

Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry

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A method using liquid chromatography tandem mass spectrometry (LC-MS-MS) with electrospray for the analysis of acrylamide in foods is reported. The method comprises the addition of deuterium-labelled acrylamide- d_3 , extraction with water, mixed mode solid phase extraction, ultrafiltration and a graphitised carbon column for chromatography. The transitions m/z $72 > 55$, $72 > 54$, $72 > 44$, $72 > 27$, $72 > 72$ and $75 > 58$ were recorded in multiple reaction monitoring mode for identification and quantification. In-house validation data for products from potatoes and cereals (30 to 10 000 $\mu\text{g kg}^{-1}$) are presented (accuracy 91 to 102%, relative standard deviation 3 to 21%). Interlaboratory validation data (comparison with gas chromatography mass spectrometry, 25 to 2000 $\mu\text{g kg}^{-1}$) showed excellent results ($r^2 = 0.998$).

Introduction

Findings of high amounts of acrylamide in various common cooked foods were announced jointly by the University of Stockholm and the National Food Administration at a press conference in Stockholm on April 24, 2002. At the same time analytical data for various common foods were made available on the Administration's website (www.slv.se).

Acrylamide has been classified as 'probably carcinogenic to humans' (Group 2A) by the International Agency for Research on Cancer (IARC).¹ The Swedish findings have attracted world-wide attention and there has been a huge demand for the newly developed analytical LC-MS-MS procedure behind the published concentration data.

The discovery originates from findings of a specific haemoglobin adduct of acrylamide in human subjects, later also in rats fed with fried feed, by scientists at the University of Stockholm.² The analytical method employed for acrylamide analysis in feed relied on derivatisation of acrylamide by bromine, a time-consuming work-up including solvent partitioning, and detection by gas chromatography mass spectrometry (GC-MS).² The procedure was further developed for cooked foods by the introduction of C-13 labelled acrylamide as an internal standard, and by omitting a gel permeation chromatography step.³

One important purpose of the present work was to verify the presence of acrylamide in food by using alternative methodology, *i.e.* to confirm the identity of acrylamide and to show that it was not formed as an artefact during the analytical procedure. Moreover, there was a need for a simple and fast method that could be used for extensive investigations of acrylamide in a wide range of foods. This communication presents the new LC-MS-MS procedure and preliminary validation data. A full paper including further developments and validation is planned.

Experimental

Materials

Acrylamide was supplied by Merck (Darmstadt, Germany), and deuterium-labelled acrylamide- d_3 by Polymer Source Inc. (Dorval, Quebec, Canada). The SPE-column used was Isolute Multimode 300 mg from IST (Hengoed, Mid Glamorgan, UK), HPLC-grade acetonitrile from Lab-Scan (Dublin, Ireland). Water was Milli-Q water from a Millipore purification system, the syringe driven filter was a Millex-GS, 0.22 μm , and the centrifuge spin filter a Microcon YM-3 regenerated cellulose with 3 kDa cut off, all from Millipore (Bedford, MA, USA). Stock solutions of standards, 1 mg ml^{-1} , were prepared in water and stored at -20°C . Working standard solutions, for spiking samples as well as for the standard curve, were obtained by dilutions in water. Concentrations for the standard curve were 0, 1, 10, 100 and 1000 ng ml^{-1} , all with acrylamide- d_3 at 40 ng ml^{-1} .

Equipment

Mass Spectrometry was performed using a triple quadrupole: Micromass Quattro Ultima with Z-spray and the standard electrospray probe (Micromass UK Ltd, Altrincham, Cheshire, UK). The HPLC system was a Waters Alliance 2690 (Waters Ltd, Watford, Hertfordshire, UK). The HPLC column was a Hypercarb, 5 μm , $50 \times 2.1 \text{ mm}$, with a guard column 5 μm , $10 \times 2 \text{ mm}$ (Thermo Hypersil-Keystone, www.thermohypersil.co.uk). The homogeniser was a DISP 25 (InterMed, Roskilde, Denmark).

Samples

Samples were homogenised and analysed fresh, or stored at -20°C until analysis. Mashed potatoes for the recovery experiments were made from boiled potatoes (400 g) that were mashed and mixed with rapeseed oil (40 ml). Samples of mashed potatoes and rye flour were analysed blank and spiked with acrylamide (100 to 400 μl of a standard solution 100 ng ml^{-1} to 100 $\mu\text{g ml}^{-1}$ to 4 g of sample). After mixing, these samples were allowed to stand for 30 min and then treated in the same way as the normal samples. The experiments were carried out on different occasions, thus reflecting between-day variations. Additional precision data were obtained by duplicate analysis on six different occasions of one sample each of crispbread and potato crisps, representing relatively low and high acrylamide concentrations respectively.

Sample extraction

Water (40 ml) was added to homogenised samples (potato crisps $2.0 \pm 0.02 \text{ g}$, other samples $4.0 \pm 0.04 \text{ g}$). An internal

standard was added (800 µl of a water solution of deuterium-labelled acrylamide 2.0 µg ml⁻¹) and the samples were extracted by means of a homogeniser (2 min, 9500 min⁻¹). The extracts were centrifuged (3600 g, 10 min) (extra centrifugation (16 800g, 10 min) for potato crisps after precipitation by freezing). SPE-columns were pre-treated with acetonitrile (1 ml) and water (2 × 2 ml), and supernatant (3 ml) was filtered through the column. The first portion (1 ml) was discarded and the remaining portion was collected and passed through a syringe filter (0.22 µm). The filtrate was collected and passed through a centrifuge spin filter (16 800g, 10 to 20 min) until a sufficient volume had been obtained for analysis with LC-MS-MS.

LC-MS-MS analysis

The mobile phase was water and the flow rate was maintained at 400 µl min⁻¹. The injection volume was 10 µl, and injections were made every tenth minute. As the default position the HPLC eluate was directed to waste and 20 % v/v methanol in water was pumped into the LC-MS-MS interface by means of an extra HPLC pump. Only the HPLC eluate from 1.2 to 3.2 min was directed into the interface by means of a divert valve. Samples were injected twice and standard solutions three times. After each overnight run the column was washed with 80% v/v acetonitrile in water. The LC-MS-MS was operated in the positive electrospray mode. Nitrogen was used as drying gas (600 l h⁻¹, 400 °C), as nebulising gas (fully open) and as cone gas (200 l h⁻¹). Argon was used as collision gas (2.3 10⁻³ mbar). The source temperature was maintained at 125 °C, the capillary voltage at 2 kV, the cone voltage at 20 V and the first hexapole voltage at 10 V. For the transitions m/z 72 > 55, 75 > 58, 72 > 54, 72 > 44, 72 > 72 and 72 > 27 the collision energies were set to 9, 9, 16, 20, 0 and 14 eV respectively. The dwell time for each MRM transition was 0.15 s, and the interchannel delay was 0.03 s. The instrument was operated at unit resolution.

Results and discussion

Method development

The first aim of this work was to develop a mass spectrometry method for direct detection of acrylamide, which would unequivocally verify the presence of acrylamide in a range of cooked foods. The choice was LC-MS due to the hydrophilic properties of acrylamide, and MS-MS for a high degree of verification if several transitions could be found. The second aim was to develop a method with high sample throughput and it was decided to avoid lengthy extraction steps if possible.

Positive electrospray proved to be the most sensitive mode with the present instrument. All parameters were optimised to obtain as high a signal as possible for the ion [M+1]⁺ in a water solution. By adding formic acid or acetonitrile the signal was not improved. By scanning the daughter ions the transitions with the highest response were identified. For each transition the collision energy was optimised. Only the transition m/z 72 > 55 showed a relatively high intensity while the remaining transitions m/z 72 > 54, 72 > 44 and 72 > 27 showed a relative intensity of approximately 2%, 1% and 0.2% respectively. These four transitions are suggested to correspond to the loss of ammonia, water, ethene and formamide respectively, thus representing different parts of the molecule. This was supported by the fact that acrylamide-d₃ (CD₂CDCONH₂) gave the corresponding fragments m/z 75 > 58, 75 > 57, 75 > 44 and 75 > 30, which all optimised at the same collision energy as the corresponding transitions for acrylamide. The instrument could also be set to record the trace m/z 72 > 72, giving a relative intensity of approximately 195% compared to m/z 72 > 55.

Validation and analysis of foods

For validation purposes and for the analysis of foods the instrument was used in MRM mode set to record m/z 72 > 55, 72 > 54, 72 > 44, 72 > 72 and 75 > 58. Extracts from a range of foods cleaned up as described above were injected into the system. The retention time was approximately 2.3 min (k' = 4.0) and varied slightly, mostly decreasing when running overnight. Preliminary experiments showed that this was caused both by temperature variations in the room and by column contamination. However, acrylamide-d₃ always had the same retention time as acrylamide. Problem with marked ion suppression to a varying extent was also overcome by the use of the isotopic internal standard. Thus, for quantification of acrylamide the area ratio of m/z 55/58 was used, and concentrations were calculated against a standard curve. Standard curves (1 to 1000 ng ml⁻¹) typically produced correlation coefficients of 0.999. When adding acrylamide in known concentrations (30 to 10 000 µg kg⁻¹) to blank samples of mashed potatoes or rye flour (totally 39 samples), the bias varied between 1 and 9% and the relative standard deviation (%RSD) between 3% and 9% under between-day repeatability conditions (Table 1). In addition, potato crisps and crispbread samples with mean acrylamide concentrations of 980 and 35 µg kg⁻¹ respectively were analysed in duplicate six days showing a %RSD of 6% and 21% respectively (Table 1). Preliminary estimations of limit of quantification and limit of detection for the matrices validated would not be higher than 30 µg kg⁻¹ and 10 µg kg⁻¹, respectively. However, due to varying ion suppression and background levels in other matrices no general limits could be set without further investigations.

The method's accuracy was further investigated through a small-scale inter-laboratory trial. Samples (n = 10) including potato crisps, crispbread, breakfast cereals and biscuits ranging in concentration from 25 to 2000 µg kg⁻¹ were analysed with the present LC-MS-MS method and sent to another laboratory for analysis with a GC-MS method.³ Excellent agreement (r^2 = 0.998) was demonstrated (Table 2).

For the purpose of confirming the identity of acrylamide in food samples, relative ion intensities were calculated for standard solutions as well as for a number of food extracts. The ratios varied slightly between days. For standard solutions (10, 100 and 1000 ng ml⁻¹) typical average values for m/z 54/55, 44/55, and 55/72 were 0.0230, 0.0139 and 0.512, respectively. For samples (n = 14) representing potato crisps, crispbread, french fries, breakfast cereals and biscuits ranging in concentration from 300 to 1600 µg kg⁻¹, the corresponding average values were 0.0233, 0.0133 and 0.515. Whenever the corresponding ratios could be calculated for other major peaks

Table 1 Between-day precision and accuracy results from in-house validation trial

Concentration added/µg kg ⁻¹	Average concentration found/µg kg ⁻¹	%RSD	<i>n</i>
Mashed potatoes			
0	< 10		4
30	30	6	3
50	49	5	3
100	102	8	6
1 000	1015	7	4
10 000	9807	4	4
Rye flour			
0	< 10		4
30	27	9	3
50	46	3	3
100	96	9	5
1 000	987	3	4
10 000	9819	5	4
Crispbread ^a	35	21	12
Potato crisps ^a	980	6	12

^a No acrylamide was added to these samples.

detected between 1.2 and 3.2 min they differed substantially from above. This indicates that not only the transitions m/z $72 > 55$, $72 > 54$ and $72 > 44$ but also m/z $72 > 72$ might aid in the identification of acrylamide. In a coming EU directive for confirmation of drug residues in meat with LC-MS-MS, maximum permitted tolerances for relative ion intensities are suggested.⁴ The maximum permitted tolerances for m/z 54/55, 44/55, and 55/72 would accordingly be $\pm 50\%$, $\pm 50\%$ and $\pm 20\%$, respectively. These tolerances were applied on five occasions for food analysis. For samples with an estimated concentration of more than $100 \mu\text{g kg}^{-1}$ all three ratios applied in most cases (40 out of 45). Below $50 \mu\text{g kg}^{-1}$ usually (in 13 out of 17 samples) only the ion with the highest signal to noise ratio, *i.e.*

m/z 55, could be detected. For the interval 50 to $100 \mu\text{g kg}^{-1}$ one to three ratios could confirm the presence of acrylamide (in 10 out of 13 samples). In addition, the transition m/z $72 > 27$ was recorded for some samples with high acrylamide levels, adding further evidence to the identification of acrylamide.

One purpose of monitoring the transition m/z $72 > 72$ was to provide data to be able to make a rough estimate of how specific the detection of m/z 72 would be using a single quadrupole instrument with the present work-up. Preliminary results indicate that it might be possible to use a single quadrupole especially at concentrations over $100 \mu\text{g kg}^{-1}$. However, this must be shown in true single ion monitoring experiments, and it should be stressed that it would give limited qualitative information.

Table 2 Interlaboratory comparison of the present method and GC-MS

Laboratory	A. AnalyCen AB	B. National Food Administration	B/A
Technique	GC-MS/ $\mu\text{g kg}^{-1}$	LC-MS- MS/ $\mu\text{g kg}^{-1}$	(%)
Crispbread a, (wheat)	23	25	109
Crispbread b, (rye)	50	59	118
Breakfast cereals, cornflakes, pooled samples	53	60	114
Corn crisps	184	190	103
Biscuits, pooled samples	230	224	97
Crispbread c, (rye)	560	592	106
Potato crisps a	694	649	94
Potato crisps b	1833	1631	89
Crispbread d, (rye)	1874	1744	93
Potato crisps c	2287	1993	87
		Average	101
		<i>s</i>	10.6
		%RSD	10.5

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