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Direct determination of acrylamide in food by gas chromatography–high-resolution time-of-flight mass spectrometry

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Abstract

Simple and rapid gas chromatographic (GC) method employing a high-resolution time-of-flight mass analyzer that enables direct analysis (no derivatization) of acrylamide in various heat-processed foodstuffs has been developed and validated. Co-isolation of acrylamide precursors such as sugars and asparagine, constituting the risk of results overestimation due to additional formation of analyte in hot GC injector, is avoided by the extraction with *n*-propanol followed by solvent exchange to acetonitrile (MeCN). Introduction of a novel purification strategy, dispersive solid phase extraction, based on addition of primary-secondary amine (PSA) sorbent into deffated extract in MeCN, provides a significant reduction of some abundant matrix co-extracts (mainly free fatty acids). Isotope dilution technique (d_3-acrylamide as an internal standard) is employed for compensation of potential target analyte losses and/or matrix-inducted chromatographic response enhancement. Limits of quantifications (LOQs) ranged between 15 and 40 $\mu g\,kg^{-1}$ and recoveries were between 97 and 108% depending on the examined food matrix. The repeatability of measurements (expressed as relative standard deviation, R.S.D.) was as low as 1.9% for potato crisps containing acrylamide at a level of 1 $mg\,kg^{-1}$. Slightly higher values (R.S.D. < 4.0%) were achieved for breakfast cereals and crisp bread with approximately 10 times lower content of this processing contaminant. Trueness of results generated by this new method was demonstrated via FAPAS® (Food Analysis Performance Assessment Scheme) interlaboratory proficiency tests.

Keywords: Acrylamide; Gas chromatography; High-resolution time-of-flight mass spectrometry; Food analysis

1. Introduction

The discovery of acrylamide occurrence in various heat-processed starch-rich foodstuffs in 2002 resulted in an urgent requirement for the development of a reliable analytical procedure allowing to control the levels of this "new", potentially carcinogenic, contaminant in a wide range of various food matrices.

As a consequence of serious health concerns related to the dietary intake of this hazardous chemical, implementation of relevant analytical procedure(s) became an urgent task. Because of time pressure, no interlaboratory validation of developed methods was carried out in the early stage of dietary acrylamide research. Large variation of generated data among food laboratories concerned with acrylamide issues was well documented in FAPAS® proficiency tests (Food Analysis Performance Assessment Scheme) organized by Central Science Laboratory (York, UK). For instance, interlaboratory reproducibility of data sets for test coffee sample (Series 30, Round 4), crisp bread sample (Series 30, Round 5), and breakfast cereals (Series 30, Round 6) were as high as 111, 656, and 56%, respectively, although, considering the assigned acrylamide concentrations 312, 707, and 95 $\mu g\,kg^{-1}$, respectively, the calculated R.S.D. values according to the Horwitz equation [1] should be in particular cases no more than 19, 17, and 22%, respectively.

Several reviews focused on acrylamide analysis have been published recently [2–4]. Currently, two analytical approaches are considered to represent a relevant standard for accurate determination of acrylamide in foodstuffs, and are considered

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by the European Union Joint Research Centre for international interlaboratory validation. While only a conventional GC–MS bench-top instrument employing either single quadrupole or ion trap analyzer is needed for determination of derivatized (brominated) acrylamide, tandem-in-space mass analyzer (MS/MS) has to be used for obtaining low LODs when analysis of non-derivatized acrylamide is carried out by HPLC coupled to mass spectrometry [5–12].

It should be noted that GC–MS determination of free acrylamide isolated from complex food matrices is rather troublesome. Typically, there are still some residual matrix co-extracts left even in purified extract and many of them yield low *m/z* ions interfering with acrylamide fragment ions. Due to this high chemical noise, obtaining low detection limits is obviously impossible. In addition, when using water for extraction of polar acrylamide its precursors (sugars, asparagine) are co-isolated. As far as these compounds are not completely removed from extract, risk of additional analyte formation in the hot GC injector exists [13], potentially resulting in acrylamide content overestimation. With regard to these difficulties, direct GC–MS acrylamide analysis has never become a method of routine use although few studies employing this approach were published [14,15].

As mentioned above, the majority of current GC–MS methods employ bromination to obtain a less polar, more volatile target analytical form yielding more specific ions (higher *m/z*). Instead of 2,3-dibromopropionamide, more stable 2-bromopropenamide, generated under experimental conditions *via* dehydrobromination, is employed [9,10,12,16,17]. Some laboratories, however, find using bromine uncomfortable, pointing at certain workplace hazards; moreover, the sample preparation procedure involving the derivatization step is relatively laborious and time consuming.

The distinct advantage of methods based on liquid chromatography coupled to mass spectrometry (LC–MS) is the possibility to determine acrylamide without its previous derivatization. However, even if highly selective tandem mass spectrometry is used (triple quadrupole provides typically the best detection potential), a large effort has to be spent to achieve effective sample clean-up. In most cases combination of several solid phase extraction (SPE) cartridges has to be employed for purification of crude extracts prior to the determinative step [5,9,10,18]. In general terms, also efficient HPLC separation of acrylamide from remaining matrix components is highly desirable since both the chemical noise around the analyte peak and ionization suppression caused by co-eluting matrix components have to be minimized for obtaining low detection limits. However, achieving this objective is not easy, since when using common reversed phase sorbents, the relatively polar acrylamide tends to elute close to a void column volume together with most of impurities. Higher *k* values, enabling better acrylamide separation, can be obtained by Hypercarb column [5,8,10]. Unfortunately, performance of this column is relatively rapidly deteriorated during injection of larger series of real-world samples. On this account various special reversed-phase and also normal-phase columns were tested for separation of acrylamide [6,8,9,12,18]. As an alternative to normal- and reversed-phase HPLC systems, ion-exchange chromatography represents a conceivable solution. An example of a suitable column is IonPac ICE-AS1, which combines ion exchange chromatography with size exclusion separation mechanism [19].

The aim of our study was to demonstrate the possibility to carry out reliable GC-related analysis of acrylamide in various foodstuffs by employing novel approaches both in the field of sample preparation and detection/quantification. As far as high-resolution mass spectrometric detector is available (GCT time-of-flight mass spectrometer, in particular case), a simple and rapid sample preparation procedure omitting derivatization can be used.

2. Experimental

2.1. Materials

Acrylamide (99.8%) and acrylamide (2,3,3-d_3) (98%) certified standards were purchased from Sigma–Aldrich (Munich, Germany) and Cambridge Isotope Laboratories, CIL (Andover, USA), respectively. Stock solutions of acrylamide (1 mg mL^{-1}) and d_3-acrylamide (1 mg mL^{-1}) prepared each by dissolving these compounds in MeCN were stored at 4 °C. L-Asparagine (99.5%) was obtained from Sigma–Aldrich (Munich, Germany) and D-glucose (p.a.) from Penta (Prague, Czech Republic). Primary secondary amine (PSA) sorbent was purchased from Varian (Harbor City, CA, USA). MeCN, *n*-propanol, and *n*-hexane were supplied by Merck (Darmstadt, Germany), demineralized water was obtained from a Millipore apparatus (Billerica, MA, USA).

2.2. Methods

2.2.1. Sample preparation

Food samples were homogenized by laboratory blender. Three grams of representative sample were weighed into a 45 mL centrifuge tube with a screw cap. After addition of 30 μL (50 μg mL^{-1}) of d_3-acrylamide (internal standard, added amount corresponding to a concentration of 500 μg kg^{-1}), and 4.5 mL of demineralized water, the blended sample was allowed to swell 30 min in the ultrasonic bath.

After addition of 24 mL of *n*-propanol the content of the centrifuge tube was mixed by Ultra Turrax for 5 min and then centrifuged (5 min at 11000 rpm). Ten milliliters of supernatant were transferred into a 50 mL round bottomed flask. Five drops (about 60 mg) of refined olive oil were added as a keeper to avoid possible loss of acrylamide during evaporation of a water–*n*-propanol azeotropic mixture (16:84, v/v).

The residue left on the wall of flask was re-dissolved in 2 mL of MeCN by sonication and then defatted by shaking with two portions of *n*-hexane (10 and 5 mL). 1 mL of MeCN (bottom) phase was transferred into a 14 mL centrifuge tube containing 60 mg of PSA sorbent. This suspension was mixed using a Vortex mixer for 30 s and centrifuged at 11000 rpm for 1 min; the obtained supernatant was placed into the autosampler vial for GC–HRTOF MS analysis.

2.2.2. Instrumentation

GC–HRTOF MS system, consisting of an Agilent gas chromatograph 6890 (Agilent Technologies, Palo Alto, CA, USA) coupled to a GCT high-resolution time-of-flight mass spectrometer (Micromass, Manchester, UK), was used for experiments. The gas chromatographic system was equipped with an electronic pressure control (EPC), a split/splitless injector and a PAL Combi autosampler (CTC Analytics, Zwingen, Switzerland).

2.2.2.1. Gas chromatographic separation. An Innowax (J&W Scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) used for sample separation was operated under following conditions—oven temperature program: 70 °C for 1.0 min, 20 °C min^{-1} to 240 °C (held for 10.5 min); constant helium flow rate: 1.0 mL min^{-1}; injection mode: pulsed splitless 1.0 min, 4 mL min^{-1} (223 kPa); injection temperature: 250 °C; injection volume: 1 μL.

2.2.2.2. Mass spectrometric detection. The parameter settings for the HRTOF MS detector were as follows—acquisition rate: 2 Hz; pusher interval: 33 μs (30303 raw spectra s^{-1}); inhibit push value: 14; time-to-digital converter (TDC): 3.6 GHz; mass range: *m/z* 45–500; ion source temperature: 220 °C; transfer line temperature: 240 °C; detector voltage: 2600 V.

The instrument was manually tuned using 2,4,6-tris-fluoromethyl-[1,3,5]-triazine as a reference compound. The mass resolution was calculated from the continuum data using the *m/z* 285 for this purpose and the full width at half maximum of its peak. Generally, the mass resolution exceeded 7000 FWHM (full width at half maximum) in all experiments. For exact mass calibration nine fragments (obtained in an electron ionization mode) of this reference compound in centroid mode were used. Once this calibration was made the *m/z* 284.9949 was used as an internal reference mass (so-called the lock mass). The exact mass calibration was considered relevant with maximum differences between measured and theoretical masses not exceeding 1.0 mDa. The reference compound was continuously introduced into the ion source also during the real sample analysis. MassLynx 3.5 software was employed for data processing.

3. Results and discussion

As shown in Section 1 either tandem LC–MS or unit resolution GC–MS are currently employed techniques for analysis of acrylamide in various foodstuffs (the latter one after analyte bromination). However, as far as several projects are running in an analytical laboratory simultaneously, particular instrument capacity may become a bottleneck. Initiation of extensive studies concerned with acrylamide formation under various processing conditions led to the need to implement an alternative method enabling reliable examination of test samples even under circumstances when the LC–MS triple quadrupole instrument was occupied by other analyses. Since we failed to obtain low limit of quantification (LOQ) by LC–MS system (LCQ Deca, Finnigan) employing an ion trap analyzer (insufficient selectivity of MS/MS transition of acrylamide ion), a GC method remained the only viable option to achieve required sample throughput. Under these circumstances it was tempting for us to develop a new simple method employing the potential of a novel high-resolution time-of-flight mass detector for the direct determination of acrylamide in extracts prepared by a novel approach. Steps realized during the method implementation and validation are described in the following paragraphs.

3.1. Extraction

Due to the polar nature of the acrylamide molecule, water is used for extraction of this analyte from food matrices in many studies [2–4]. In preliminary experiments aiming selection of an optimal sample handling strategy, we prepared aqueous extract from the crisp bread distributed as a test material within the FAPAS® test (Series 30, Round 01). With regard to the availability of highly selective detection mode we attempted to analyze this extract only after minimal clean-up. However, introduction of deffated (partition of centrifuged aqueous supernatant with *n*-hexane) sample into the hot splitless GC injector resulted in the determination of a higher (5240 μg kg^{-1}) acrylamide content as compared to the assigned value declared in the FAPAS® report (1213 μg kg^{-1}). The formation of additional acrylamide during the splitless period under high temperature from co-extracted (polar) acrylamide precursors (in addition to sugars and asparagine also other Maillard intermediates are conceivable) obviously occurred. Attempting to document this phenomenon, several injections of an aqueous L-asparagine–D-glucose mixture (each 50 μg mL^{-1}) were also carried out. As shown in Fig. 1 the amount of *de novo* originated acrylamide raised with increasing injection temperature.

It should be noted that totally selective isolation of acrylamide from its precursors contained in the aqueous extracts is hardly feasible. Nevertheless, when using LC–MS, residual sugars and asparagine that may be contained in SPE purified extracts do not pose a serious problem (these compounds not retained on the analytical columns used for separation); similarly, conversion of acrylamide into brominated derivative for subsequent GC–MS analysis eliminates this problem. Under these conditions the only solution to avoid the risk of results over-

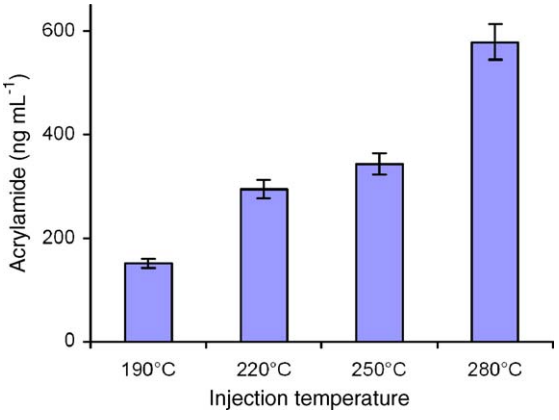

Fig. 1. Formation of acrylamide in the hot splitless GC injector (error bars are ± S.D.; $n = 3$). A 1 μL of aqueous solution of L-asparagine and D-glucose (each 50 μg mL^{-1}) injected. d_3-Acrylamide used for quantification.

estimation when using the direct GC–MS method, was to change the concept of sample preparation. *n*-Propanol, an organic solvent possessing still enough polarity for acrylamide extraction but largely discriminating co-isolation of such polar (and/or ionized) substances such as sugars and asparagine was chosen for this purpose (similar approach was employed by Biedermann et al. [14]). Compared to turbid aqueous extracts (matrix hydrocolloids), obtained in the first phase of this study, those obtained by *n*-propanol were transparent. In addition, the evaporation within the concentration step was somewhat faster. Further, the final solvent exchange to MeCN (solvent used for GC injection) avoids the transfer of isolated acrylamide precursors into the sample due to a low solubility of those compounds in this organic solvent.

3.2. Clean-up

In line with many other studies employing LC–MS for quantification [4], the use of SPE for removal of matrix interferences from the crude extract might be seen as a method of choice. However, typically two or even more purification principles have to be combined to achieve low LOD [4]. For example, the subsequent use of Oasis HLB + Bond Elut-Accucant (mixed mode: C8, SAX, and SCX) cartridges were employed as an effective way of sample clean-up in some LC–MS studies [6,8].

With respect to labor and cost demands associated with these means of purification, and considering the fact that none of them were designed for clean-up of *n*-propanol extracts (generic approach hardly possible), we decided to apply a rather different strategy: dispersive SPE for removal of non-target compounds employing an anion exchange sorbent—primary-secondary amine (PSA). A similar approach simplifying analysis of pesticide residues, so-called QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), has been recently introduced for elimination of most matrix components occurring in a crude MeCN extracts in various food commodities [20]. In our experiments, addition of different amounts (20, 40, 60, and 80 mg) of this sorbent to 1 mL of MeCN extract of crisp bread sample containing equivalent of 0.5 g mL^{-1} of original matrix, was tested to recognize the extent of co-extracts elimination. An example of clean-up effectiveness is shown in Fig. 2 addition of 60 mg of PSA led to a distinct reduction of many (semi)volatile matrix components. Among abundant compounds extensively eliminated from sample extracts (see intensive peaks in Fig. 2) were mainly free fatty acids represented by caproic, palmitic, oleic, and linoleic acid. Also significant reduction of furan-2-carbaldehyde derivative (peak no. 7 in chromatogram) occurred; however, other *O*-containing heterocyclic products of the Maillard reaction such as maltol, a compound yielding similar fragments like acrylamide, were not removed.

Although the novel clean-up approach removed only a minimum of chemical noise in the area of acrylamide elution (only a slight decrease of the LOD was obtained), the overall performance of the method such as stability of analyte response within long sample sequences was largely improved. Also the demand for instrument maintenance (cleaning of ion source) was substantially reduced.

It should be noticed that linearity of the GCT instrument is limited and distortion of signal of abundant sample components

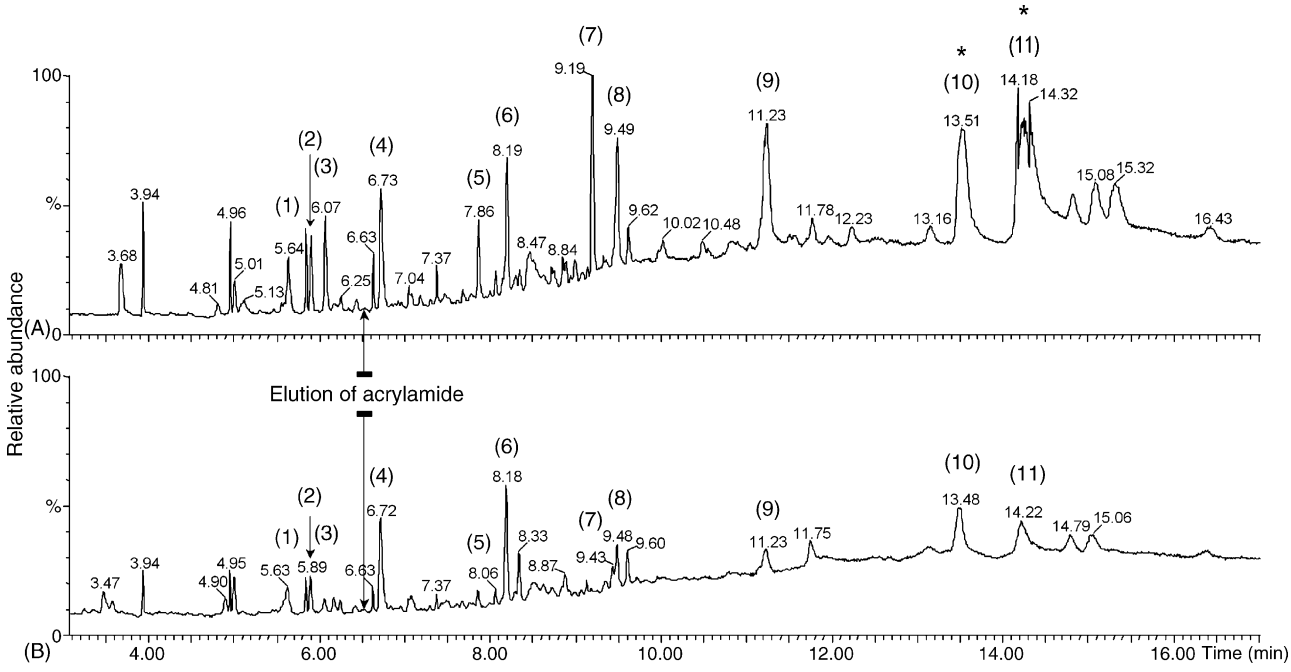

Fig. 2. Elimination of some matrix components from the MeCN extract of crisp bread samples by addition of PSA sorbent. GC–HRTOF MS chromatograms (TIC) of (A) extract without dispersive-SPE clean-up; (B) extract after dispersive-SPE clean-up using PSA sorbent (60 mg mL^{-1}). Identified co-extracts: (1 and 2) deca-2,4-dienal isomers; (3) hexanoic acid (caproic acid); (4) 3-hydroxy-2-methyl-pyran-4-one (maltol); (5) 1-(2-hydroxy-5-methyl-phenyl)ethanone; (6) 3,5-dihydroxy-2-methyl-5,6-dihydropyran-4-one; (7) 5-(hydroxymethyl)furan-2-carbaldehyde; (8) 3-ethoxy-4-methoxy-benzaldehyde; (9) hexadecanoic acid (palmitic acid); (10) octadec-9-enoic acid (oleic acid); (11) octadeca-9,12-dienoic acid (linoleic acid). Samples injected in the split mode 1:5. The asterisks denote peaks outside the GCT TOF MS linear range.

occurs due to a saturation of the TDC. For unbiased assessment of the clean-up efficiency of dispersive SPE employing PSA, MeCN extracts were analyzed prior and after sorbent addition by means of GC–FID technique (records not shown here).

3.3. Identification and quantification

As already mentioned, direct analysis of acrylamide in such a complex matrix as food is not an easy task since the *m/z* 71 and *m/z* 55 ions yielded by EI fragmentation are of low value, and nonspecific. Intensive chemical noise at low *m/z* range does not allow obtaining low detection limit and adequate precision when using commonly available unit mass resolution instruments (in this context bromination provides improved detectability of analyte). Under these circumstances the only solution for GC analysis of free acrylamide is the use of a high-resolution mass spectrometric detector.

In general terms, a GCT TOF MS analyzer with 7000 FWHM resolution enables partial or complete resolution of interfering matrix components, yielding ions with close nominal masses to those of the target analyte. In this way improvement of signal/noise ratio can be effectively achieved in high-resolution detection mode [21,22]. Using a narrow mass window setting (0.02 Da in this study) both acrylamide and d_3-acrylamide (internal standard) can be unequivocally identified by the ions *m/z* 71.036 (55.018) and *m/z* 74.056 (58.039), respectively (see Fig. 3). Isotope dilution technique was used for acrylamide quantification. Improved accuracy (both precision and trueness) by reducing the problems with calibration and matrix effects can be obtained in this way. d_3-Acrylamide used as an internal standard possessing very similar properties as those of target analyte (hence mimicking its behavior during analysis) is eluted under particular GC conditions at the same retention time, nevertheless, thanks to higher *m/z* value of deuterated ions, it can be easily spectrometrically resolved from native analogue. A known amount of d_3-acrylamide is added to each sample aliquot

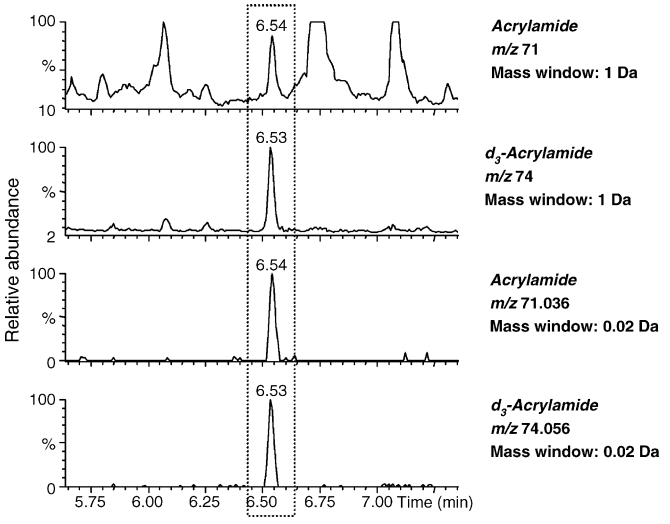

Fig. 3. GC–HRTOF MS analysis of acrylamide in crisp bread samples (450 μg kg^{-1}) under conditions of different mass window settings (1 and 0.02 Da) for extraction of target ions.

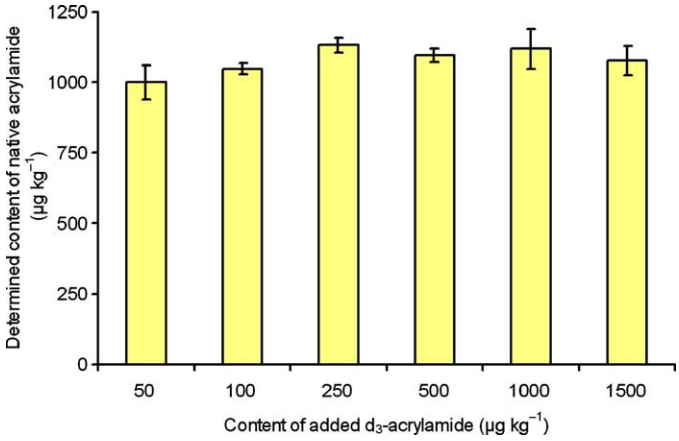

Fig. 4. Results of native acrylamide determination in bread extract to which increasing amounts of d_3-acrylamide (internal standard) were added for quantification (error bars are ± S.D.; $n = 3$).

before the extraction step (see discussion in the next section). The amount of native acrylamide in the sample is simply calculated by comparison its peak areas and deuterated standard.

3.4. Performance characteristics

As documented in Fig. 4, the response of d_3-acrylamide was linear over the whole tested concentration range (50–1500 μg kg^{-1} of internal standard were added to the bread containing 1100 μg kg^{-1} of native acrylamide). For the follow-up experiments, addition of d_3-acrylamide at concentration 500 μg kg^{-1} was chosen. As far as samples with high acrylamide content beyond the linear range are to be analyzed, dilution of respective extract still allows obtaining intensive analytical signal of d_3-acrylamide employed for quantification of native acrylamide. Since the response factors of acrylamide and d_3-acrylamide are identical, the concentration of 1500 μg kg^{-1}, that is the upper linear range of d_3-acrylamide, also represents the upper linear range of native acrylamide.

As discussed in our earlier study concerned with critical assessment of HRTOF MS performance in residue analysis [21], common strategy, *i.e.* estimation of LOD by extrapolation based on the data obtained for the lowest measured concentration and pre-defined target S/N ratio is not always applicable when using narrow mass window setting for analyte identification, since almost complete elimination of chemical noise occurs in the case of higher *m/z* ions. This was not the case in this study; nevertheless, a pronounced enhancement of the S/N parameter was obtained by high-resolution detection compared to GC–MS analyses performed in unit mass resolution mode. Since all the heat processed matrices examined in our study unavoidably contained some acrylamide, the LOQ value was estimated based on analyses of low contaminated samples and taking in account not only the target S/N ratio (>10) but also absolute signal intensity and, regarding the HRTOF MS detector setting, obtaining at least three data points above the baseline per chromatographic peak. LOQ values obtained in this way were only slightly higher compared to those attainable by GC–MS preceded a bromination step (2–25 μg kg^{-1}) [4].

Table 1
Performance characteristics of GC–HRTOF MS method, various food matrices ($n = 6$)

Matrix	Mean acrylamide level ($\mu g\,kg^{-1}$)	Added amount of acrylamide ($\mu g\,kg^{-1}$)	Mean determined acrylamide level in spiked sample ($\mu g\,kg^{-1}$)	Recovery (%)	R.S.D. (%)	LOQ ($\mu g\,kg^{-1}$)
Potato crisps	1048	500	1679	108	1.9	15
Breakfast cereals	88	100	201	107	3.6	25
Crisp bread	117	100	211	97	3.2	40

Table 2
External quality control of data generated by new GC–HRTOF MS method: results obtained in FAPAS® proficiency tests concerned with acrylamide analysis

Series/round	Matrix	Assigned value ($\mu g\,kg^{-1}$)	z-Score
Series 30/round 5	Crisp bread	707	0.8
Series 30/round 7	Oven chips	1843	1.0
Series 30/round 9	Baby rusk	711	−0.3
Series 30/round 11	Potato crisps	1404	0.2

The optimized analytical method was evaluated within a validation study involving six replicates of different kinds of matrices (potato crisps, breakfast cereals, crisp bread). For potato crisps the repeatability (expressed as relative standard deviation) was as low as 1.9% at acrylamide level of 1048 $\mu g\,kg^{-1}$. A slightly higher value (<4.0%) was obtained for the cereal-based samples with approximately 10 times lower content of this processing contaminant.

Recovery ($n = 6$) of the method was determined for each of the previously mentioned matrices. Acrylamide standard was added to each batch of samples at a level close to native acrylamide concentration in the examined samples. The average recoveries ranged between 97 and 108% depending on food matrices (see Table 1).

As already mentioned, external quality control was realized by participation in FAPAS® interlaboratory tests. The trueness of data generated by this newly developed GC–HRTOF MS method was documented, all the z-scores were in the satisfactory range $|z| \leq 2$ in four subsequent rounds (see Table 2).

While the majority of laboratories involved in those proficiency tests employ analytical strategies based on LC–MS(-MS) or GC–MS with derivatization of acrylamide, only a few of them reported the use of direct GC–MS analysis. Worth to notice that those results were overestimated, probably due to a formation of secondary acrylamide from its precursors left in poorly prepared extracts.

4. Conclusions

Extraction by *n*-propanol followed by solvent exchange to MeCN avoids co-isolation of acrylamide precursors that could yield additional analyte in the hot splitless GC injector. Extensive reduction of matrix components in sample extracts, hence improvement of method robustness, was obtained by a novel approach employing PSA sorbent for dispersive-SPE. Thanks to the development of a such simple clean-up and the elimination of a derivatization step, sample throughput was increased and at the same time the labor demands were significantly reduced compared to the commonly used GC procedure. Considering rapid development of TOF MS technology and growing number of its users, we believe that this method will find applicability into laboratories concerned with food safety control in the near future, since it meets all criteria stated in the EU Directive [23] and Eurachem Guide [24].

Acknowledgements

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