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Review

Occurrence and analytical methods of acrylamide in heat-treated foods
Review and recent developments

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Abstract

In early 2002, Swedish National Food Administration (SNFA) and University of Stockholm together announced that certain foods that are processed or cooked at high temperature contain relatively high levels of acrylamide. The occurrence of acrylamide is derived from heat-induced reactions between the amino group of asparagine and the carbonyl group of reducing sugars during baking and frying. Corresponding chromatographic methods are used to determine various structural groups present during this process. Gas chromatography (GC)–mass spectrometry (MS) and liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis are both acknowledged as the major useful and authoritative methods for the acrylamide determination and other chromatographic methods are also briefly introduced. The aim of this review is to summarize the state-of-the-art about the occurrence, analytical methods, and extraction and clean-up procedures of acrylamide. Special attention is given to chromatographic techniques applied for the occurrence and determination of acrylamide.

Keywords: Reviews; Acrylamide; Occurrence; Analytical methods; GC–MS; LC–MS/MS; Heat-treated foods

Contents

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1. Introduction

In early 2002, Swedish National Food Administration (SNFA) and University of Stockholm together announced that certain foods that are processed or cooked at high temperatures contain relatively high levels of acrylamide [1]. Meanwhile, an earlier feeding study in rats demonstrated a relationship between acrylamide in fried animal foods and specific hemoglobin adducts [2]. Long-term exposure to acrylamide may cause damage to the nervous system both in humans and animals to a certain extent [3,4], and acrylamide is also considered as a potential genetic and reproductive toxin [5,6] with mutagenic and carcinogenic properties in experimental mammalians both in in vitro and in vivo study [7]. Meanwhile, the risk assessment of acrylamide evaluated by the Scientific Committee on Toxicity Ecotoxicity and the Environment (CSTEE) of the European Union (EU) demonstrated that the exposure of acrylamide to humans should be kept as low as possible with regard to the inherent toxic properties of acrylamide (neurotoxicity, genotoxicty to both somatic and germ cells, carcinogenicity and reproductive toxicity) [8].

These findings have attracted considerable interest and wide attention all over the world. Earlier toxicological studies suggested that acrylamide vapours irritate the eyes and skin and cause paralysis of the cerebrospinal system, and it has been demonstrated to have carcinogenic properties in animals [9,10]. The International Agency for Research on Cancer (IARC) has therefore classified it as “potential carcinogenic to humans” [11]. As a result of this classification, the maximum work place concentration list defined it as a Category III A2 substance. On the other hand, the Swedish findings about the high level of acrylamide in heat-treated foods were quickly confirmed by a series of government agencies through official website notifications [12].

With the development of the state of affairs on such new contaminant, all available research data on acrylamide have been reviewed at an international level (e.g., by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (UN) [13], Joint Institute for Food Safety and Applied Nutrition/National Center for Food Safety and Technology (JIFSAN/NCFST) Workshop [14] by expert Working Groups). Meanwhile, JIFSAN established special website as a global resource and inventory of ongoing research on acrylamide in foods. This website includes formal research, surveillance/monitoring and industry investigations of acrylamide, etc. [15]. Under such situation, researchers from all over the world can publish their latest research data on various study, investigation, sample analysis and statistic results of acrylamide in different kinds of heat-treated foods.

In general, thermal processes during the production of foodstuffs are complex, and the initial results on acrylamide level do not seem to indicate a common pattern, except that carbohydrate-rich foods seem to generate relatively more acrylamide [16]. Another important aspect is that low water content seems important for the reactions, and acrylamide is nearly not detected in boiled foods containing starch. Deep frying or roasting seems to be propitious to the formation of acrylamide [17]. Several months after the Swedish announcement, a number of research groups simultaneously discovered that acrylamide is formed during the Maillard reaction, and the major reactants leading to the formation of acrylamide are reducing sugars and the amino acid asparagine [18–21]. Although significant progress has been made already, many gaps in the scientific community are evident and should be addressed. For instance, in the analytical field the performance of current methods used for the determination of acrylamide is not adequate for the more “difficult” food matrices, such as cocoa, coffee, and high salt flavorings. Having sensitive and robust methods that provide reliable data in the different food categories is of crucial importance for intake assessments. Staple foods, such as bread that contains acrylamide only at trace amounts (in the low part-per-billion range), may,

nevertheless, contribute significantly to the overall dietary intake. On the other hand, intensive activity began examining many different types of food, and thousands of analytes have been undertaken world wide since the discovery of acrylamide formation in cooked foods in 2002 following that measurement data have been published in many different types of media. Within this flood of publications, there were only a limited number of articles concerned with the technical aspects of the measurements. Few researchers focused on the generalization and summarization of the analytical methods for the determination of acrylamide in foods. Although gas chromatography (GC)–mass spectrometry (MS) and liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis are acknowledged as the most useful and authoritative method for acrylamide determination [22], their method validation, extraction and clean-up procedures need to be optimized.

Review article is an approbatory medium to list and summarize the above aspects for the determination of acrylamide by citing corresponding references. Consequently, a review article about acrylamide determination in heat-treated foods is important since the quantificational methods have a large impact on the results of the analysis. This review summarizes the state-of-the-art about the analytical methodology for the determination of acrylamide in heat-treated foods and this review paper can also be considered as a basis for further investigations. Special attention is given to chromatographic methods and instrumental conditions applied for the determination of acrylamide.

2. Occurrence of acrylamide and corresponding chromatographic methods

2.1. Formation of acrylamide in food stuffs and Maillard browning reactions

The major mechanistic pathway for the formation of acrylamide in foods so far established is via the Maillard reaction [18–21,23]. The Maillard reaction is a complex desired process, generating a plethora of important flavor and aroma compounds. Thus, any concepts established to minimize the formation of acrylamide should take into account that the organoleptic properties of the cooked foods are not negatively affected [24]. Amino-carbonyl and related reactions of food constituents involve the organoleptic and flavor changes commonly termed browning reactions. Specifically, heat-induced reactions of amines, amino acids, peptides, and proteins with reducing sugars and Vitamin C (nonenzymatic browning) and quinones (enzymatic browning catalyzed by polyphenol oxidase) cause deterioration of foods during storage and processing [25]. One of adverse effects is the formation of acrylamide during corresponding reactions which may further reduce the nutritional value and safety of foods.

Recent studies [19,21] suggested that acrylamide in foods is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars such as glucose during baking and frying. Foods rich in both of these precursors are largely derived from plant sources such as potatoes and cereals (barley, rice, wheat) but apparently not animal foods such as poultry, meat, and fish. Widely consumed processed foods with high amounts of acrylamide include French fries, potato chips, tortilla chips, bread crust, crispbread, and various baked goods and cereal formulations [26]. However, the observed wide variations in levels of acrylamide in different food categories as well as in different brands of the same food category (e.g., French fries; potato crisps) appear to result not only from the amounts of the precursors present but also from variations in processing conditions (e.g., temperature; time; nature of frying oil; nature of food matrix) [27]. Large research data showed that the most acrylamide-rich heat-treated food is potato crisp or slice [28]. However, the formation of acrylamide was not affected by the presence of phenolic antioxidants from cranberry and oregano extracts in the potato slices. The acrylamide content on the contrary increased when exogenous phenolics were present [29]. This indicates that acrylamide formation is non-oxidative in nature, therefore rules out the possibility of its formation via lipid oxidation.

2.2. Mechanism of acrylamide occurrence

Recently, increasing free radical and mechanistic studies [29–31] have focused on the generating mechanism of acrylamide in heat-treated foods because of its neurotoxic effects and latent carcinogenesis. Once the generating mechanism of acrylamide is well understood one day, it is more possible for scientists to obtain an effective pathway for reducing the occurrence of acrylamide during different kinds of heat processing technology by controlling critical steps of food processing. To date, studies clearly suggest that the amide amino acid asparagine is mainly responsible for acrylamide formation in cooked foods after condensation with reducing sugars or a carbonyl source, i.e. so-called asparagine pathway, the main intermediate product and molecular rearrangement products of which are shown in Fig. 1. Moreover, the sugar–asparagine adduct, *N*-glycosylasparagine, generated high amounts of acrylamide, suggesting the early Maillard reaction as a major source of acrylamide [21]. In addition, decarboxylated asparagine (3-aminopropionamide) can generate acrylamide in the absence of reducing sugars [32]. Other possible pathways involve the Strecker reaction of asparagine, with the Strecker aldehyde as the direct intermediate [19], or a mechanism via acrylic acid [18,33–35]. In fact, a recent report [36] on model reaction systems demonstrates that acrolein together with asparagine may generate appreciable levels of acrylamide under certain conditions, suggesting a critical role of acrolein in the formation of acrylamide in lipid-rich foods.

Structural considerations dictated that asparagine alone might be converted thermally into acrylamide through decarboxylation and deamination reactions. However, the main

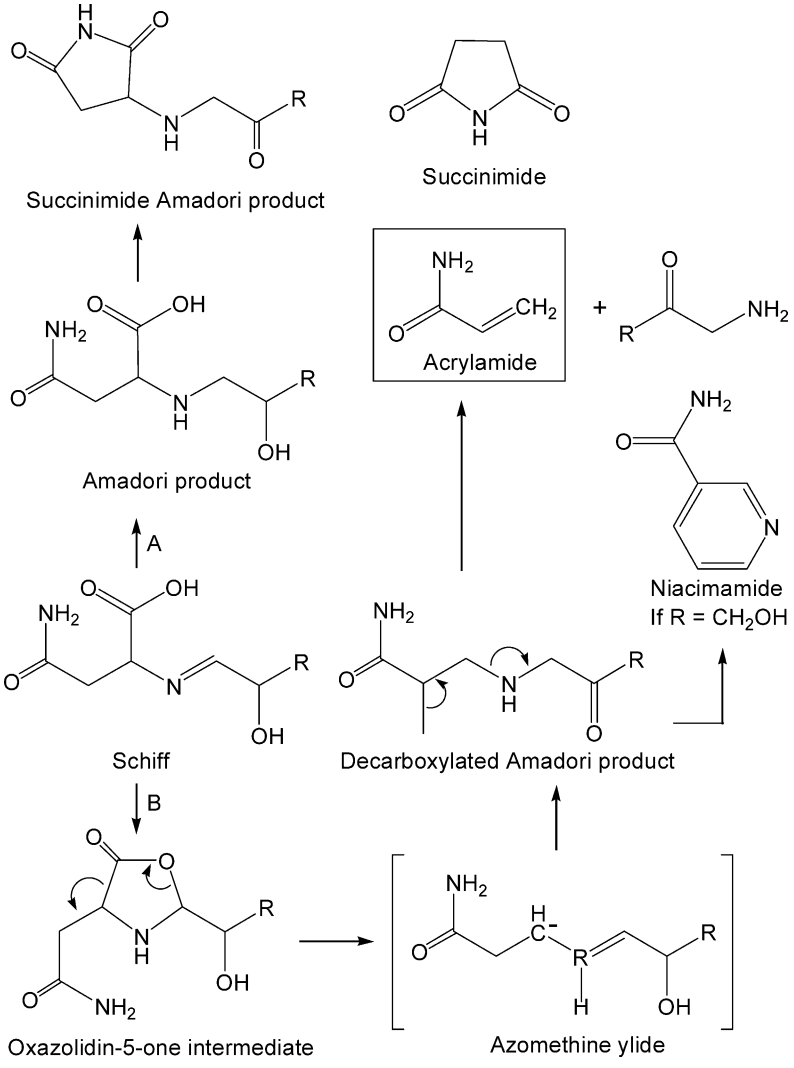

Fig. 1. Mechanism of acrylamide formation from a decarboxylated Amadori product of asparagines. R = H or $[CH(OH)]_nCH_2OH$; $n = 0–3$.

product of the thermal decomposition of asparagine is maleimide, mainly due to the fast intramolecular cyclization reaction that prevents the formation of acrylamide. On the other hand, asparagine, in the presence of reducing sugars, was able to generate acrylamide in addition to maleimide [37]. Good evidence supporting the early Maillard reaction as a main reaction pathway involving early decarboxylation of the Schiff base, rearrangement to the resulting Amadori product, and subsequent β-elimination to release acrylamide, has been presented [38]. Experimental results which corroborate this route are presented, as well as other potential pathways to acrylamide.

2.3. Corresponding chromatographic methods

A number of chromatographic methods for determination and detection of acrylamide in heat-treated foods has been developed. These assays are based on different principles and used to determine various structural groups present during the formation of acrylamide. Gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) techniques are used widely for both determination and detection of acrylamide. It is necessary to apply these chromatographic techniques for confirmation of each intermediate product or molecular rearrangement reasonably in order to elucidate the chemical structure of relevant compounds as described in Fig. 1. Structure elucidation is often achieved using combination of GC and HPLC with mass spectrometric analysis, as well as other relevant techniques.

2.3.1. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

Pyrolysis-gas chromatography/mass spectrometry is a technique very well suited for the characterization of polymers, wood extractives and deposits from the pulp and papermaking processes. Pyrolysis techniques are based on the principle of increasing the amount of heat energy in the system thus leading to thermal cracking of bonds of non-volatile organic compounds. Because of the large potential of the Py-GC/MS technique, it is highly recommended

for troubleshooting and evaluation of complex samples. In contrast to the majority of other analytical techniques, usually no pre-treatment is necessary for this analysis. Polar compounds such as acrylamide can be derivatized directly on the filament in the pyrolysis chamber. Most of the organic content of the sample is analyzed and the amount necessary for a sequence of analysis is very small. If the sample also contains inorganic components the ash remaining after the pyrolysis can be characterized by Environmental scanning electron microscopy coupled with energy dispersive X-rays analysis (ESEM-EDXA).

To testify the amide amino acid asparagine is mainly responsible for acrylamide formation in cooked foods, ^{13}C isotope tracer technique is necessarily applied for the generating mechanistic study of acrylamide. Py-GC/MS is also a cost-effective and convenient method to perform experiments with labeled reactants [39]. Although under pyrolytic conditions a higher number of products are formed compared with aqueous reactions, most of the products identified in aqueous systems are also formed under pyrolytic conditions, albeit in different amounts. In addition, experimental evidence was provided that the position and label distribution in the common products observed in the same model systems, between aqueous and pyrolytic reactions, are identical [40]. This indicates the similarity of mechanisms of formation of acrylamide under both conditions. Consequently, mechanistic conclusions derived from label incorporation in the products observed under pyrolytic conditions, which are common to both systems, have relevance to the aqueous reactions.

Yaylayan et al. [37] obtained important evidence and corresponding data in favor of the formation of the decarboxylated Amadori intermediate coming from identification of niacinamide by Py-GC/MS assays with ^{13}C-labeled glucose in the reaction mixture of the asparagine/glucose model system. Niacinamide can be formed only from the decarboxylated Amadori product of asparagine with glyceraldehyde through cyclization and dehydration reactions. On the other hand, the Amadori product will quickly undergo intramolecular cyclization and form a succinimide intermediate, which is unable to form niacinamide due to its altered structure [29]. Furthermore, Maillard model systems containing glucose are known to produce free glyceraldehyde and glyceraldehyde Amadori product [41] through retro-aldol reactions. In fact, when glyceraldehyde was pyrolyzed in the presence of asparagine, both acrylamide and niacinamide were detected in higher amounts relative to the glucose model system. During Py-GC/MS assays, it is easy for researchers to confirm chemical structures of each intermediate or final product according to the mass spectrometric data compared to the US National Institute of Science and Technology (NIST) library in the software of Py-GC/MS. Fig. 2 shows the pyrograms of sorbitol/asparagine and glucose/asparagine model systems by Py-GC/MS assay and acrylamide is found only in the glucose/asparagines model system and clearly indicates the presence of a significant peak due to CO_2 release in the case of the glucose/asparagine model system and its complete absence in the case of the sorbitol/asparagine model system.

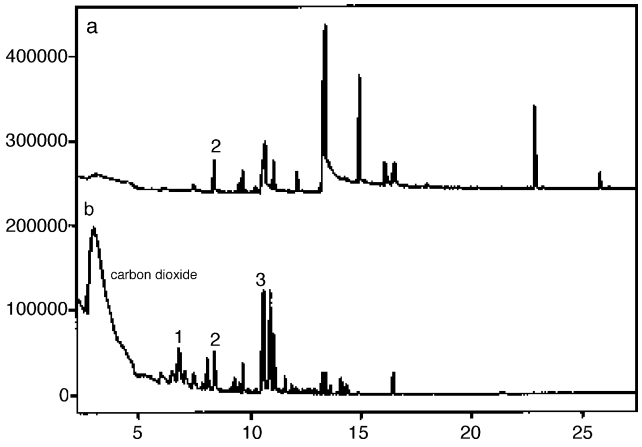

Fig. 2. Pyrograms of (a) sorbitol/asparagine and (b) glucose/asparagine model systems. Peaks: 1, acrylamide; 2, maleimide; 3, succinimide (reprinted from [36], reproduced with permission from ©ACS).

2.3.2. *Fourier transform infrared (FT-IR) analysis*

Fourier transform infrared is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". FT-IR is also most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture. It can be applied to the analysis of solids, liquids, and gasses. The term FT-IR refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. Today's FT-IR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments.

When glucose/asparagine model reactions are performed using FT-IR analysis, the facile formation of a decarboxylated asparagine Amadori product is obtained from infrared spectral analysis of a mixture of asparagine and glyceraldehydes in methanol. Within 15 min of incubation at room temperature, the Amadori product has already started to form as indicated by the appearance of the absorption band at 1737 cm^{-1}, which is assigned to the carbonyl band [42,43] of the Amadori product (see Fig. 3b). This assignment is based on the fact that the Amadori product is a derivative of dihydroxyacetone [42]. Further incubation of the sample at 40 °C for 6 days caused the disappearance of both symmetrical and antisymmetrical stretching frequencies of the carboxylate bands at 1493 and 1580 cm^{-1} (see Fig. 3c), indicating decarboxylation reaction and formation of a decarboxylated Amadori product. The spectral properties of this mixture does not change upon further incubation at 40 °C; however, when the temperature of the mixture is raised to 180 °C and the spectrum is acquired, the data show the disappearance of the amide I (1674 cm^{-1}) and amide II (1615 cm^{-1}) absorption bands of the amino acid, indicating cleavage of the asparagine moiety.

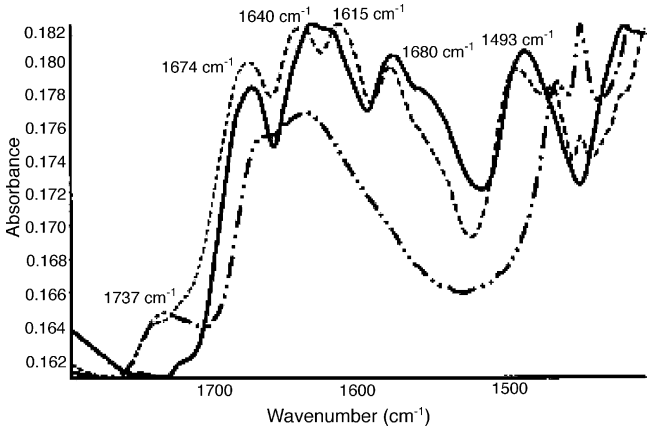

Fig. 3. Overlaid infrared spectra (1400–1800 cm^{-1}) of (a) asparagine (solid line), (b) asparagine/glyceraldehyde after 15 min of incubation at room temperature (dotted line), and (c) asparagine/glyceraldehyde after 6 days of incubation at 40 °C (dash-dot line) (reprinted from [36], reproduced with permission from ©ACS).

2.3.3. Other chromatographic methods

Beside the chromatographic technique (Py-GC/MS) and the spectroscopic technique (FT-IR) described above, other methods including high-performance liquid chromatography with ultraviolet diode array detection (HPLC–UV-DAD) [44,45], liquid chromatography with tandem mass spectrometry (LC-MS/MS) [46–48], time-of-flight mass spectrometry (TOF-MS) [49] also play an important role in the elucidation of generating mechanism of acrylamide during heat processing. The critical tools of these three techniques are UV scan spectrograms, multiple reaction-monitoring mode (MRM) quantitative analysis and precise mass assignment, respectively. Hitherto, many new findings that contribute towards a better understanding of the formation and presence of acrylamide in foods are presented. Rational use of chromatographic techniques will be in favor of establishing integrated and accepted mechanistic elucidation of acrylamide occurrence.

3. Chromatographic analysis of acrylamide

3.1. General introduction of applied chromatographic techniques

A great number of methods have been developed in the past years to quantificationally analyze the acrylamide monomer, especially in sugar [50], field crops [51] and mushrooms [52]. The majority are classical methods based on HPLC or GC technique. However, because of the complexity of food matrices, these methods do not suffice for the analysis of acrylamide in heat-treated foods at trace levels. Particularly, they lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule, such as acrylamide, in a complex food matrix.

Rosén and Hellenäs [53] firstly reported the analysis of acrylamide in different heat-treated foods using the isotope dilution LC–MS technique. They developed a mass spectrometry method for direct detection of acrylamide, which would unequivocally verify the presence of acrylamide in a range of heat-treated 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. From then on, a sequence of analytical methods dealing with the analysis of acrylamide in heat-treated foods have been published in peer reviewed journals, reported by specific research groups or presented at international scientific conferences [22]. In other words, most of published analytical methods are mainly based on MS as the determinative technique, coupled with a chromatographic step either by LC [53–56] or GC with [35,57–60] and without [61,62] derivatization of the analyte.

Hitherto, it can be summarized from recent methodological studies that GC–MS and LC–MS/MS appeared to be acknowledged as the most useful and authoritative method for acrylamide determination. Therefore, the following sections of this review mainly focus on the parameter optimization and correlative study of these two main techniques and sample extraction and clean-up procedures for the determination of acrylamide in heat-treated foods. Although chromatographic techniques other than GC–MS and LC–MS/MS appear to have been less widely employed, they will also be discussed here.

3.2. Extraction and clean-up procedures

3.2.1. Extraction of acrylamide

The whole extraction and clean-up procedures generally summarized from many peer-reviewed papers before sample injection of GC–MS or LC–MS/MS analysis are shown in Fig. 4. Water at room temperature has been used to extract acrylamide from many kinds of sample matrices in most of analytic methods published so far because acrylamide is a good hydrophilic small molecule [2,53,63]. Besides water as an extractant, methanol also can be used to extract acrylamide for the convenience of rotatory evaporation and concentration [62,64]. Young et al. [65] suggested that acrylamide could be extracted from sample matrices by using NaCl aqueous solution with a relative high level in order that the emulsification process during sample pre-treatment was obviously inhibited and the high recovery of analytes was demonstrated. Moreover, one of the laboratories that took part in the proficiency test about acrylamide used a mixture of water and acetone as extractant [66]. In addition, a research group of the National Institute of Health Sciences of Japan chose this solvent composition for acrylamide extraction [67]. Heating or ultrasonicating during the extraction step may as well be avoided because this may generate large amounts of slight particles that can saturate the solid-phase extraction (SPE) cartridges used in further clean-up steps and reduce the efficiency of clean-up and the operating life of SPE cartridges. However, water that had been previously heated to 80 °C has been used with no extracting problems

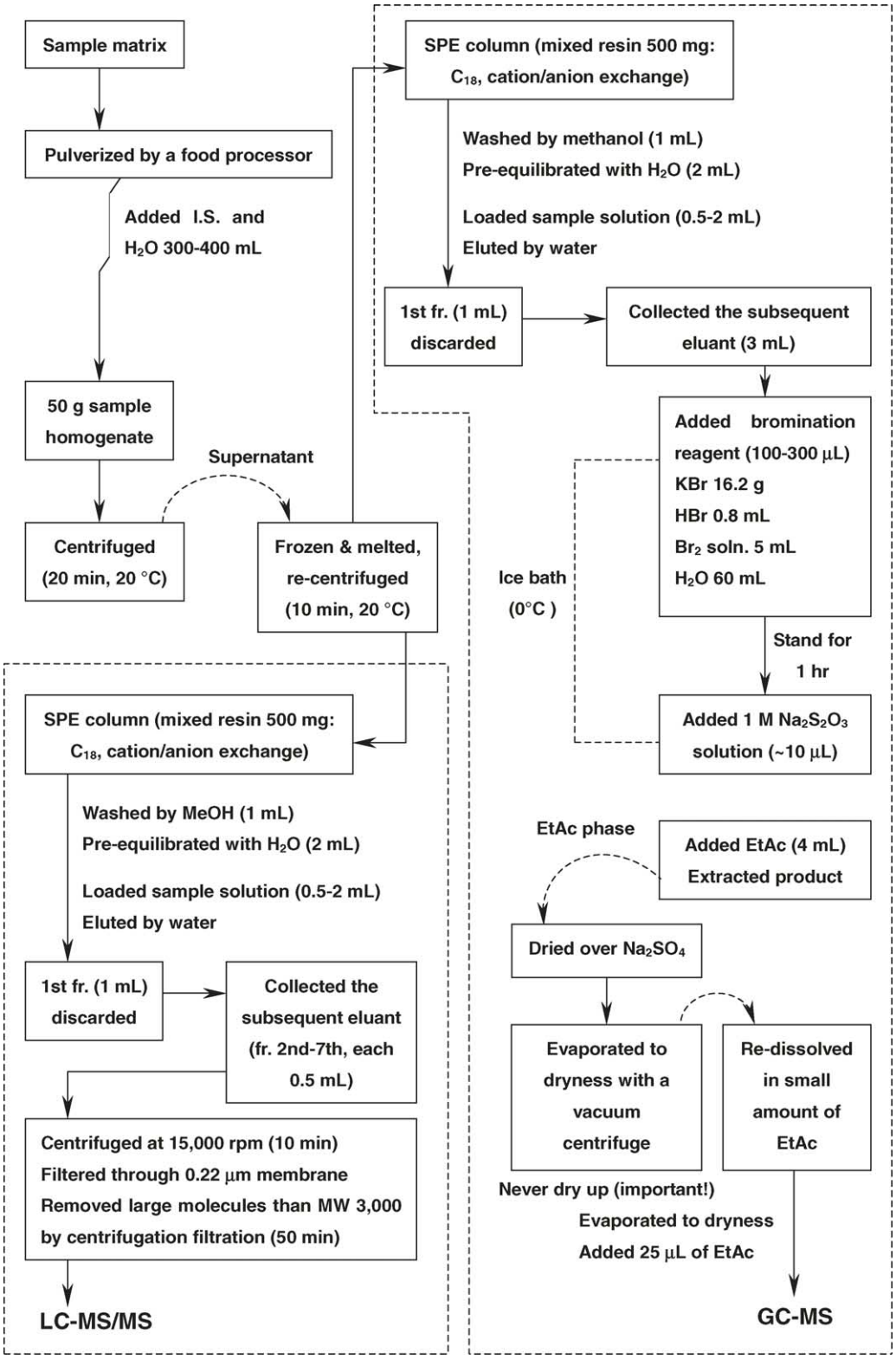

Fig. 4. The whole extraction and clean-up pre-treatment of acrylamide before GC–MS or LC–MS/MS. I.S., internal standard; fr., fraction; EtAc, ethyl acetate; MW, molecular weight.

because acrylamide is relatively stable at such temperature [68].

As for the extract method of acrylamide, some corresponding studies [69,70] referred to pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction). PLE is a technique for extracting solid and semisolid samples with liquid solvents. PLE uses conventional liquid solvents at elevated temperatures and pressures to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, while elevated pressure keeps the solvent below its boiling point, thus enabling safe and rapid extractions. Therefore, the ASE method provides a fast and efficient extraction of acrylamide from various food samples.

In order to control the recoveries and keep track of possible losses occurring during the whole sample pre-treatment, an internal standard was added to the sample matrix after homogenization. Most of published studies used [$^{13}C_3$] acrylamide produced by Cambridge Isotope Labs. (Andover, MA, USA) as internal standard [54,60,63,71–73]. Besides this, [$^{13}C_1$]acrylamide [67,74], [$^{2}H_3$]acrylamide [58,75], *N*,*N*-dimethylacrylamide [2,60], methacrylamide [68] and propionamide [76] also have ever been published to be used as acrylamide internal standard.

Some researches about acrylamide analysis have included a defatting step before or in combination with the extraction step. This was carried out by extraction with hexane, petroleum ether or cyclohexane. Moreover, some protein-rich sample matrices need to have a deproteinating step. This was carried out by adding methanol, acetonitrile or saline solution with a relatively high level. Delatour et al. [77] achieved the visual protein precipitation step within <1 min by addition of 1 mL of a 0.68 M potassium hexacyanoferrate(II) trihydrate solution (Carrez I) and 1 mL of a 2 M zinc sulfate heptahydrate solution (Carrez II) that were added under continuous swirling (1–2 min). The similar protein precipitation step was achieved by Gertz and Klostermann [35]. Whether the extraction step of acrylamide needs to have a defatting or deproteinating step should be carried out according to sample matrices.

After extraction, the aqueous phase was centrifuged before continuing with the clean-up procedure. However, centrifugation conditions seem different between different laboratories. Andrzejewski et al. [56] and Becalski et al. [63] combine centrifugation with filtration by using a 5 kDa cut-off Centricon Plus-20 and 0.45 μm PVDF filters (Millipore, Billerica, MA, USA), respectively. Moreover, multistep centrifugation (first run: 4000 rpm, 10 min at 10 °C; centrifuge GR 412, Jouan, Unterhaching, Germany; second run: 10,000 rpm, 10 min at 10 °C; Beckmann J2-HS, München, Germany) also has ever been reported by Granvogl et al. [46].

3.2.2. Clean-up of acrylamide

Most clean-up procedures consisted of the combination of several solid-phase extractions. One approach was to combine Oasis HLB (Waters, Milford, MA, USA) and Bond Elut-Accucat (mixedmode: C_8, SAX and SCX) (Varian, Palo Alto, CA, USA) cartridges. Becalski et al. [63] used a combination of three different cartridges: Oasis MAX (mixed-mode anion exchange) (Waters), Oasis MCX (mixed-mode cation exchange) and ENVI-Carb (graphitized carbon) (Supelco, Bellefonte, PA, USA). A similar combination of SPE cartridges consisting of Bond Elut C_{18}, Bond Elut Jr-PSA (anion exchange) and Bond Elut Accucat (all Varian) were chosen for clean-up of samples, which were measured by LC–MS with column switching [67]. Another group of laboratories used Isolute M-M 300 mg cartridges (mixed-mode: C_{18}, SAX and SCX) (International Sorbent Technology, Hengoed, UK) combined with filtration and/or ultracentrifugation to avoid blockage of the chromatographic system.

Oasis HLB cartridge, the main supporter of which is a hydrophilic–lipophilic balance and water-wettable reversed-phase sorbent for all compounds and all of general SPE needs produced by Waters (Milford, MA, USA), is in common use during the SPE clean-up step of acrylamide analysis. Many researches [45,78,79] reported the application of this cartridge before the chromatographic analysis of acrylamide. Oasis MCX cartridge, the main supporter of which is a mixed-mode cation-exchange reversed-phase sorbent for bases with a high selectivity for basic compounds, also can be used during the SPE clean-up step. Good accuracy and high recovery of chromatographic method validation during the acrylamide analysis can be achieved when reasonable and effective clean-up procedures are well designed. Young et al. [65] divided the SPE protocol for determination of acrylamide into two steps. For SPE step A, a vacuum manifold was used with the vacuum level set for a flow rate of 2–4 mL/min for the Oasis HLB cartridge. No vacuum was required for SPE step B because a large-particle (60 μm) Oasis MCX cartridge was used for the pass-through clean-up of the extract. The whole SPE clean-up procedures were presented in Fig. 5. Under such clean-up conditions, the inter-day study of their research showed good accuracy and precision of the method over a 3-day period with a recovery of 98% and a relative standard deviation (RSD) of 9.5% (n = 15).

3.2.3. Summarizations in the current extraction and future requirements

Many researchers have focused on the extraction efficiency of acrylamide in different sample matrices using water, methanol or other solvents. Bai et al. [80] discussed the process of extraction of solutes from sample tissues to solution. According to their study, extraction conditions and particle size and microstructure affect the process. Pedersen and Olsson [81] have sketched the extraction of acrylamide from sample particles by methanol using the stepwise model established by Bai et al. [80]. Acrylamide is extracted from a particle into the methanol solvent in five major steps: (i) entry of the solvent into the particle; (ii) redistribution of solvent and expansion of the solid matrix; (iii) dissolution of acrylamide depending on solvent properties and temperature; (iv) diffusion of acrylamide to

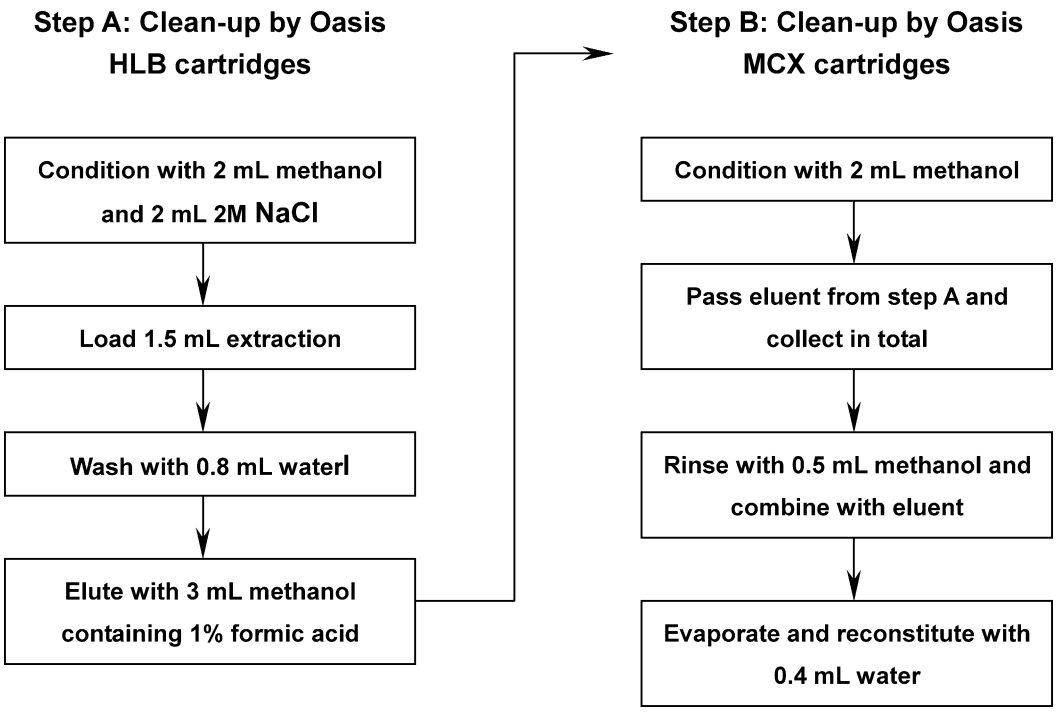

Fig. 5. Routine SPE protocol for determination of acrylamide.

the exterior of the particle; and (v) migration of the extracted acrylamide from the particle surface into the bulk solution of the methanol solvent. More detailed procedures on the extraction, clean-up and chromatographic techniques during the acrylamide analysis are summarized in Table 1.

With the increase of published papers on the analytical methods for the determination of acrylamide, optimization of extractive conditions needs to be taken into account and operated before chromatographic analysis. The factors which need to be optimized may contain grinding under dry or wet conditions and variable temperatures, extraction with various solvents (water, with or without organic solvents); swelling (e.g. of starch containing materials), extraction temperature, extraction time, number of extraction cycles and special needs such as defatting prior to the extraction. As for the clean-up procedures, SPE plays an important role during the clean-up of sample matrices. Corresponding factors such as choice of SPE cartridges (such as Oasis HLB, MAX, MCX, etc.), choice of eluents and filtration through a nylon filter also need to be optimized.

3.3. Chromatographic analysis based on GC–MS

3.3.1. GC–MS analysis with derivatization

Although acrylamide can be analyzed as such, without derivatization, when using GC–MS, the molecule is normally brominated to form a derivative that has improved GC properties. The acrylamide derivative is identified by its retention time and by the ratio of characteristic MS ions [13]. During the routine analysis of acrylamide by GC–MS, the derivatization step may usually be operated before the clean-up step [91,92].

Bromination is most frequently applied in the derivative treatment during GC-related analysis of acrylamide [57,74,93–95]. The advantage of acrylamide bromination is that a relative 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 hard and time-consuming derivatization process. In the recent derivative method reported in peer-review journals, conversion of acrylamide to 2,3-dibromopropionamide is performed according to the protocol originally described by Hashimoto [96], which involves addition of potassium bromide (KBr), hydrobromic acid (HBr) and a saturated Br_2 solution. The excess bromine is then removed by addition of sodium thiosulfate until the solution becomes colorless so that the derivative reaction is terminated. Under such conditions, the yield of 2,3-dibromopropionamide is nearly invariant when the reaction time is more than 1 h [97]. 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. [98] 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

Table 1
Some pre-treatment procedures for determination of acrylamide in heat-treated foods

Sample matrix	Extraction procedures	Clean-up procedures	Chromatographic methods and internal standard	Ref.
Breakfast cereals, crackers	Extraction with water, homogenization using a dispersing tool, centrifugation, mixed with acetonitrile to precipitate co-extractives, acetonitrile evaporation	Preconditioning with methanol and water (isolute multimode, 2, 2 × 2 mL; Accubond II SCX 1, 1 mL), residual water removal, extract (2 mL) loaded and collected, collected (1 mL) extract charged onto the latter cartridge, effluent collected, filtration through a syringe filter unit	LC–ESI-MS/MS; I.S.[a]: [$^{13}C_3$]acrylamide	Riediker and Stadler [54]
Coffee	Spiked with I.S., extraction with HPLC grade water, centrifuge tubes capped and shaken/vortexed (30 s), centrifugation, aliquot transfer to a Maxi-Spin PVDF[b] filtration tube (0.45 μm) and centrifugation	Conditioning (Oasis HLB 6cc cartridge) with methanol and water (3.5 mL each), filtered extract (1.5 mL) loaded, water (1.5 mL) elution and effluent transfer onto the second cartridge (Bond Elut-Accucat), a mark placed on the outside of the tube at a height equivalent to 1 mL of liquid above the sorbent bed, conditioning with methanol and water (2.5 mL each), sample allowed to pass through the column until it reached the mark previously placed on the outside, effluent collected	LC–MS/MS; I.S.: [$^{13}C_3$]acrylamide	Andrzejewski et al. [56]
Baked good foods	Mixed with an electric blender, defatted with hexane, residual solvent removal, extraction with methanol (50 mL), stirred (15 min) and shaken (1 min) in an ultrasonic bath, centrifugation	Concentrated in a rotary evaporator to <2 mL, diluted to a constant volume (2 mL) and fast filtered	GC–MS	Tateo and Bononi [62]
French fries, potato chips, wheat snacks, crisp bread	Extraction with water or water with formic acid (10 mM), using PLE	Filtration using a 0.22 μm nylon filter	IC-UV; IC-MS	Cavalli et al. [69]
Cereal-based foods	Weighted (15 g) into a 250 mL centrifuge bottle, spiked with I.S., sample suspended in water (150 mL) and homogenized (30 s), suspension acidified to pH 4–5 by addition of glacial acetic acid (∼1 mL), treated successively with Carrez I and II[c] (2 mL each), centrifugation (16,000 × *g*, 15 min), bromination	Extract transferred onto a glass chromatography column containing calcinated sodium sulfate and activated Florisil (5 g each), using small aliquots taken from hexane (50 mL), acrylamide derivative eluted with acetone (150 mL), evaporated to ∼2 mL and then to dryness (N_2), re-dissolved in EtAc[d] (400 μL), triethylamine added (40 μL)filtered through a 0.2 μm microfilter	GC–MS; I.S.: [$^{13}C_3$]acrylamide	Pittet et al. [72]
Chocolate powder, cocoa, coffee	I.S. (5 μg/mL) added, deproteinating with Carrez I and Carrez II (1 mL each), dichloromethane (5 mL) added, centrifugation, supernatant transfer into a Nalgen tube containing NaCl (1.8 g), extraction with EtAc (13 mL), organic phase transfer into amber vial containing water (2 mL), organic phase evaporation (N_2)	Conditioning (Isolute Multimode cartridge) with methanol (3 mL) and water (2 × 3 mL), aqueous extract loaded, elution and rinse with water (1 mL each), both fractions collected, extract volume reduction to ca. 500 μL (N_2), methanol (90 μL) added to extract (180 μL)	LC–MS/MS; I.S.: [$^2H_3$]acrylamide	Delatour et al. [77]
French fries	Extraction with water, centrifugation, spiked with internal standard	Preconditioning (strata-X-C cartridge) with methanol (2 × 1 mL) and water (2×1 mL), extract loaded, SPE[e] sorbent dried under vacuum, elution with water (1 mL), effluent collected	HPLC–UV; LC–MS; I.S.: [$^2H_3$]acrylamide	Peng et al. [82]
Potato chips, French fries	Spiked with I.S., incubation at room temperature (30 min), extraction with water, ultrasonication (10 min), centrifugation	Filtration using a 0.45 μm nylon filter	LC–ESI-MS; I.S.: [$^{13}C_1$]acrylamide	Inoue et al. [83]
Crisp bread, butter cookies	Sample homogenization, I.S. added, exposure (30 min), admixed with water (20 mL), ultrasonic extraction (60 °C, 30 min)	Purification with acetonitrile (20 mL), Carrez I and Carrez II (500 μL each), centrifugation, filtration through a membrane filter	LC–MS/MS; I.S.: [$^2H_3$]acrylamide	Wenzl et al. [84]
More than 130 samples, such as bread, fish and pizza, etc.	Homogenized and analysed fresh, or stored at −20 °C until analysis, spiked with deuterium-labeled I.S., extraction with water, centrifugation	SPE clean-up according to the method reported by Rosén and Hellenäs [52], filtrates collected and passed through a centrifuge spin filter until a sufficient volume had been obtained	LC–MS/MS; I.S.: deuterium-labeled acrylamide	Svensson et al. [85]
Baby food	Sample homogenization, spiked with I.S., extraction with water (50 mL) in an ultrasonic bath (60 °C, 30 min)	At first addition of Carrez I and Carrez II and *n*-hexane (30 mL) to the aqueous solution, afterwards centrifugation (45,000 × *g*, 10 min), saturation of the aqueous phase with NaCl and extraction with 50 mL EtAc (twice), the organic phases combined, dried over Na_2SO_4 and evaporated to 1 mL	GC–CI-MS/MS; I.S.: [$^2H_3$]acrylamide	Wiertz-Eggert-Jörisson (WEJ) GmbH [86]
Tomatoes	Sample homogenization, blended with I.S., centrifugation (2000 × *g*, 15 min) and re-extraction with water (25 mL), re-centrifugation, derivatize combined supernatants, extraction with EtAc (1 × 20 mL + 1 × 10 mL), centrifugation (1500 × *g*, 5min) drying of EtAc over Na_2SO_4, evaporation of the extract to 100 mL	Fractionation over preconditioned Bond-Elut silica gel column (1: 2.5 mL EtAc/hexane = 1/4, 2: 5 mL EtAc/hexane = 35/65), first 2 mL discard, next 2 mL collected, addition of I.S.2 (50 μL (72 μg/mL)), evaporation to 50 mL	GC–MS; I.S.1: methacrylamide; I.S.2: 2,3-dibromo-*N*,*N*- dimethylpropion- amide	Castle et al. [87]
Crisps, chips, rye bread, Dutch rusk, toast	Sample homogenization in a household food cutter, spiked with deuterium-labeled I.S., extraction with 5% methanol aqueous solution (100 mL), standing at room temperature (1 h), extract (1.5 mL) centrifugation (13,000 × *g*, 5 min)	No further clean-up steps, direct injection	LC–MS/MS; I.S.: deuterium-labeled acrylamide	Konings et al. [88]
Various food products	Weighed into a filter, placed on a Witt'scher pot, equipped with a vacuum pump, defatted by adding *iso*-hexane (80 mL), spiked with I.S., incubation (30 min), extraction with water (20 mL) in an ultrasonic bath (60 °C, 30 min)	Purification by adding acetonitrile (20 mL), Carrez I and Carrez II (500 μL each), centrifugation (4500 × *g*, 10min), supernatant filtration through a membrane filter	HPLC–MS/MS; GC–MS/MS; I.S.: [$^2H_3$]acrylamide	Hoenicke et al. [89]
Coffee	Spiked with I.S., extraction with water, centrifugation (3500 × *g*, 10 min)	Conditioning (Isolute Multimode cartridge) with acetonitrile (1 mL) and water (2 × 2 mL), extract (500 μL) applied to SPE cartridges and pushed through the column to waste by 2 mL air at a flow of 6 mL/min, extract (next 400 μL) collected directly in Mini uniprep PTFE[f] filter HPLC vials	LC–MS/MS; I.S.: [$^2H_3$]acrylamide	Granby and Fagt [90]

[a] I.S., internal standard.
[b] PVDF, polyvinylidene fluoride.
[c] Carrez I, potassium hexacyanoferrate (II) trihydrate solution; Carrez II, zinc sulfate heptahydrate solution.
[d] EtAc, ethyl acetate.
[e] SPE, solid-phase extraction.
[f] PTFE, polytetrafluoroethylene.

Table 2
GC-based analysis for the determination of acrylamide in heat-treated foods

Sample matrix	Internal standard	Derivatization	GC Column	Temperature programme and injection	LOD/LOQ and WR[a]	MS parameters	Ref.
Fried food	*N*,*N*-Dimethyl-acrylamide	Bromination (7.5 g KBr, HBr till pH 1–3, bromine water, 4 °C, 18 h)	HP PAS 1701 column, 25 m × 0.32 mm i.d., 0.25 μm film thickness (Emulation, Kungsporten 4A S-427 50 Billdal Sweden)	65 °C held for 1 min, ramped at 15 °C/min to 250 °C, held for 10 min; Inj.[a]: 2 μL, splitless	GC–MS; LOD: 5 μg/kg; WR: 5–500 μg/kg	AA[a]: *m/z* = 152, 150, 108, 106; I.S.[a]: *m/z* = 180, 178	Tareke et al. [2]
Various foods	[$^{13}C_3$]Acrylamide	Bromination using 200–300 mL of brominating reagent (0 °C, 1 h) to 2-BPA[b]	Stabilwax Crossbond Carbowax column, 15 m × 0.25 mm i.d., 0.25 μm film thickness (Thames Restek, High Wycombe, UK)	40 °C ramped at 30 °C/min to 220 °C; Inj.: 1 μL, splitless	GC–MS; LOD: 5 μg/kg; WR: 5–1000 μg/kg	AA: *m/z* = 167, 169; I.S.: *m/z* = 170, 172	Robarge et al. [57]
63 processed foods	[$^{2}H_3$]Acrylamide	Bromination (15.2 g KBr, 0.8 mL HBr, 5 mL bromine water, 60 mL water, 4 °C, 1 h) to 2,3-DBPA[b]	CP-Sil 24 CB Lowbleed/MS column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Varian, Palo Alto, CA, USA)	85 °C held for 1 min, ramped at 25 °C/min to 175 °C, held for 6 min, ramped at 40 °C/min to 250 °C, held for 7.52 min	GC–MS; LOD: 0.2 ng/mL; WR: 20–1000 ng/mL	AA: *m/z* = 152, 150; I.S.: *m/z* = 155, 153	Ono et al. [58]
Protein-rich and carbohydrate-rich foods	I.S.1: *N*,*N*-dimethyl-acrylamide; I.S.2: [$^{13}C_1$]acrylamide	Bromination (7.5 g KBr, HBr till pH 1–3, 10 mL bromine water, 4 °C, overnight)	BPX-10 column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Varian)	65 °C held for 1 min, ramped at 15 °C/min to 250 °C, held for 10 min; Inj.: 2 μL, splitless	GC–MS; LOD: 5 μg/kg; WR: 5–500 μg/kg	AA: *m/z* = 152, 150, 106; I.S.1: *m/z* = 180; I.S.2: *m/z* = 155	Tareke et al. [60]
Baked good foods	–	–	Supelcowax column, 30 m × 0.25 mm i.d., 0.20 μm film thickness (Agilent, Palo Alto, CA, USA)	60 °C held for 1 min, ramped at 10 °C/min to 240 °C; Inj.: 1 μL, splitless	GC–MS; LOD: 2 μg/kg; WR: 100–4000 μg/L	AA: *m/z* = 71, 55, 27	Tateo and Bononi [62]
Toasted bread, fried chips, grilling and baking potatoes	Methacrylamide	Bromination (15.2 g KBr, 0.8 mL HBr, 5 mL bromine water, 60 mL water, 4 °C, overnight)	DB 17 capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent)	85 °C held for 1 min, ramped at 25 °C/min to 175 °C, held for 6 min, ramped at 40 °C/min to 250 °C, held for 5 min; Inj.: 1 μL, splitless	GC–MS; LOD: 25 μg/kg	AA: *m/z* = 152, 150, 108, 106; I.S.: *m/z* = 122, 120	Ahn et al. [68]
Cereal-based foods	[$^{13}C_3$]Acrylamide	Silylation using BSTFA[c] which was drawn through a septum into a 250 μL gastight, glass syringe and added through the PTFE[c]/silicone septa of the 4 mL vial to the acrylamide in acetonitrile solution (70 °C, 1 h)	Varian CP-Sil 8 CB column, 30 m × 0.26 mm i.d., 0.25 μm phase thickness (Varian)	50 °C ramped at 3 °C/min to 100 °C, and then ramped at 25 °C/min to 250 °C; Inj.: 1 μL, splitless (0–2 min) and 100:1 split (after 2 min)	GC–MS; LOD: 0.9 μg/kg; WR: 4–6700 μg/kg	AA: *m/z* = 200, 128; I.S.: *m/z* = 203, 131	Lagalante and Felter [71]
Cereal-based foods	[$^{13}C_3$]Acrylamide	Bromination (7.5 g calcinated KBr, HBr till pH 1–3, 10 mL saturated bromine water, 0 °C, >1 h) to 2-BPA	ZB-WAX capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent)	65 °C held for 1 min, ramped at 15 °C/min to 170 °C, 5 °C/min to 200 °C, 40 °C/min to 250 °C, held for 15 min; Inj.: 2 μL, splitless	GC–MS; LOD: 2 μg/kg; WR: 5–500 μg/kg	AA: *m/z* = 149, 70; I.S.: *m/z* = 154, 110	Pittet et al. [72]
Various foods	[$^{13}C_1$]Acrylamide	Bromination to 2,3-DBPA	–	–	GC–MS; LOD: 9 μg/kg	AA: *m/z* = 150, 106, 70; I.S.: *m/z* = 154, 110	Nemoto et al. [74]
Drinking water, coffee, snuff	[$^{2}H_3$]Acrylamide	*N*-(2-Carbamoylethyl)valine, incubated with pentafluorophenyl isothiocyanate to give a PFPTH[d] derivative (45 °C, 90 min)	DB5 MS fused silica capillary column, 30 m × 0.32 mm i.d., 1 μm phase thickness (Agilent)	100 °C for 1 min, 20 °C/min to 240 °C, 10 °C/min to 320 °C, and finally isothermal at 320 °C for 5 min; Inj.: 1 μL, splitless	GC–MS/MS; LOD: ~0.003 μg/L in water	AA: *m/z* = 375 > 319, 304; I.S.: *m/z* = 378 > 319, 304 according to Bergmark [101] and Pérez et al. [102]	Pérez and Osterman-Golkar [75]
Potato chips	–	–	BP 21 capillary column, 15 m (GC–FID) or 30 m (GC–MS) × 0.25 mm i.d., 0.25 μm film thickness (Agilent)	75 °C held for 2 min, ramped at 10 °C/min to 230 °C; Inj.: 2.5 μL, splitless	GC–FID; GC–MS	AA: *m/z* = 71, 55, 27	Pedersen and Olsson [81]
Cereal-based foods	[$^{2}H_3$]Acrylamide	–	DB-WAX capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent)	70 °C held for 1 min, ramped at 20 °C/min to 230 °C, held for 10 min; Inj.: 1 μL, splitless	GC–MS/MS; LOQ: 30 μg/kg	AA: *m/z* = 89 > 72, 55; I.S.: *m/z* = 92 > 75	Hoenicke et al. [89]
Various foods	–	Bromination (7.5 g KBr, HBr till pH 1–3, 2.5 mL saturated bromine water, 4 °C, >1 h) to 2,3-DBPA	GC glass column, 2 m × 3 mm i.d., 5% FFAP on 60–80 mesh acid washed Chromosorb W, or equivalent (Quadrex, Woodbridge, CT, USA)	165 °C held for 15 min; Inj.: 5 μL, splitless	GC–ECD; LOD: 0.032 μg/L; WR: 0–5 μg/L	–	US EPA [97]
Crisp bread, potato chips, sweet cookies, butter cookies, rusk	[$^{2}H_3$]Acrylamide	–	FFAP[e] fused silica capillary column, 30 m × 0.32 mm i.d., 0.25 μm film thickness (Quadrex)	60 °C held for 2 min, ramped at 10 °C/min to 240 °C, held for 15 min; Inj.: 2 μL, splitless	GC–MS; LOD: 4 μg/kg	AA: *m/z* = 71, 55, 27; I.S.: not specified	Jezussek and Schieberle [100]

[a] LOD, limit of detection; LOQ, limit of quantification; WR, working range of concentrations; Inj.: injection volume; AA, acrylamide; I.S., internal standard.
[b] 2-BPA, 2-bromopropionamide; 2,3-DBPA, 2,3-dibromopropionamide.
[c] BSFTA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; PTFE, polytetrafluoroethylene.
[d] PFPTH, pentafluorophenylthiohydantoin.
[e] FFAP, free fatty acid polyester.

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 [98].

Another bromination recipe based on the use of KBr-potassium bromate ($KBrO_3$) has recently been proposed in the literature [74]. This approach indicates that the bromine molecule can be produced by an oxidation-reduction reaction between KBr and $KBrO_3$ in order to offer the advantage of eliminating the handling of elemental bromine. Although it has not been evaluated whether low bromination yields have been reported under such circumstances [74], it is a more safe and less hazardous operation method for the derivative treatment of acrylamide.

As for the brominating reaction time, bromination is frequently carried out from 1 h to overnight at temperatures approximately at the freezing point of water reported by various corresponding studies [72,74]. Castle [99] recently reported many differences in the reaction kinetics of the bromination reaction of acrylamide and methacrylamide at Joint European Commission–Swedish National Food Administration Workshop on "Analytical Methods for Acrylamide Determination in Food" (Oud-Turnhout, Belgium, 28–29 April 2003). It was also stated that the application of isotopically labelled internal standards allowed a reduction in the reaction time from overnight to 1 h.

Besides the bromination of acrylamide, a few studies [71,100] reported other derivative methods and their results on method validation and sample recovery. Lagalante and Felter [71] reported a method for quantitative analysis of acrylamide which has been developed for use with headspace solid-phase microextraction (SPME). In the method, acrylamide is derived by silylation with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) to form the volatile *N*,*O*-bis (trimethylsilyl)acrylamide (BTMSA). Once formed, BTMSA is easily extracted from the headspace over the silylation reaction using a 100 μm poly(dimethylsiloxane) SPME fiber. A series of experiments in their study was performed to optimize the amount of BSTFA, the silylation reaction temperature, the silylation reaction duration, and SPME sampling duration to maximize the analytical sensitivity for BTMSA before GC–MS analysis. An analytical working curve was constructed and found to be linear over the 4–6700 μg/kg acrylamide range investigated with a limit of detection of 0.9 μg/kg. In a word, whatever derivative method has been used before chromatographic analysis, the target of derivatization is to reduce the polarity of acrylamide, improve the retention time and peak regularization, and ensure the optimization of corresponding indexes or parameters to a certain extent.

3.3.2. Quantification and chromatographic analysis of acrylamide

The ions monitored for identification of the analyte, 2-bromopropenamide, are $[C_3H_4NO]^+ = 70$, $[C_3H_4{}^{79}BrNO]^+ = 149$, and $[C_3H_4{}^{81}BrNO]^+ = 151$, using mass to charge ratio (*m*/*z*) 149 for quantification. The ions monitored for identification of the internal standard, if 2-bromo($^{13}C_3$)propenamide, are $[^{13}C_2H_3{}^{81}Br]^+ = 110$ and $[^{13}C_3H_4{}^{81}BrNO]^+ = 154$, using *m*/*z* 154 for quantification. The separation of acrylamide analyte after derivatization was performed on standard GC capillary columns of middle to high polarity with a length of 30 m and an internal diameter of 0.25 mm (standard in GC–MS). A total of 1–2 μL sample was injected in splitless mode. Initial oven temperature settings were in the range 60–85 °C and in most cases, the heating rate was 15 °C/min. The final oven temperature was about 250 °C.

Quantification was initially achieved using the external reference method. However, the deficiency of this initial method including the method validation, recovery and limit of detection (LOD) became obvious because of the trace content of acrylamide in most of heat-treated foods. Tateo and Bononi [62] reported 2 μg/kg of LOD and 10 μg/kg of limit of quantification (LOQ) determined by external reference method. With the development of analytical technology, quantification was then achieved using the internal reference method in order to reduce pre-treatment loss and improve recovery in the condition of ensuring LOD/LOQ during the GC–MS analysis. The acrylamide internal standard should refer to whose physicochemical properties are similar to acrylamide standard. The original method [87] using GC–MS with bromination was based on the addition of methacrylamide as an internal standard into the homogenized sample and the derivative 2,3-dibromo-2-methylpropionamide was detected by GC–MS, or based on the addition of *N*,*N*-dimethylacrylamide as a surrogate. However, under such situation, satisfactory repeatability of the results of the analysis could not be achieved until isotopically labeled acrylamide was used because the properties of *N*,*N*-dimethylacrylamide are obviously too different from acrylamide. However, the relative standard deviation of the acrylamide recovery of spiked samples decreased from 26 to 7.5% when using [$^{13}C_3$]acrylamide [60]. This could be due to the differing stability of the compounds.

In the method reported by Biedermann et al. [61], the determinative step is either positive ion chemical ionization in the selected ion monitoring (SIM) mode or electron impact ionization, achieving a level of LOD of around 50 and <10 μg/kg, respectively, for potato products. Better sensitivity (LOQ = 5 μg/kg) can be achieved in the tandem (MS/MS) mode using a high-resolution mass spectrometer. As described above, 2,3-dibromopropionamide could be produced during the derivatization. Corresponding studies suggest that the bromination of acrylamide to 2,3-dibromopropionamide has multiple advantages, which include (i) improved selectivity, (ii) increased volatility, (iii) removal of potentially interfering co-extractives, and (iv) better sensitivity. Usually the ions *m*/*z* 150/152 $[CH_2–CHBr–CONH_2]^+$ and *m*/*z* 106/108 $[CH_2{=}CHBr]^+$ (Fig. 6a) are monitored in the SIM mode. Some analysts, however, choose to convert the rather labile di-bromo derivative to 2-bromopropenamide by

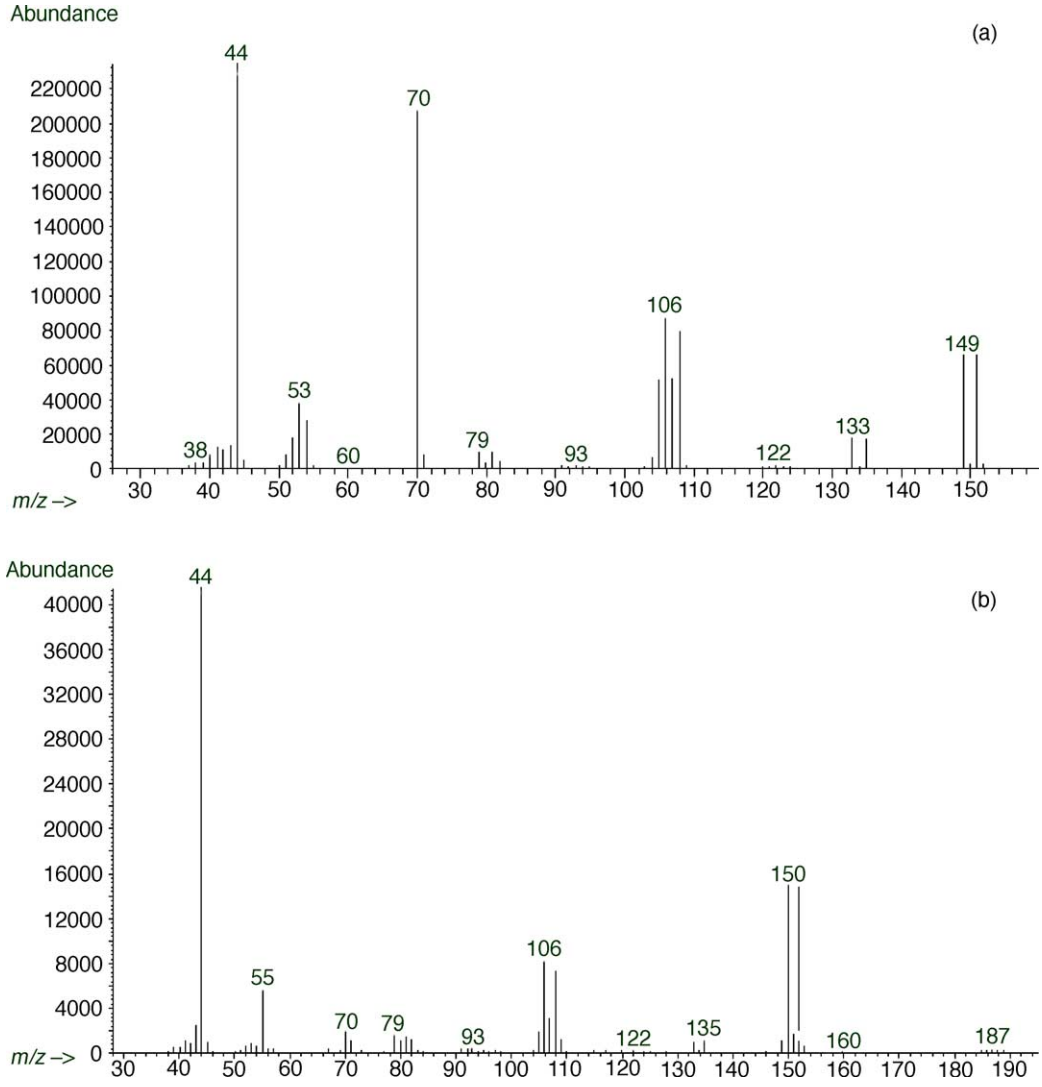

Fig. 6. Mass spectra of acrylamide derivatives: (a) 2-bromopropenamide; (b) 2,3-dibromo-propenamide.

treatment with triethylamine. This additional step avoids the risk of dehydrobromination in the injector or the ion source of the MS and has no impact on the selectivity or sensitivity of the method. Compared to 2,3-dibromopropionamide, the ions *m/z* 149/151 $[CH_2{=}CBr{-}CONH_2]^+$ and *m/z* 106/108 $[CH_2{=}CHBr]^+$ (Fig. 6b) are chosen in the SIM mode in this case. Therefore, GC–MS after bromination is probably the best choice for the analysis of acrylamide in foods with a detection level at or less than 10 μg/kg.

3.3.3. GC–MS analysis without derivatization

Considering GC–MS for acrylamide analysis as a whole, derivatization especially bromination technology has already been accepted by increasing scientists in order to obtain reasonable results via this chromatographic method. However, some researches [61,62,86] reported the acrylamide determination using GC based chromatographic methods without derivatization. Owing to the higher polarity of the non-derivatized acrylamide, columns with polar phases, e.g. polyethylenglycol, have to be used. Temperature programmes do not differentiate much from those in methods applying derivatization. The major drawback of GC analysis without derivatization is lack of characteristic ion peaks in the mass spectrum of non-derivatized acrylamide. In electron ionization mode, the major fragment ions are at *m/z* 71 and 55, respectively. These ions are also used for quantification. Co-extracted substances such as maltol or heptanoic acid produce almost the same fragmentation pattern and may therefore interfere [61]. More detailed information about chromatographic conditions including GC and MS parameters during the acrylamide analysis are summarized in Table 2.

3.4. Chromatographic analysis based on LC–MS/MS

Besides GC–MS for the determination of acrylamide, recent studies pay more attention to assays employing LC–MS/MS techniques for the routine analysis [103,104] or exposure survey of acrylamide [105] because this chromatographic technique applied for the quantitative analysis of

acrylamide has high sensitivity and avoids the derivatization step.

3.4.1. Chromatographic separation of acrylamide

For the chromatographic separation of acrylamide, most researchers use reversed-phase chromatography. Hypercarb column (5 μm, Thermo Electron, San Jose, CA, USA) [2,53,63] has been the most frequently used column. However, it is difficult to choose appropriate mobile phase to achieve good analyte elution with a reasonable retention time because of the high polarity of acrylamide. Most chromatographers have experienced problems retaining and separating polar compounds, just as acrylamide, using conventional reversed-phase chromatography. These difficult-to-analyze compounds either pass through the column unretained or, if retained at all, co-elute at the beginning of the chromatogram. Waters Atlantis columns (Waters, Milford, MA, USA) are designed for these types of challenging separations. Atlantis dC_{18} columns are a silica-based line of difunctionally bonded C_{18} columns that provide the optimal balance of retention for polar and non-polar compounds in reversed-phase chromatography. Atlantis dC_{18} columns are intelligently designed to provide full LC/MS compatibility, superior peak shapes and excellent column-to-column reproducibility. Ono et al. [58] used Atlantis dC_{18} reversed-phase chromatographic analytical column to achieve good separation and elution of acrylamide because it provided sufficient retention of acrylamide to minimize matrix interference while allowing a 10 min chromatographic cycle time.

An alternative to reversed-phase columns is ion-exchange chromatography [59,69]. In this case, an IonPac ICE-AS1 column (Dionex, Sunnyvale, CA, USA) is used that combines ion exchange with size exclusion chromatography. The advantage is that there is a significant increase of the k' value compared with reversed-phase columns, leading to good separation of acrylamide from matrix compounds even of untreated sample extracts. Based on this chromatographic technique, recently Cavalli et al. [106] reported a new method based on the combination of ion-exclusion chromatographic separation and MS detection to achieve the determination of acrylamide in drinking water. Samples of drinking water have been directly injected in the microbore ICE-AS1 column and detected in the selected-ion monitoring mode by a single quadrupole system with electrospray ionization. Chromatographic conditions, such as eluent composition and flow rate, have been optimized by a central composite design experiment. Moreover, their previous works [69,107] suggested that the use of an ion exclusion column instead of a classical reversed-phase column can have the advantage to separate acrylamide by potential interferences by exploiting the multiple retention mechanism of the stationary phase. Furthermore, the high-capacity characteristic allows large-volume injection to overcome sensitivity limitations. A microbore version of this column, which is more apt to coupling with electrospray ionization system, has been used to gain further sensitivity.

3.4.2. Detection of acrylamide

For the detection of acrylamide after LC separation, tandem mass spectrometry was most used to detect the characteristic ions of acrylamide.

LC–MS/MS has a high selectivity when working in MRM mode, in which the transition from a precursor ion to a product ion is monitored. MRM means that the transition from a precursor ion, which is separated in the first quadrupole, to a product ion, generated by collision with argon in the second quadrupole, is monitored in the third quadrupole. As for the acrylamide monomer detection using MRM, injected samples from the liquid introduction system enter the ionization source at atmospheric pressure. These ions are sampled through a series of orifices and ion optics into the first quadrupole where they are filtered according to 72 of mass to charge ratio (m/z) of acrylamide. The mass separated ion $[CH_2{=}CHCONH_2]^+$, then passes into the ion tunnel collision cell, with axial field, where they either undergo collision induced decomposition (CID), or pass unhindered to the second quadrupole. The fragment ions of acrylamide after collision using argon as collision gas, i.e. $[CH_2{=}CHC{=}O]^+$ for m/z 55 and $[CH_2{=}CHC{=}NH]^+$ for m/z 54, are then mass analyzed by the second quadrupole. Finally, the selected and transmitted ions are detected by a conversion dynode, phosphor, and photomultiplier detection system. The output signal is amplified, digitized, and presented to the data system (e.g. MassLynx NT). The whole detection procedure of acrylamide using MRM mode is demonstrated in Fig. 7. The transition 72 > 55 is always selected to quantify acrylamide because it shows high relative abundance [18,53,68]. Other transitions, such as 72 > 54, 72 > 44 and 72 > 27, have been used in some cases for the analyte confirmation of acrylamide. The transition 72 > 72 has also been observed with a relative intensity of approximately 195% compared with 72 > 55 [53]. For the detection of the isotopically labeled acrylamide used as internal standard, the monitored transitions are 75 > 58 for $[^2H_3]$- and $[^{13}C_3]$acrylamide and 73 > 56 for $[^{13}C_1]$acrylamide.

Although the high selectivity offered by MS/MS shows much more clear analyte elution than chromatograms based on other chromatographic techniques, interference can still inevitably occur [63] and similar problems also occur during GC–MS analysis [108]. Peaks showing identical retention time to acrylamide and deuterated acrylamide have been observed. On the other hand, similar to the experiences of private and official food control laboratories, problems have been encountered in the analysis of difficult matrices due to interfering compounds in the characteristic acrylamide transitions (either for the internal standard or the analyte). A promising approach is to extract the analyte into a polar organic solvent, such as ethyl acetate. Sanders et al. [20] employed ethyl acetate to extract acrylamide from the aqueous phase (removing interfering constituents such as salt, sugars, starches, amino acids, etc.). The ethyl acetate extract could then be concentrated and analyzed by LC–MS/MS. Becalski et al. [63] also reported the existence of an early eluting compound that interferes when transition 72 > 55 was used for the

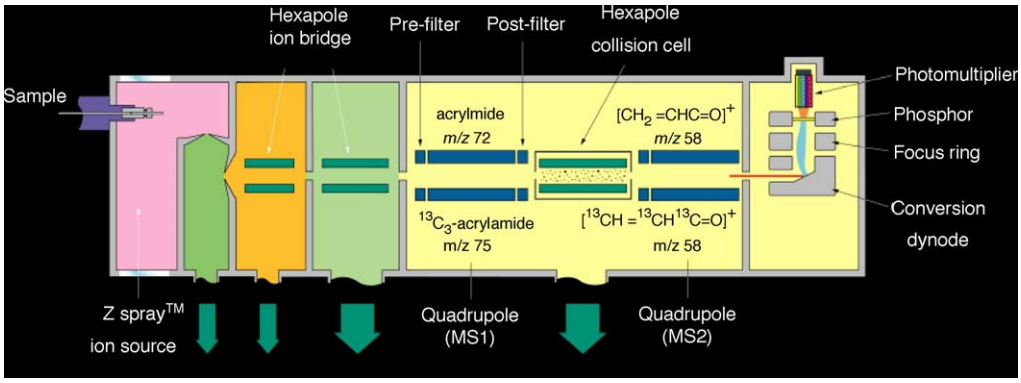

Fig. 7. The whole detection procedure of acrylamide by LC–MS/MS using MRM mode. Molecular ion peak can be detected as $[M+H]^+$.

detection of acrylamide. Increasing the column length from 100 to 150 mm and applying Isolute Multimode cartridges during sample preparation eliminated the problem well.

Jezussek and Schieberle [100] developed a new LC–MS method for the quantification of acrylamide based on a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid. In stable isotope dilution assays, the differentiation between acrylamide and its internal standard was done by recording the respective molecular masses and/or mass fragments. Due to the low molecular weight of acrylamide, and therefore, also due to its resulting low mass fragment ions, background interference may impede the analysis. Better confirmation of the analyte and selectivity can be achieved with a two-stage mass spectrometer, i.e. double quadrupole, by monitoring more than one characteristic mass transition [60,63,68,109]. However, triple quadrupole mass spectrometers are quite expensive and the cheaper single-stage LC mass spectrometers are not sensitive enough for the direct analysis of acrylamide. Such isotope dilution assay involving derivatization of acrylamide with 2-mercaptobenzoic acid prior to MS analysis could be useful for not only improving the sensitivity of acrylamide analysis, but also reducing analytical cost by achieving the transition from two-stage mass spectrometer to single-stage mass spectrometer [100]. Further details on the chromatographic conditions and the optimum parameters used for LC-based methods especially the LC–MS/MS technique are fully given in Table 3.

3.5. *Official and fully validated methods for the determination of acrylamide*

During the past years, numerous methods have been developed to determine the acrylamide monomer in different heat-treated sample matrices. Besides the analytical method of acrylamide reported by journal publication, many corresponding official institutions, government departments and graduate schools standardized and published their fully validated methods for the determination of acrylamide, most of which focused on LC–MS/MS analysis. The details of some validated methods for the analysis of acrylamide and corresponding research institutes were described as follows:

3.5.1. *US Food and Drug Administration Center for food safety and applied nutrition [114]*

The samples were homogenized, and 9 mL of water and 1 mL of the internal standard [$^{13}C_3$]acrylamide (200 ng/mL in 0.1% formic acid), were added to 1 g of homogenized sample. The mixture was shaken for 10 min on a rotating shaker and centrifuged at 9000 rpm for 30 min. A 5 mL aliquot of supernatant was placed in a Maxi-Spin filter tube, 0.45 μm PVDF (Alltech Associates, IL, USA) and centrifuged at 9000 rpm for 4 min. An Oasis HLB 6 mL SPE cartridge (200 mg; Waters, Milford, MA, USA) was conditioned with 5 mL of methanol and 5 mL of water. A 2 mL aliquot of the extract was loaded onto the Oasis SPE cartridge, 2 mL of water was added and the eluent was collected. A Bond Elute-Accucat (mixed mode C_8, SAX and SCX) 3 mL SPE cartridge (Varian, Palo Alto, CA, USA) was conditioned with 3 mL of methanol and 3 mL of water. The first two portions of the eluent from the previous steps were discarded and the rest was passed through the second SPE cartridge.

The samples were analyzed by LC–MS/MS (ESI+) using Aqua C_{18} column (250 mm × 2 mm, 5 μm; Phenomenex, Torrance, CA, USA). The HPLC parameters had the following settings: mobile phase, 0.5% methanol in aqueous 0.1% acetic acid; flow rate, 0.2 mL/min; injection, 20 μL; column temperature, 26 °C. The electrospray source had the following settings (with nitrogen): probe temperature, 240 °C; source temperature, 120 °C; collision gas pressure, 1 Torr.

Acrylamide was identified by MRM mode. The precursor ion $[M+H]^+ = 72$ was fragmented, and product ions $[H_2C{=}CHC{=}O]^+ = 55$ and $[H_2C{=}CH]^+ = 27$ were monitored. The ion *m/z* 55 was used for quantification. Monitored product ions for the internal standard were $[^{13}C_3H_3O]^+ = 58$ and $[^{13}C_2H_3]^+ = 29$ from precursor ion $[M+H]^+ = 75$. The collision energy was 19 eV.

3.5.2. *Swiss Federal Office of Public Health [70]*

The homogenized sample (5 g) was spiked with a deuterated acrylamide solution (50 μL, corresponding to ca. 5 μg [$^2H_3$]acrylamide) and well mixed to 4 g of hydromatrix. The mixture was placed into a 22 mL PLE cartridge filled previously with 0.5 g of hydromatrix material. The material was

Table 3
LC-based analysis for the determination of acrylamide in heat-treated foods

Sample matrix	Internal standard	LC column	LC parameters	MS parameters	LOD/LOQ, WR[a] and recovery	Ref.
French fries	[$^{13}C_3$]Acrylamide	Aquasil C_{18} column, 250 mm × 2.1 mm i.d., 5 μm (Thermo Hypersil-Keystone, San Ramon, CA, USA)	Mobile phase: methanol/1 mM aqueous ammonium formate = 16/84, 0.175 mL/min; Inj.[a]: 10–20 μL	Cone voltage: 34 V; source temperature: 120 °C; Desolvation temperature: 250 °C; *m/z* transitions (collision energy); AA[a]: 72 > 55 (11 eV), 54 (11 eV), 44 (14 eV), 27 (16 eV)	LC–MS/MS; WR: 10–500 ng/mL; recovery: >95%	Becalski et al. [47]
Fried potatoes	Methacrylamide	Hypercarb column, 50 mm × 2.1 mm i.d., 5 μm (Thermo Electron, San Jose, CA, USA)	Mobile phase: 0.1% formic acid/acetonitrile = 98/2, 0.3 mL/min; Inj.: 10 μL	Capillary voltage: 4.5 kV; capillary temperature: 300 °C; *m/z* transitions (collision energy); AA: 72 > 55, 54, 44 (14 eV); I.S.: 86 > 69, 58, 41 (16 eV)	LC–MS/MS; LOD[a]: 1 ng/mL; LOQ[a]: 3 ng/mL; WR: 1–10,000 ng/mL; recovery: 95–105%	Taubert et al. [48]
Cooked foods	[$^2H_3$]Acrylamide	Hypercarb HPLC column, 50 mm × 2.1 mm i.d., 5 μm (Thermo Hypersil-Keystone, San Ramon, CA, USA)	Mobile phase: methanol/water = 20/80, 0.4 mL/min; Inj.: 10 μL	Capillary voltage: 2 kV; cone voltage: 20 V; source temperature: 125 °C; desolvation temperature: 400 °C; *m/z* transitions (collision energy); AA: 72 > 72 (0 eV), 55 (9 eV), 54 (16 eV), 44 (20 eV), 27 (14 eV); I.S.: 75 > 58 (9 eV)	LC–MS/MS; LOD: <10 μg/kg; LOQ: <30 μg/kg; WR: 25–2000 μg/kg	Rosén and Hellenäs [53]
Breakfast cereals and crackers	[$^{13}C_3$]Acrylamide	Shodex RSpak DE-613 polymethacrylate gel column, 150 mm × 6 mm i.d. (Showa Denko, Kawasaki, Japan)	Mobile phase: 0.01% aqueous formic acid/methanol = 6/4, 0.75 mL/min split to 0.35 mL after the LC column using a PEEK[b]; T-piece, run time: 12 min; Inj.: 50 μL	Capillary voltage: 3.1 kV; cone voltage: 22 V; source temperature: 100 °C; desolvation temperature: 350 °C; *m/z* transitions (collision energy); AA: 72 > 55 (11 eV), 54 (20 eV), 27 (20 eV)	LC–ESI-MS/MS; LOQ: 45; WR: 0; recovery: 58	Riediker and Stadler [54]
Coffee	[$^{13}C_3$]Acrylamide	Synergi Hydro-RP 80 Ǻ column, 250 mm × 2 mm i.d., 4 μm (Phenomenex, Torrance, CA, USA)	Mobile phase: 0.5% methanol in water, 0.2 mL/min, run time: 10 min; Inj.: 20 μL	Capillary voltage: 4.1 kV; cone voltage: 20 V; source temperature: 120 °C; desolvation temperature: 250 °C; *m/z* transitions (collision energy); AA: 72 > 72 (5 eV), 55 (10 eV), 27 (19 eV); I.S.: 75 > 75 (5 eV), 58 (10 eV), 29 (19 eV)	LC–MS/MS; LOD: 10 μg/kg; WR: 8–3600 ng/mL	Andrzejewski et al. [56]
Commercial processed foods	[$^2H_3$]Acrylamide	Atlantis dC_{18} column, 150 mm × 2.1 mm i.d., 3 μm (Waters, Milford, MA, USA)	Mobile phase: methanol/water = 10/90, 0.1 mL/min, run time: 10.2 min; Inj.: 2 μL	Ion spray voltage: 5.2 kV; Turbo gas temperature: 450 °C; *m/z* transitions (collision energy); AA: 72 > 55 (18 eV); I.S.: 75 > 58 (18 eV)	LC–MS/MS; LOD: 0.2 ng/mL; LOQ: 0.8 ng/mL; WR: 20–1000 ng/mL	Ono et al. [58]
Toasted bread, fried chips, grilling and baking potatoes	[$^2H_3$]Acrylamide	Primesphere $5C_{18}$-HC 100A column, 250 mm × 3.2 mm i.d., 5 μm (Primesphere, Rue Jos Felten, Luxembourg)	Mobile phase: 0.1% acetic acid (in water)/0.1% acetic acid (in 7:3 water: acetonitrile) = 93/7, 0.5 mL/min; Inj.: 50 μL	AA: 72 > 55, 44, 27; I.S.: 75 > 58	LC–MS/MS; WR: 0.01–100 mg/L	Ahn et al. [68]
Chocolate powder, cocoa, coffee	[$^2H_3$]Acrylamide	Shodex RSpak DE-413L polymethacrylate gel column, 150 mm × 6 mm i.d. (Showa Denko)	Mobile phase: methanol/water/formic acid = 30/70/0.007, 0.6 mL/min, run time: 12 min; Inj.: 60 μL	According to Riediker and Stadler [53]	LC–MS/MS; CCα[c]: 9.2 mg/kg; CCβ[c]: 12.5 mg/kg; WR: 10–2500 μg/kg	Delatour et al. [77]
Various foods	[$^{13}C_3$]Acrylamide	Synergi Hydro-RP 80 Å column, 250 mm × 2 mm i.d., 4 μm (Phenomenex)	Mobile phase: 0.5% methanol/ 0.1% acetic acid in water, 0.2 mL/min, run time: 10 min; Inj.: 20 μL	The same as Andrzejewski et al. [55]	LC–ESI-MS/MS; LOD: 10 μg/kg; WR: 8–3600 ng/mL; recovery: 94.8 ± 5.6%	Roach et al. [78]
French fries	[$^2H_3$]Acrylamide	Synergi Hydro-RP column, 250 mm × 3 mm i.d., 4 μm (Phenomenex)	Mobile phase: water/methanol (both contain 0.1% formic acid) = 96/4, <0.5 mL/min, run time: 10 min; Inj.: 25 μL	AA: 72, 55; I.S.: not specified	LC–MS; WR1: 10; WR2: 100; recovery: 78	Peng et al. [82]
Potato chips, French fries	[$^{13}C_1$]Acrylamide	Mightysil RP-18 GP column, 100 mm × 2 mm i.d., 5 μm (Kanto, Portland, OR, USA)	Mobile phase: water/methanol = 80/20, 0.2 mL/min, run time: 25 min; Inj.: 20 μL	Capillary voltage: 3.5 kV; fragmentor voltage: 100 V; desolvation temperature: 350 °C; AA: 72; I.S.: 73	LC–ESI-MS; LOD: 1 ng/mL; LOQ: 5 ng/mL; WR: 5–1000 ng/mL; recovery: 93.0–102.2%	Inoue et al. [83]
Fried potato products, bread	[$^2H_3$]Acrylamide	Alltima C_{18} LC column, 150 mm × 3.2 mm i.d., 5 μm (Alltech Associates, Deerfield, IL, USA)	Mobile phase: acetonitrile/5 mM aqueous formic acid = 5/95, 0.3 mL/min, retention time: 4.5 min; Inj.: 5 μL	Capillary voltage: 2.5 kV; cone voltage: 31 V; source temperature: 70 °C; desolvation temperature: 400 °C; *m/z* transitions (collision energy); AA: 72.1 > 55.2, 44.4; I.S.: 75.1 > 58.2, 47.4	LC–MS/MS; LOQ: 30 μg/kg; WR: 50–2000 μg/kg; recovery: 96–112%	Konings et al. [88]
Potato chips, crisp bread, cereals, cookies	[$^2H_3$]Acrylamide	Merck LiChrospher 100 CN column, 250 mm × 4 mm i.d., 5 μm (Merck, Whitehouse station, NJ, USA)	Mobile phase: 50% acetonitrile in 1% acetic acid isocratic for 5 min, following rinsing with 100% acetonitrile for 5 min, 0.7 mL/min (Spilt 1:5), run time: 10 min; Inj.: 10 μL or 40 μL[d]	Electrospray voltage: 5.5 kV; source temperature: 350 °C; *m/z* transitions (collision energy); AA: 72 > 72, 55, 44 (18 eV); I.S.: 75 > 75, 58, 44 (18 eV)	LC–MS/MS; LOD: <10 μg/kg; LOQ: <30 μg/kg; WR: 5–1500 μg/kg; recovery: 80–110%	Hoenicke et al. [89]
Ground/instant coffee	[$^2H_3$]Acrylamide	Hypercarb HPLC column, 50 mm × 2.1 mm i.d., 5 μm (Thermo Hypersil-Keystone)	Mobile phase: 0.1% formic acid in water or 0.5% methanol in water, 0.2 mL/min; Inj.: 10 μL	Capillary voltage: 3 kV; cone voltage: 31 V; source temperature: 120 °C; desolvation temperature: 400 °C; *m/z* transitions (collision energy); AA: 72 > 55 (10 eV); I.S.: 75 > 58 (10 eV)	LC–MS/MS; LOD: 2 μg/L; WR: 2–30 μg/L; recovery: 84–117%	Granby and Fagt [90]
Crisp bread, potato chips, sweet cookies, butter cookies, rusk	[$^{13}C_3$]Acrylamide	Luna Phenyl-Hexyl HPLC column, 250 mm × 4.6 mm i.d., 5 μm (Phenomenex)	Mobile phase: acetonitrile/1 g/L acetic acid in water (gradient programmed from 30/70 to 60/40), 0.8 mL/min, run time: 15 min; Inj.: 20 μL	Spray needle voltage: 5 kV; Capillary voltage: 16 V; Capillary temperature: 200 °C; AA: 226; I.S.: 229	LC–MS; derivatization with 2-mercaptobenzoic acid; LOD: 6.6 μg/kg; LOQ: 19.6 μg/kg; recovery: 96–112%	Jezussek and Schieberle [100]
Heat processed foods	[$^2H_3$]Acrylamide	–	–	AA: 72 > 55; I.S.: 75 > 58	LC–MS/MS; LOD: 2 ng/mL; WR: 2–20,000 ng/mL	Tsutsumiuchi et al. [110]

[a] LOD, limit of detection; LOQ, limit of quantification; WR, working range of concentrations; Inj.: injection volume; AA, acrylamide; I.S., internal standard.
[b] PEEK, polyether ether ketone.
[c] CCα, decision limit; CCβ, detection capability (Both calculated according to [111–113]).
[d] 10 μL of Inj. for ethyl acetate extract, 40 μL of Inj. for aqueous extract.

subjected to a two-step extraction procedure with an ASE-200 instrument from Dionex (Sunnyvale, CA, USA). The sample was first degreased three times during 5 min with hexane (PLE experimental set-up: 40 °C, 100 bar static, 100% eluent). The residual hexane was flushed with nitrogen for 5 min and the sample was extracted three times 20 min (85/15% acetonitrile/water, 40 °C, 100 bar static, 150% eluent). The complete extraction program lasted ca. 1.5 h per sample. A portion (20 mL) of the obtained extract (ca. 45 mL) was concentrated to 1–2 mL in a rotative evaporator at 40 °C. The extract was taken in 15 mL water, basified by addition of 1 mL buffer solution (0.1 M trisodium phosphate in water) and shaken 1 min in an ultrasonic bath. The mixture was poured onto a 20 mL Chemelut cartridge (Varian) with the 5 mL of water used to rinse the flask. After a delay of 15 min, the extraction was carried on with 100 mL of ethyl acetate. The eluent was concentrated to a final volume of ca. 0.5 mL in a rotative evaporator and transferred into an autosampler vial. Turbid solutions were filtered additionally through a syringe nylon filter (Titan Nylon Promax, 0.45 μm, Scientific Resources, Eatontown, USA).

The samples were analyzed by LC–MS/MS (ESI+) using Hypercarb column (125 mm × 2.1 mm, 5 μm; Thermo Electron, San Jose, CA, USA). The HPLC parameters had the following settings: mobile phase + gradient, 100% A to 25% A in 10 min, recondition at 100% A for 5 min (A: 0.01 M formic acid in water); flow rate, 0.2 mL/min; injection, 10 μL; column temperature, 20 °C. The electrospray source had the following settings (with nitrogen): source temperature, 350 °C.

Acrylamide was identified by MRM mode. The precursor ion *m*/*z* 72 was fragmented, and product ion *m*/*z* 55 was monitored and used for quantification. Monitored product ion for the internal standard was *m*/*z* 58 from precursor ion *m*/*z* 75.

3.5.3. Scientific Institute of Public Health-Louis Pasteur [115]

The samples were minced, and 9.5 mL of water and 0.5 mL of the internal standard [$^2H_3$]acrylamide (1 μg/mL in water), were added to 1 g of homogenized sample. The mixture was shaken for 10 min on a rotating shaker in the darkness. The mixture was centrifuged at 10,000 rpm for 10 min at 5 °C. A 3 mL aliquot of supernatant was filtered on a 0.45 μm nylon membrane (Euroscientific, Lint). The clean-up procedure of samples in this research institute was similar to that in US FDA (see Section 3.5.1). Finally, the eluent was collected and filtered on a 0.45 μm nylon membrane.

The samples were analyzed by LC–MS/MS (ESI+) using μ-Bondapak RP18 column (300 mm × 3.9 mm, 10 μm; Waters). The HPLC parameters had the following settings: mobile phase, aqueous 0.1% acetic acid (isocratic); flow rate, 0.6 mL/min; injection, 100 μL; column temperature, room temperature. The electrospray source had the following settings (with nitrogen): cone voltage, 20 V; capillary voltage, 4 kV; source temperature, 120 °C; desolvation temperature, 300 °C; desolvation gas flow, 150 L/h; collision gas pressure, 3×10^{-3} mbar.

Acrylamide was identified by MRM mode. The precursor ion *m*/*z* 72 was fragmented, and product ions *m*/*z* 55, 44 and 27 were monitored and used for quantification. Monitored product ion for the internal standard was *m*/*z* 58 from precursor ion *m*/*z* 75.

3.6. Other analytical methods for the determination of acrylamide

3.6.1. High-performance anion-exchange chromatography analysis

Chromatographic analysis of precursors and products of the Maillard reaction can be substantially improved by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). This technique takes advantage of the affinity between the ionized group of sugars at alkaline pH and a pellicular quaternary amine stationary phase [116], thus offering excellent resolution, and also the highly selective and sensitive detection of nonderivatized Maillard reaction products at picomole levels with minimal sample clean-up. HPAEC should also offer good resolution for Amadori compounds. Indeed, phenylalanine, glucose, and the corresponding Amadori compound were analyzed by HPAEC using a CarboPac PA-1 column (Dionex) with a PAD to determine the sugar and an ultraviolet (UV) detector to monitor the amino acid and Amadori product [117]. However, this method was applicable only to aromatic amino acids and their Amadori compounds. Amino acids and Amadori compounds were determined quantitatively by PAD using a gradient of acetonitrile and phosphate buffer on an aminopropyl column [118]. Davidek et al. [119] simultaneously analyzed Maillard reaction precursors, products and acrylamide by HPAEC. Their research can be considered as an important transition from the determination of Maillard reaction products to the analysis of acrylamide.

3.6.2. HPLC and GC analysis

Besides HPLC or GC combined with MS technique, some researchers reported robust analytical methods for determination of acrylamide using simplex HPLC or GC technique, the advantages of which are acknowledged as easy generalization, lower cost and strong maneuverability compared to those combined with MS technique. Terada and Tamura [45] reported the analytic method of acrylamide using HPLC systematically. The HPLC system consisted of two pumps, two 6-port-2-position valves, two columns and a UV detector. At first, the sample solution was chromatographed on an ODS column with a mobile phase of water, then the flow of the mobile phase was switched using a 6-port-2-position valve, and the acrylamide peak fraction was introduced into an aqueous gel permeation column (analytical column). The fraction was chromatographed again on the analytical column with a mobile phase of water, and the eluate was monitored with a UV detector (205 nm). The recoveries of acrylamide from potato chips, fried potato, croquette and instant noodle fortified at levels of 50–1000 mg/kg were 93.1–101.5% and

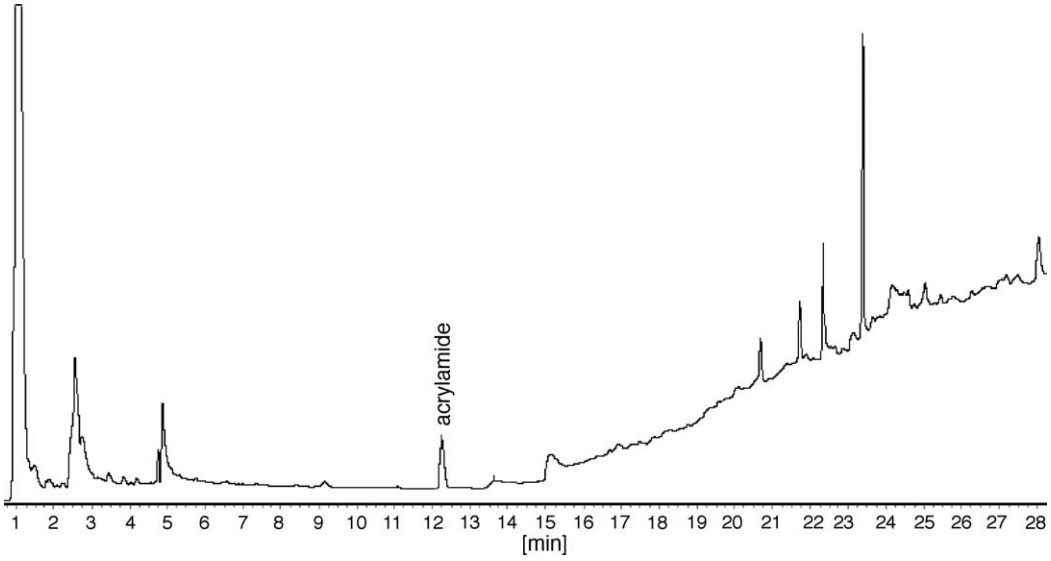

Fig. 8. GC with electron capture detection chromatogram of acrylamide in potato crisps.

the coefficient of variation was 1.5–5.2%. The limit of detection corresponded to 10 mg/kg in processed foods. On the other hand, GC analysis of acrylamide detected by hydrogen flame ionization detection (FID) [81] or electron-capture detection (ECD) [97] was also published. In our laboratory, the amount of acrylamide in potato crisps was analyzed by GC–ECD (Fig. 8). During the pre-treatment of sample matrices, acrylamide was extracted and purified without solid-phase extraction. The GC chromatogram (Fig. 8) shows that the interference of unknown peaks for the analytes could negligible because these impurity peaks did not overlay with the peak of brominated acrylamide analyzed.

3.6.3. Proton transfer reaction mass spectrometry (PTR-MS) analysis

Proton transfer reaction mass spectrometry has been considered as a suitable method for rapid and on-line measurements of volatile compounds of headspace samples [92,120]. It combines a soft, sensitive, and efficient mode of chemical ionization, with a quadrupole mass filter. The four key features of PTR-MS can be summarized as follows: (a) it is fast, and time dependent variations of headspace profiles can be monitored with a time resolution of about 0.1 s; (b) the volatiles are not subjected to work-up or thermal stress and little fragmentation is induced by the ionization step, hence, mass spectral profiles closely reflect genuine headspace distributions; (c) mass spectral intensities can be transformed into absolute headspace concentrations; (d) it is not invasive. All these features make PTR-MS particularly suited to investigate fast dynamic processes, such as formation of aroma and volatile contaminants in Maillard reactions [121].

The application of the PTR-MS approach for monitoring on-line the formation of acrylamide was evaluated in real food systems using thermally treated potatoes as an example [122]. The mass trace at *m*/*z* 72 indicated the presence of acrylamide in the headspace obtained by heating potato at 170 °C. The mass at *m*/*z* 72 was found to be homogeneous, without interference with other volatile compounds, using an off-line coupling method. Retention index and EI mass spectrum were identical with those of the acrylamide reference compound, and only one peak with the mass at *m*/*z* 72 was detected by PTR-MS. The EI spectrum of the compound eluting 49.6 m was conclusively identified by the Wiley EI database as acrylamide.

4. Conclusion

During the past years since acrylamide first found in heat-treated foods in early 2002, many published papers and reviews [16,22,25,49,122,123–126] reported chromatographic techniques applied in the occurrence and analytical methods of acrylamide in heat-treated foods. During these years, the analytic methods of acrylamide have also been applied in the field of bioassay [127–129]. Some other analytic methods including chromatographic techniques, such as microemulsion electrokinetic chromatography (MEEKC) [130] and capillary gas–liquid chromatography (GLC) with alkali flame-ionization detection [50], and non-chromatographic techniques, such as determination using respiratory activity of microbial cells [131–133], are also developed. However, a challenge still remaining today is the development of stable, reliable and robust methods for difficult food matrices, such as cocoa powders, coffee, and high salt flavorings. Another challenge is the demonstration of methods according to inter-laboratory proficiency test in order to eliminate subjective preconception of experimenters and objective factors of experiment conditions. Moreover, additional concern may as well focus on the cross-sectional sampling, which should

be statistically representative for a product and, therefore, demands adequate procedures to be put in place.

As for the recognition of methods, the mainly two methods of analysis (LC–MS/MS or GC–MS) are used by laboratories all over the world, and based on the early indications of proficiency tests, it is difficult to say that one is more reliable than the other. GC–MS after bromination is the best approach because this method is a relative mature coupled technique. This approach has the advantage of adequate sensitivity with multiple ion confirmation. Application of GC–MS/MS or coupling to a high resolution MS would even further lower the detection limit of certain foods, approaching the range of 1–2 μg/kg. On the other hand, determination of acrylamide using LC–MS/MS can avoid derivatization and have rather high sensitivity and stability but a clean-up procedure especially the SPE technique may as well be reasonably designed and carefully operated.

To date, there is little evidence that the applied methods have been systematically validated according to international guidelines, although there have been reports of in-house validation. It is also quite obvious that there are significant differences between laboratories in the clean-up procedures applied for both LC and GC methods.

The key requirements in the analytical field are the establishment of exigent in-house quality control procedures for all relevant matrices tested. This can be done by inclusion of a standard sample or, if available, reference sample in each analytical series, setting acceptable ranges by using statistical control charts. Furthermore, interlaboratory proficiency tests have not been conducted on samples with relatively "low" concentrations of acrylamide. Method reproducibility in the 10–30 μg/kg range has not yet been assessed and should be considered a priority, especially for those foods containing amounts that are a significant part of the staple diet.

Finally, research should also be focused on cheap, convenient and rapid screening methods that are reliable and robust. These could then be employed in a quality control environment closer to production in a manufacturing facility or factory environment, for instance, in an on-line laboratory or enzyme linked immunosorbent assay (ELISA), allowing more efficient control and enabling more rapid response if needed.

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