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Research Article

Acrylamide levels in Finnish foodstuffs analysed with liquid chromatography tandem mass spectrometry

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Sample clean-up and HPLC with tandem mass spectrometric detection (LC-MS/MS) was validated for the routine analysis of acrylamide in various foodstuffs. The method used proved to be reliable and the detection limit for routine monitoring was sensitive enough for foods and drinks (38 μg/kg for foods and 5 μg/L for drinks). The RSDs for repeatability and day-to-day variation were below 15% in all food matrices. Two hundred and one samples which included more than 30 different types of food and foods manufactured and prepared in various ways were analysed. The main types of food analysed were potato and cereal-based foods, processed foods (pizza, minced beef meat, meat balls, chicken nuggets, potato-ham casserole and fried bacon) and coffee. Acrylamide was detected at levels, ranging from nondetectable to 1480 μg/kg level in solid food, with crisp bread exhibiting the highest levels. In drinks, the highest value (29 μg/L) was found in regular coffee drinks.

Keywords: Acrylamide / Exposure / Food / Food intake / LC-MS/MS

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1 Introduction

Acrylamide is a reactive compound which is used in the synthesis of various polymers. It is also used in water purification processes and occasionally detected at low levels in drinking water [1]. The maximum residue limit has been set at 0.1 μg/L in drinking water by the European Union [2]. Acrylamide is also a constituent of tobacco smoke [3]. Other common users of acrylamide are the mining, papermaking, cosmetics and paint industries as well as biochemical laboratories [4, 5].

DNA damage, neurological disturbance and reproductive and carcinogenic effects have been reported in experimental animals exposed to acrylamide [6]. The International Agency for Research on Cancer (IARC) has classified acrylamide as 'probably carcinogenic to humans' (Group 2A), but its carcinogenicity has not been demonstrated in humans in epidemiological studies [4, 6]. The mode of action in chemical carcinogenesis at least in experimental settings seems to be *via* the formation of DNA adducts and indeed various adducts have been identified both *in vitro* and *in vivo* [7, 8].

Over the past few years, acrylamide contamination was reported in several fried and baked foodstuffs [9–12]. Acrylamide was demonstrated to be formed during cooking (>100°C) with the Maillard reaction being the most probable mode of its formation in food [13]. The Maillard reaction involves mainly asparagine and glucose [13–16]. Soon reports of several other studies in different countries confirmed the initial acrylamide observation and human biomonitoring studies were initiated using a variety of approaches including analysis of haemoglobin adducts and urine mercapturic acid measurements [17–23].

Polarographic, HPLC and GC techniques have been used to analyse acrylamide. These methods have mainly been used for environmental samples [1, 24–26] and methods suitable for acrylamide analysis in foods have only recently been described [14, 17, 18, 27–29]. These methods involve various kinds of sample processing, a chromatographic separation with HPLC or GC and detection with a mass spectrometer (MS) or preferentially with a tandem mass spectrometer (MS/MS). Recent results from interlaboratory trials indicate that the HPLC/MS/MS is applicable [30, 31]. Recently, a simple and rapid method was reported using LC

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with DAD for the determination of acrylamide in potato-based foods [32].

Some data of the acrylamide content of foodstuff have already been published and a database established [33]. Databases or individual studies of acrylamide contents in various foodstuffs are used for calculating the intakes of human populations and these can be utilised in the risk estimation [34–36].

In this communication, we describe acrylamide levels in food and drink samples collected from the Finnish food market. We also determined the effects of various cooking practices to the acrylamide levels. The samples were analysed with HPLC/MS/MS instrumentation using an in-house validated method.

2 Materials and methods

Acrylamide was purchased from Sigma (St. Louis, MO, USA) and deuterated acrylamide-d3 was obtained from Polymer Source (Quebec, Canada). The stock solutions were prepared in water (2 mg/mL). Working solutions were diluted from the stock solutions with water to concentrations ranging from 1 to 100 μg/mL. These solutions were used for standard preparation and for spiking. ACN and methanol were purchased from J.T. Baker (Deventer, The Netherlands). All solvents were HPLC-grade. Water was purified using a Millipore Milli-Q Plus system. The SPE columns Isolute Multimode Mixed-mode extraction column (300 mg/6 mL and 300 mg/3 mL) were purchased from IST International Sorbent Technology (Hengoed, Mid Glamorgan, UK), Oasis HLB 200 mg/6 mL columns were purchased from Waters (Milford, MA, USA) and Bond Elute Accucat 600 mg columns were purchased from Varian (Walnut Creek, CA, USA). The filters (Centriplus YM-3, Centrifugal Filter Devices 3000 Da) were from Millipore Corporation (Bedford, MA, USA).

Food samples were homogenised with a food mixer (La Moulinette, Moulinex, Italy). The chromatographic separation was achieved with an HPLC (Waters 2695 Alliance Separations module, Waters). The analytical column was a graphite carbon column (Hypercarb, 100 mm × 2.1 mm id, 5 μm particle size) connected to a guard column (Hypercarb, 10 mm × 2.0 mm id, 5 μm particle size, Thermo Hyprsil-Keystone, Cheshire, UK). A Croco-CilTM column oven was used (CILCluzeau, Sainte-Foy-la-Grande, France) with the temperature maintained at 26 ± 1°C. The analytes were detected with a triple quadropole mass spectrometer operating with Mass Lynx software (Micromass Quattro Micro, software version V3.5, 2000, Micromass Manchester, UK).

2.1 Samples

Samples were purchased from local retail shops and fast food restaurants 1–5 days before the analysis. They were stored in the laboratory according to the instructions on the packaging labels. Acrylamide was measured in rye crisp breads, fresh rye breads, toasts, French fries, potato chips, potato-ham casseroles, fried bacons, meat balls, pizzas, chicken nuggets, minced meat beefs, rye flour, oat flakes, biscuits, muesli, breakfast cereals, popcorn, coffee powder, coffee drinks, instant coffee drink, tea drinks, chocolate drink and cow milk. A total of 201 food samples which included 30 different types of food were analysed. The food samples included from 1 to17 different brands of each type of food.

Toasts (wheat, oats and rye bread) were heated in a toaster using short (40 s) toasting time or long (180 s) toasting time. Some slices were not heated. Potato-ham casseroles were heated in an oven (RK210W Rosenlew, Vantaa, Finland) at 180°C for 40 min or in a microwave oven (Grilli Mikro automatic 992D, Rosenlew) at full power according the cooking instructions. Five potato-ham casseroles were analysed as such, bacon slices were mildly and strongly fried and coffee was prepared in a coffee maker (60 g of coffee powder in 1 L of water) (Bravilor Bonamat TH-20, Japan). Instant coffee was prepared by weighing instant coffee powder (1 g), boiling water was added (100°C, 200 mL) and the drink was mixed carefully. Two tea bags were brewed in hot water (100°C, 200 mL) for 3 min and bulk tea leafs (2 g) were brewed in hot water (100°C, 80 mL) for 5 min. The chocolate drink was made by weighing chocolate drink powder (7 g) into a cup and mixing carefully with hot water (100°C, 100 mL).

2.2 Sample preparation, clean-up and analysis

The method of sample preparation was an in-house modification of the method published previously [29]. A homogenised sample (4 g) was extracted with water (30 mL) for 1 h in a horizontal shaker (270 min^{-1}) at room temperature and samples were protected from the light. Before extraction an internal standard (20 μg acrylamide-d3/mL in water, 150 μL) was added to the sample. In the case of liquid sample, the sample amount was 30 mL, in which the internal standard (50 μL) was added. Samples were frozen at –20°C for 40 min (cereal- and potato-based samples) or for 60 min (meat-based, coffee powder sample) and subsequently centrifuged (25 000 × *g*, 15 min at −5°C, Beckman model J2-21M Induction Drive Centrifuge, Beckman Instruments, Palo Alto, CA, USA). During the centrifugation, the samples thawed which allowed us to collect supernatants for further purification [39]. The SPE columns were preconditioned first with methanol (2 mL) followed by water (2 × 2 mL). Subsequently a sample (3 mL) was introduced into the column. Sample purification performed at the a flow rate of one drop *per* second and the first part of the effluent (1.5 mL) was discarded to prevent sample dilution. The rest of the sample (about 1.5 mL) was collected from the SPE columns and filtered (Centriplus YM-3, Centrifu-

gal Filter Devices 3000 Da) in a centrifuge ($3200 \times g$, 60 min at 20°C, Multifuge 3 L-R Heraeus, Kendro Laboratory Products, Hanau, Germany). Coffee, tea and chocolate drink samples were not frozen before SPE extraction and the chocolate drink was analysed without centrifugation. Meat-based samples, coffee, tea and chocolate samples were further purified using the second, similar SPE column. All samples were prepared and analysed in duplicate.

2.3 HPLC/MS/MS analysis

The HPLC/MS/MS method was an in-house modification of the published methods [28, 29]. In brief, the HPLC was operated using an isocratic elution with a methanol–water (5 + 95) mobile phase at a flow rate of 0.2 mL/min. Samples (10 µL) were introduced into an analytical column using an autosampler. The total time of the analysis was 10 min and the retention time of acrylamide and acrylamide-d3 was about 5 min. The LC-MS/MS was operated in the positive ion electrospray mode in which the source and cone temperatures were set to 120 and 25°C, respectively. Nitrogen was used as a nebulising (700 L/h) and a cone gas (42 L/h). The temperature to achieve the nebulisation was set at 300°C. The capillary voltage was set to 3.6 kV and the cone voltage to 30 V. Multiple reaction monitoring (MRM) mode was used for ion detection and the transitions from 72 to 55 *m/z* of acrylamide and from 75 to 58 *m/z* for the deuterated analogue were monitored. The collision gas was argon at 2.70×10^{-3} mbar and the collision energy was 15 eV. The dwell time for the MRM transitions was 0.20 s and the interchannel delay was 0.05 s.

Quantification was performed by using the internal standard and a calibration curve with 5 standards from10 to 1000 ng/mL in water (corresponding to 75–7500 µg/kg). The amount of internal standard was 100 ng/mL. The standard solutions were injected both at the beginning and at the end of each batch of samples.

Calculations were based on a calibration function constructed for each analyte. The functions were calculated *via* a weighted least squares regression using analyte to internal standard peak area ratios.

2.4 Validation and quality assurance

The method was tested for selectivity, linearity, stability, recovery, precision and accuracy. To test the selectivity, matrices which did not contain acrylamide were analysed (*e.g.* one potato chip sample containing not detected amounts of acrylamide). The blank sample was tested for interference compounds using an identical extraction procedure, as well as the same chromatographic and spectroscopic settings. These chromatograms were compared with aqueous standard solutions. Linearity was tested by performing two statistical *F* tests for the calibration curves.

Precision was assessed within a batch and between the batches. Repeatability was tested within-batch for different matrices. Reproducibility was determined using a set of 21 datapoints of a control sample (crisp bread) analysed over a period of approximately 2 years and using a crisp bread test material obtained from FAPAS ($n = 9$). The accuracy was assessed by proficiency tests and recovery trials. Stability tests were carried out by analysing two control samples eight times within a period of 50 days. Within this validation process, the limit of quantification and detection was based on the calibration curve.

3 Results and discussions

The efficiency of an SPE column to clean-up a particular sample depended strongly on the volume and the matrix of the sample. In this exercise, certain particular food items such as some coffee and bread samples were difficult to analyse with the applied method because the SPE was not selective enough (data not shown). This was particularly the case for rye bread, in which the background caused not only ion suppression but also retention time shifting. Therefore, we tested some column combinations for their ability to clean-up the problematic extracts and settled on a compromise of using the combination of the Oasis HLB/Accucat in further studies. This combination was able to overcome most of the sample matrix problems (Fig. 1). However, some coffee products exhibited interfering peaks, which made positive identification impossible. The background transitions were observed in masses 72>55, 72>27, 75>58 and these data were not included in Table 1. Recently, an improved sample clean-up procedure applied especially for coffee samples was introduced greatly helping into work on this area [37].

The LOQ was 68 µg/kg and the LOD was 34 µg/kg for solid samples except for the bacon samples in which the corresponding figures were LOQ 300 µg/kg, LOD 150 µg/kg, respectively. In liquid samples LOQ and LOD were 10 and 5 µg/L, respectively. In this study, the LOD and LOQ figures are higher than others reported in the literature. The difference is probably attributable to the instrumentation used [29]. In the spiked samples, the recovery varied from 98 to 109% being matrix dependent and the assay was linear from 34 to 3750 µg/kg. The samples were stable over 50 days (RSD of 5.5%) which is in line with the recent reports of the stability of acrylamide in various matrices [38]. The RSD of the inter and intra batch variation were from 6 to 7% and from 2 to 4%. Reproducibility within one batch was determined by using a set of 20 datapoints of a control sample (crisp bread) which were analysed over a period of approximately 2 years. The RSD value for the dataset was 4.7%. The RSD values for the reproducibility in crisp bread, biscuits, French fries and potato chips were 7, 5, 9 and 14% ($n = 6$ for each type of

Table 1. Statistical data on the concentrations of acrylamide (μg/kg) in food and drink samples in Finnish foodstuffs

Sample	Number of samples				Statistical figures of acrylamide (μg/kg)			
	Total	nd	<LOQ	Mean	SD	Med	Min	Max
Potato chips	15	–	2	539	374	440	100	1470
French fries and oven baked sliced potatoes	17	1	4	286	145	270	nd	570
Powdered mashed potatoes	2	2	–	–	–	–	nd	–
Corn snacks	2	–	–	195	–	–	180	210
Crisp breads	23	–	9	674	556	645	<68	1480
Fresh rye bread	3	–	3	–		–	<68	–
Rye flour	1	1	–	–		–	nd	–
Oat flakes	1	1	–	–		–	nd	–
Muesli, breakfast cereals	10	–	8	100	–	<68	<68	100
Sweet biscuits	15	–	1	443	380	310	<68	1150
Salted biscuits	2	–	–	179	–	–	134	224
Toast (nontoasted)	5	4	1	–	–	–	nd	<68
Toast (slightly toasted)	5	–	5	–	–	–	<68	–
Toast (strongly toasted)	5	–	2	111	10	109	nd	115
Sweet wheat buns	2	2	–	–	–	–	nd	–
Popcorn	3	–	–	300	46	290	260	350
Minced meat beef	5	1	4	–	–	–	nd	<68
Meat balls	5	4	1	–	–	–	nd	<68
Chicken nuggets	5	3	2	–	–	–	nd	<68
Pizza	10	10	–	–		–	nd	–
Potato-ham casserole (nonheated)	5	–	5	–	–	–	<68	–
Potato-ham casserole (heated)	5	1	2	151	–	–	nd	223
Bacon (slightly fried)	5	5	–[a)]	–	–	–	nd	–
Vegeburger	2	–	–	115	–	–	<68	115
Infants powdered gruel and porridge	13	13	–	–	–	–	nd	–
Bacon (strongly fried)	5	3	2[a)]	–	–	–	nd	<150
Coffee powder (regular)	1	–	–	400		–	–	400
Coffee drink (regular)	25	–	–[b)]	25[c)]	3[c)]	24[c)]	20[c)]	29[c)]
Coffee drink (instant)	1	–	1[b)]	–	–	–	<10[c)]	–
Tea drink	2	–	2[b)]	–	–	–	<10[c)]	–
Chocolate drink	1	–	1[b)]	–	–	–	<10[c)]	–

nd = not detected.
LOQ = limit of quantification (75 μg/kg).
a) LOQ for bacon samples 150 μg/kg.
b) LOQ for coffee samples 10 μg/L (corresponding to 75 μg/kg).
c) Unit μg/L.

food), respectively. The RSD of day-to-day variation was 9% measured using a crisp bread test material obtained from FAPAS ($n = 9$). The target value of acrylamide in this test material (1276 μg/kg) was 5% higher than the mean value (1213 μg/kg) determined in our laboratory [38]. The validation and intercalibration data demonstrate the reliability of the method. The method performance obtained is in good agreement with others [14, 17, 18, 29, 39]. Typical chromatograms of some of the samples are shown in Fig. 2.

To obtain a general perspective of the acrylamide levels in Finnish food items, a large number of food products ($n = 30$) were analysed, *i. e.* the total number of samples was over 200. In addition, some products were prepared with different degrees of toasting or heating (Table 1). Samples were randomly selected from local grocery stores in Helsinki, Finland during 2002 and 2004 and thus they do not necessarily reflect the national food supply.

Potato chips, rye crisp bread and some biscuits were the types of food which contained the highest levels of acrylamide (>1000 μg/kg). Fresh rye bread which is an important component of the Finnish diet, appeared to contain only low levels of acrylamide. Similarly, the processed foods (pizza, minced ground beef, meat balls, chicken nuggets, potato-ham casserole and fried bacon) contained only low or not detectable levels of acrylamide. Acrylamide is a product which is formed at elevated temperatures as a reaction product in the Maillard reaction. Therefore, it is not surprising that the acrylamide content in certain products increased if the materials were heated up or fried. However, it seemed that if one adheres to the cooking instructions, the acryl-

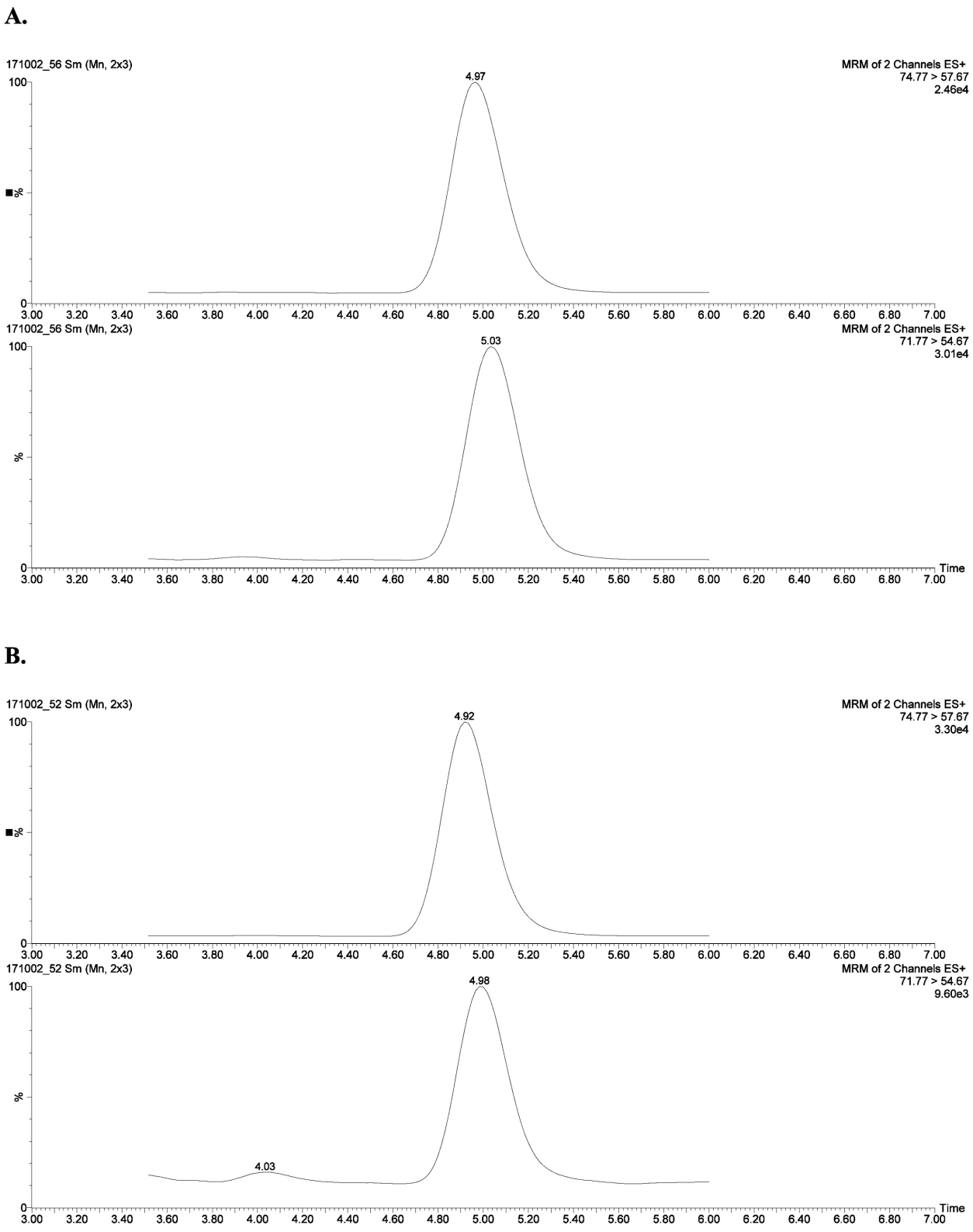
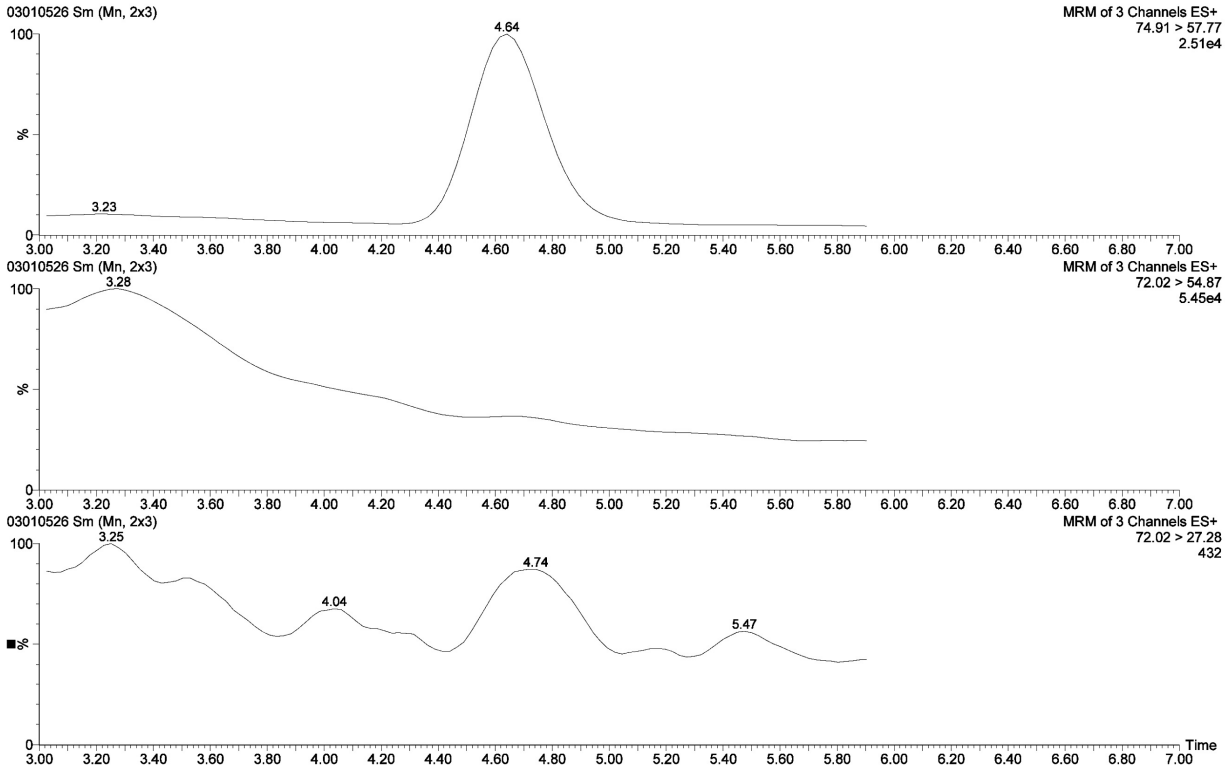
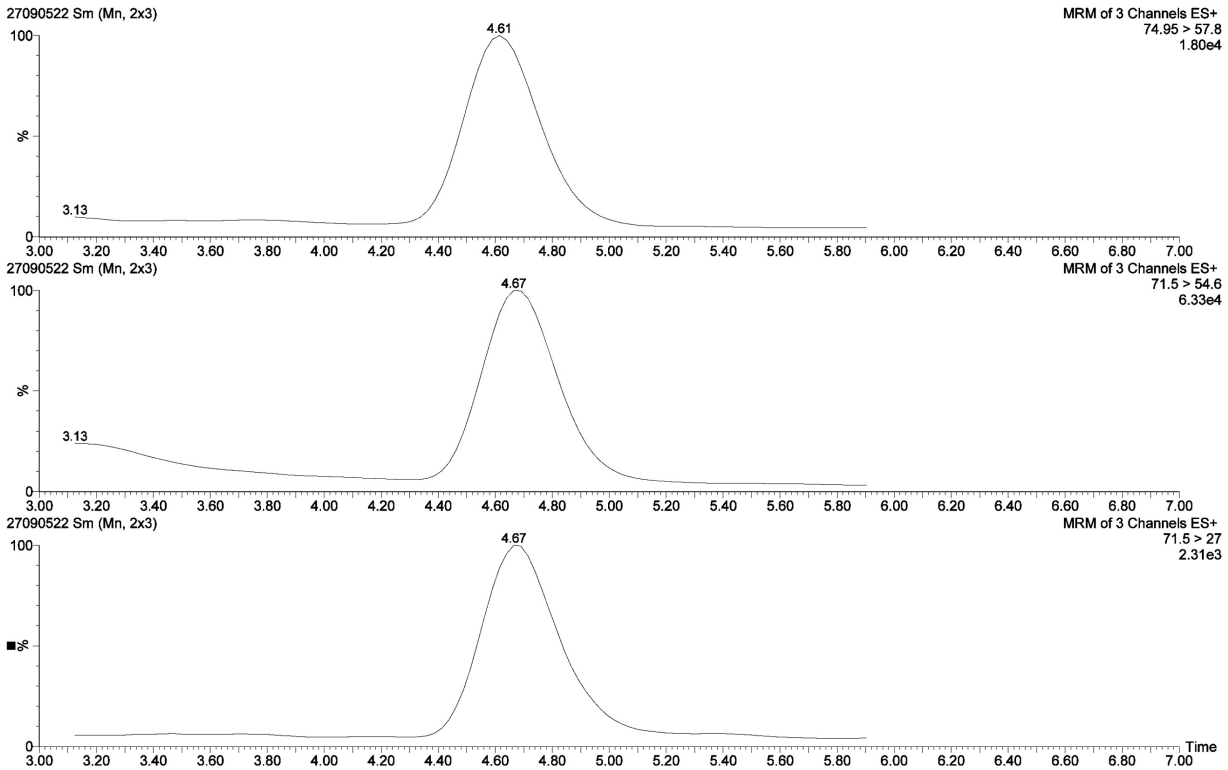


Figure 1. HPLC/MS/MS chromatograms of a spiked sample (A) and a typical coffee sample (B). In the case of coffee samples transition 75–58 *m/z* of d3-acrylamide and transition 72–55 *m/z* of acrylamide were monitored.

A.



B.



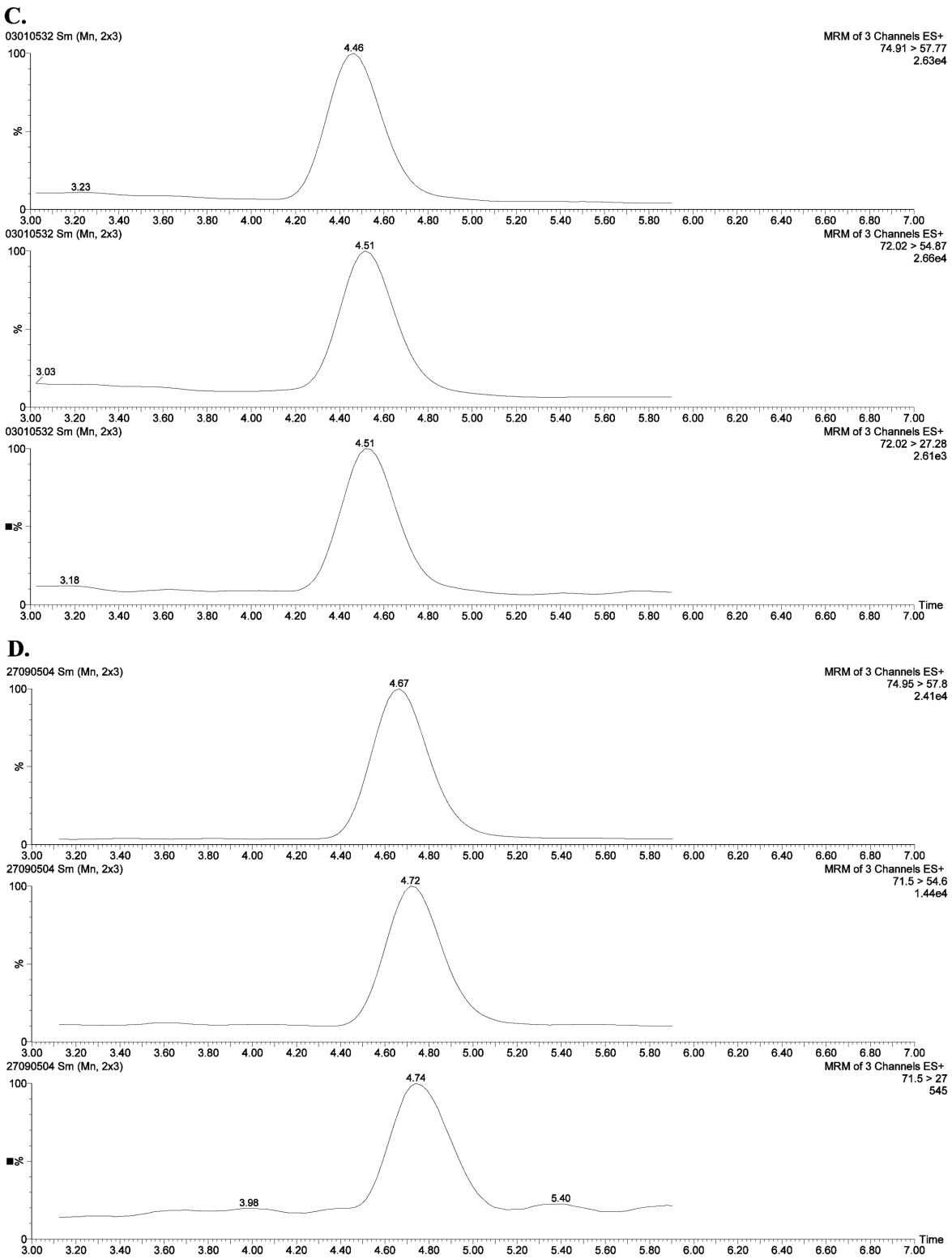


Figure 2. Typical HPLC/MS/MS chromatograms of various foodstuffs and the internal standard d3-acrylamide are shown. On the top trace the transition of 75–58 *m/z* of d3-acrylamide is shown, in the middle the corresponding transition of a particular sample is shown and on the bottom a transition of 72–27 *m/z* of a sample is shown. The matrices were oven baked potatoes not detected (A), crisp bread at a level of 1082 μg/kg (B), biscuits at a level of 1150 μg/kg (C) and the acrylamide and acrylamide d3-standard at the levels of 50 and 100 ng/mL, respectively (D).

amide levels remain low. In our samples, the level of acrylamide varied extensively between the groups of foodstuff. Some items, *e. g.* unfried bacon, did not contain acrylamide at all but in contrast elevated levels of acrylamide were present in all popcorn samples. This observation is in line with the previous results [14, 39, 40]. Coffee powder and all coffee, tea and chocolate drink samples contained acrylamide; the highest levels of acrylamide were found in chocolate powder and the lowest levels in some instant coffee powders. There is known to be variation in the levels of acrylamide in many items, *e. g.* acrylamide concentrations have been reported to vary from 170 to 3700 μg/kg in potato chips, from 800 to 1200 μg/kg in crisp bread, from 30 to 1346 μg/kg in breakfast cereals from 200 to 12 000 μg/kg in French fries and 170 to 351 μg/kg in coffee powders [18, 40]. The levels of acrylamide in these particular foodstuffs from the Finnish market were at the same level as those described above.

The acrylamide concentration in most of the cases refers only to a single randomly selected sample of each specific product. The data are a general guide to acrylamide concentrations in selected segments of the Finnish food supply. The mean concentrations of acrylamide with min, max and median values are summarised in Table 1 and the data highlight the extensive variation in the acrylamide levels between the different brands of each food groups, as can easily been seen from the min and max values. The large variation of the data in potato chips, French fries, crisp bread and sweet biscuits is mainly a result of the different processing temperatures and the variable raw materials used in their manufacture as noted by others [41].

4 Concluding remarks

In the view of its daily consumption, bread may represent one of the most important contributors to dietary exposure of acrylamide. The baking temperature is high enough to produce acrylamide on the crust of the product. Recently it was reported that bread crust contains the majority of acrylamide and the crumb is practically free of acrylamide [42]. However, many of the products which contained moderately high levels of acrylamide are popular snacks among young people. To decrease the overall exposure to these side products of food processing, attempts should be made to devise improved food making processes, perhaps using new raw materials, to prevent the formation of food-borne hazards.

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5 References

[1] WHO, Guidelines for Drinking-water Quality, Health Criteria and Other Supporting Information, Vol. 2, *International Programme on Chemical Safety*, 2nd Edn., World Health Organization, Geneva 1996.

[2] The Council of the European Union, *Council directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption*, European Union 1998.

[3] Bergmark, E., Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers, *Chem. Res. Toxicol.* 1997, *10*, 78 – 84.

[4] IARC, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Industrial Chemicals*, Vol. 60, International Agency for Research on Cancer, World Health Organization, Geneva 1994.

[5] European Union, Opinion on the results of the risk assessment of acrylamide (Human Health and the Environment)-CAS No. 79 – 06 – 1 – EINECS No. 201 – 173 – 7, Report version: October 2000 carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances 2001. Opinion expressed at the 22nd CSTEE plenary meeting, Brussels, 6/7 March 2001.

[6] Grivas, S.,Jägerstad, M., Lingnert, H., Skog, K. *et al*., Acrylamide in food – mechanisms of formation and influencing factors during heating of foods, Report of Swedish Scientific Expert Committee 2002.

[7] Doerge, D. R., da Costa, G. G., McDaniel, L. P., Churchwell, M. I. *et al*., DNA adducts derived from administration of acrylamide and glycidamide to mice and rats, *Mutat Res*. 2005, *580*, 131 – 141.

[8] Maniere, I., Godard, T., Doerge, D. R., Churchwell, M. I. *et al.*, DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide, *Mutat Res.* 2005, *580*, 119 – 129.

[9] European Commission, Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food, SCF/CS/CNTM/CONT/4 Final, 3 July 2002. Scientific Committee on Foods, European Commission 2002.

[10] FAO/WHO, Health implications of acrylamide in food, 25 – 27 June 2002, Geneva, Switzerland, Consultations and workshops, Food Safety Programme, Department of Protection of the Human Environment, World Health Organization 2002.

[11] WHO, Outcome of the Joint FAO/WHO expert consultation on health implications of acrylamide in foods held from 25 to 27 June 2002 in WHO Headquarters, Food Safety News, World Health Organization 2002.

[12] WHO, Additional research on acrylamide in food essential, scientists declare, Joint WHO/FAO Press Release/51, World Health Organization 2002.

[13] Stadler, R., Blank, I., Varga, N., Robert, F. *et al.*, Acrylamide from Maillard reaction products, *Nature* 2002, *419*, 449 – 450.

[14] Becalski, A., Lau, B. P.-Y., Lewis, D., Seaman, S. W., Acrylamide in foods: Occurrence, sources and modeling, *J. Agric. Food Chem.* 2003, *51*, 802 – 808.

[15] Yaylayan, V. A., Wnorowski, A., Perez Locas, C., Why asparagine needs carbohydrates to generate acrylamide, *J. Agric. Food Chem.* 2003, *51*, 1753 – 1757.

[16] Yaylayan, V. A., Stadler, R. H., Acrylamide formation in food: A mechanistic perspective, *J. AOAC Int*. 2005, *88*, 262 – 267.

[17] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M., Analysis of acrylamide, a carcinogen formed in heated foodstuffs, *J. Agric. Food Chem.* 2002, *50*, 4998 – 5006.

[18] Ono, H., Chuda, Y., Ohnishi-Kameyama, M., Yada, H. *et al.*, Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods, *Food Addit. Contam.* 2003, *20*, 215 – 220.

[19] Leung, K. S., Lin, A., Tsang, C. K., Yeung, S. T. K., Acrylamide in Asian foods in Hong Kong, *Food Addit. Contam.* 2003, *12*, 1105 – 1113.

[20] Croft, M., Tong, P., Fuentes, D., Hambridge, T., Australian survey of acrylamide in carbohydrate-based food, *Food Addit. Contam.* 2004, *21*, 721 – 736.

[21] Boettcher, M. I., Schettgen, T., Kutting, B., Pischetsrieder, M., Angerer, J., Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population, *J. Mutat Res.* 2005, *580*, 167 – 76.

[22] Li, C., Hu, C., Wu, K., Quantification of urinary N-acetyl-S-(propionamide)cysteine using an on-line clean-up system coupled with liquid chromatography/tandem mass spectrometry, *J. Mass Spectrom*. 2005, *40*, 511 – 515.

[23] Schettgen, T., Rossbach, B., Kutting, B., Letzel, S. *et al.*, Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population, *J. Int. J. Hyg. Environ. Health* 2004, *207*, 531 – 539.

[24] Skelly, N. E., Husser, E. R., Determination of acrylamide monomer in polyacrylamide and in environmental samples by high performance liquid chromatography, *Anal. Chem.* 1978, *50*, 1959 – 1962.

[25] U.S. EPA (1996), Acrylamide by gas chromatography, Method 8032A, U.S. Environmental Protection Agency, Washington D. C. White paper on acrylamide (2003), Outcome of the acrylamide workshop held in Brussels on March 28, 2003.

[26] Kawata, K., Ibaraki, T., Tanabe, A., Yagoh, H. *et al.*, Gas chromatographic-mass spectrometric determination of hydrophilic compounds in environmental water by SPE with activated carbon filter, *J. Chromatogr. A* 2001, *911*, 75 – 83.

[27] Clarke, D. B., Kelly, J., Wilson, L. A., Assessment of performance of laboratories in determining acrylamide in crispbread, *J. AOAC Int.* 2002, *85*, 1370 – 1373.

[28] Musser, S. M., Detection and quantitation of acrylamide in foods, 2002, U.S. Food and Drug Administration, USA. http://www.cfsan.fda.gov.

[29] Rosén, J., Hellenäs, K.-E., Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry, *Analyst* 2002, *127*, 880 – 882.

[30] Klaffke, H., Fauhl, C., Mathar, W., Palavinskas, R. *et al.*, Results from two interlaboratory comparison tests organized in Germany and at the EU level for analysis of acrylamide in food, *J. AOAC Int*. 2005, *88*, 292 – 298.

[31] Owen, L. M., Castle, L., Kelly, J., Wilson, L., Lloyd, A. S., Acrylamide analysis: Assessment of results from six rounds of Food Analysis Performance Assessment Scgem (FAPAS) proficiency testing, *J. AOAC Int*. 2005, *88*, 285 – 291.

[32] Gökmen, V., Senyuva, H. Z., Acar, J., Sarioglu, K., Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography, *J. Chromatogr.* 2005, *1088*, 193 – 199.

[33] Lineback, D., Wenzl, T., Ostermann, O. P., de la Calle, B. *et al.*, Overview of acrylamide monitoring databases, *J. AOAC Int*. 2005, *88*, 246 – 252.

[34] Hilbig, A., Freidank, N., Kersting, M., Wilhelm, M., Wittsiepe, J., Estimation of the dietary intake of acrylamide by German infants, children and adolescents as calculated from dietary records and available data on acrylamide levels in food groups, *Int. J. Hyg. Environ. Health* 2004, *207*, 463 – 471.

[35] Matthys, C., Bilau, M., Govaert, Y., Moons, E. *et al.*, Risk assessment of dietary acrylamide intake in Flemish adolescents, *Food Chem. Toxicol.* 2005, *43*, 271 – 278.

[36] Boon, P. E., de Mul, A., van der Voet, H., van Donkersgoed, G. *et al.*, Calculations of dietary exposure to acrylamide, *Mutat Res.* 2005, *580*, 143 – 155.

[37] Senyuva, H., Gökemen, V., Study of acrylamide in coffee using an improved liquid chromatography mass spectrometry method: Investigation of colour changes and acrylamide formation in coffee during roasting, *Food Addit. Contam.* 2005, *22*, 214 – 220.

[38] Hoenicke, K., Gatermann, R., Studies on the stability of acrylamide in food during storage, *J. OAC Int.* 2005, *88*, 268 – 273.

[39] FAPAS, Acrylamide, FAPAS Series 30 Round 1, September 2002, report No. 3001. Central Science Laboratory, MAFF, Sand Hutton, York, UK 2002.

[40] Food Standards Agency, Summary of known activity on acrylamide in food, Food Standards Agency, UK 2003.

[41] Taeymans, D., Wood, J., Ashby, P., Blank, I. *et al.*, A review of acrylamide: An industry perspective on research, analysis, formation, and control, *Crit. Rev. Food Sci. Nutr.* 2004, *44*, 323 – 347.

[42] Senyuva, H., Gökmen, V., Survey of acrylamide in Turkish foods by an in-house validated LC-MS method, *Food Addit. Contam.* 2005, *22*, 204 – 209.