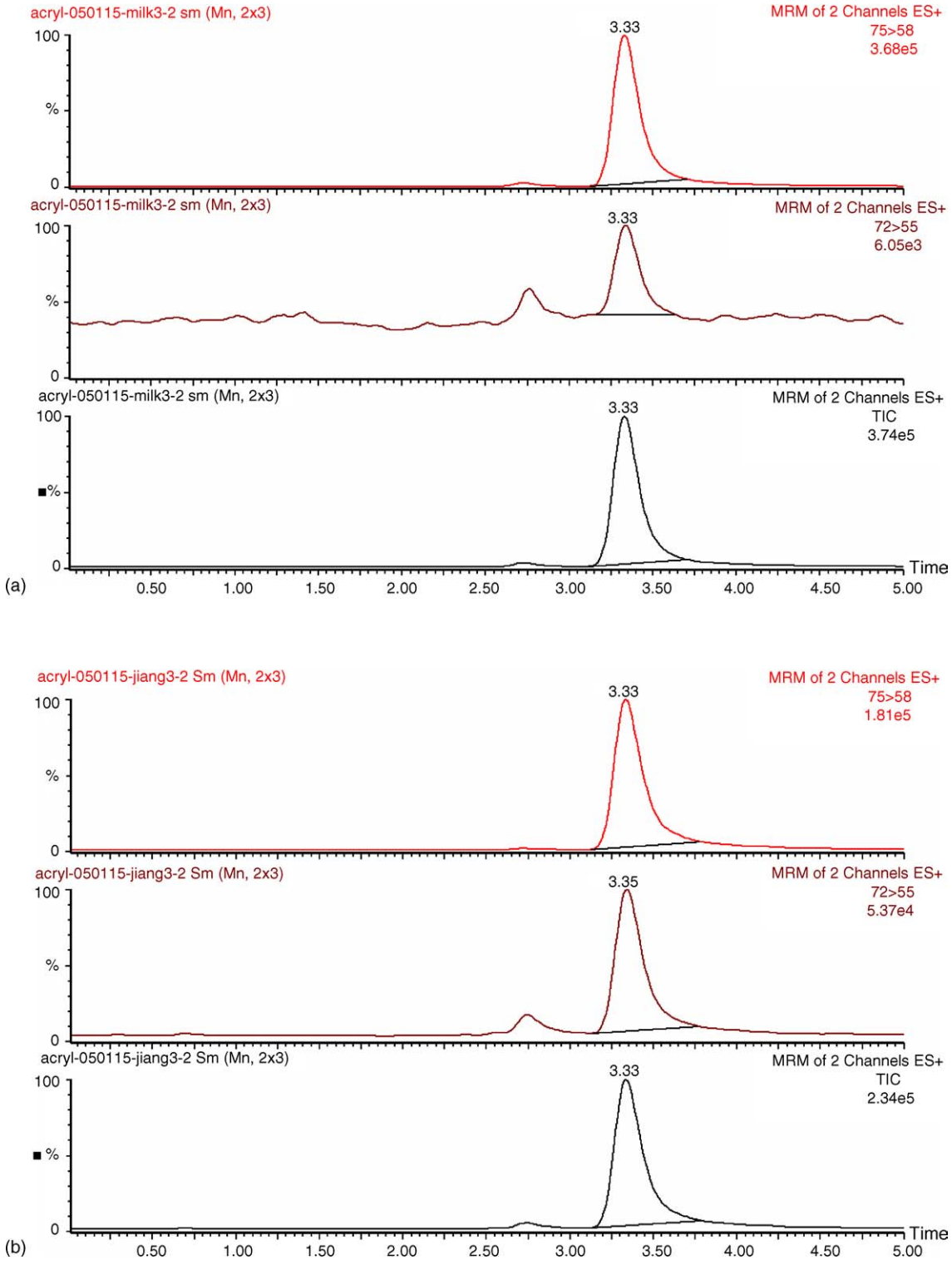

Fig. 1. Representative chromatograms of the $^{13}C_3$-acrylamide (75 > 58) and acrylamide (72 > 55) extract of (a) infant powdered milk and (b) pork liver/medlar mash.

3.4. Quantitative analysis of acrylamide in infant/baby foods

Food groups were chosen for analysis in this study if they represented a significant part of infant diet. Product sampling was not representative of all brands. Many of the food types are represented by only one product in the survey. Fig. 1 showed representative chromatograms of the extracts of both infant powdered milk and baby foods in jars. These chromatograms also showed a non-identified peak at the retention time different from that of acrylamide. This unknown compound, which could not be easily removed according to the extraction and clean-up methods in the present work, was present in the acrylamide extract. Fortunately, the interference of this response for the analyte could negligible because the retention time of this impurity peak did not overlay with the response of both acrylamide and its isotope internal standard analyzed.

Quantitative analysis of infant powdered milk samples and baby foods in jars rendered the concentration range of 3.21–9.06 $\mu g\,kg^{-1}$ (RSD = 2.9–8.8%, $n = 6$) and 6.80–124.93 $\mu g\,kg^{-1}$ (RSD = 3.3–5.4%, $n = 6$) for acrylamide, respectively. The detailed concentrations of acrylamide in above-mentioned infant foods were summarized in Table 2. On the other hand, the accuracy of a confirmatory method should be 80–110% for samples according to the EU legislation [11,12]. Recoveries in this work were 86–97%, with RSD of 1.9–3.4% (Table 1), which fulfilled the legislation requirements. A routine and robust LC–MS/MS method that worked well for the detection and quantification of acrylamide in a wide variety of infant foods was developed in the present study.

4. Conclusion

This work developed a LC–MS/MS methodology for the trace quantitative analysis of acrylamide in infant powdered milk and baby foods in jars. Especially, this new method is successfully applied to the trace quantification of acrylamide in infant/baby foods, the content of which is less than 10 $\mu g\,kg^{-1}$. Further method developments such as the amelioration of pretreatment procedures in protein-rich samples, the analytical investigation of acrylamide levels in Chinese traditional heat-treated foods and the application of ASE technique [13,14] during the analyte extraction are now being conducted in our laboratory. Meanwhile, the risk assessment of acrylamide exposure levels derived from different foods for infant daily dietary will be communicated in due course.

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