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REVIEW

Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food

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Abstract Heat-induced food contaminants have attracted attention of both the scientific community and the public in recent years. The presence of substances considered possibly or probably carcinogenic to humans has triggered an extensive debate on the healthiness of even staple foods. In that respect, acrylamide, furan and chloropropanols are the main substances of concern. Their widespread occurrence in processed food, which concomitantly causes considerable exposure to humans, led either to the setting of maximum limits (for some chloropropanols) or at least the initiation of monitoring programmes in order to put risk assessment on a solid data basis. Acrylamide, furan and chloropropanols are small molecules with physicochemical properties that make their analysis challenging. Their amount in food ranges typically from below the limit of detection to hundreds of micrograms per kilo or even milligrams per kilo. However, a number of recently published scientific reports deal with the analysis of these substances in different kinds of food. The aim of this publication is to give an overview of analytical approaches for the determination of acrylamide, furan and chloropropanols in foodstuffs.

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Introduction

Heat-induced food contaminants, in particular three substances or groups of substances, have gained widespread attention recently: these are acrylamide, furan and chloropropanols, with 3-monochloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropan-2-ol (1,3-DCP) as the most prominent representatives. The occurrence of chloropropanols and furan in food has been known since the late 1970s, whereas acrylamide was detected in food only a few years ago [1, 2]. The reason why the former substances have attracted renewed attention is that they were recently detected in food consumed in high quantities, such as bread, and in food dedicated to the most vulnerable group of consumers—baby food [3].

The precursors and generation mechanisms of the aforementioned contaminants are different, but they are all formed during processing of food. Extraneous sources seem to have no or only little importance for the content of these substances in food, which triggered questions concerning the appropriateness of the term "contaminant" for these substances [4]. They could also be regarded as an intrinsic and hardly avoidable consequence of food processing [4].

Another common characteristic of them is that they are considered as probably or potentially carcinogenic to humans. The International Agency for Research on Cancer (IARC) classified acrylamide as probably carcinogenic to humans (group 2A), whereas furan was classified as possibly carcinogenic to humans (group 2B) [5, 6]. IARC has not dealt with chloropropanols yet. However, the European Commission's Scientific Committee on Food concluded that there is sufficient evidence for carcinoge-

nicity of 3-MCPD and classified it as an undesirable contaminant in food [7].

As a response to the occurrence of these substances in food, European risk managers recently set a maximum limit for the 3-MCPD content of some foods and initiated multi-annual monitoring campaigns for acrylamide and furan in order to evaluate potential mitigation [8].

This topical paper aims to review analytical methods for the determination of acrylamide, furan and chloropropanols in food. Due to the different nature of these substances and special provisions on analytical methods, this review is divided into three subsections containing general information as well as analytical details for the respective analyte. The main characteristics of the methods of analysis are also presented in tabular form. However, it has to be noted that the level of detail of discussion of a particular method neither indicates any preferences of the authors nor its relevance for the particular field of analysis.

Acrylamide

Since its discovery in food, acrylamide has nearly become a synonym for heat-induced food contaminants. This is just the consequence of the great attention the public has paid to this substance, which can be explained by the essentially unavoidable exposure of the individual to it.

The average intake via food has been estimated by the Joint FAO/WHO Experts Committee on Food Contaminants (JECFA) [9] for the general population as well as by different national organizations for the inhabitants of their countries. A daily intake of 1 μg kg^{-1} body weight was estimated by JECFA for the average consumer, which could rise to 4 μg kg^{-1} body weight for consumers of specific food items. Intake estimations at national level could deviate from these values, which could be a consequence of different eating habits but also of different composition of the addressed population or differences of methods applied to modelling [10–12]. A detailed review on the dietary intake of acrylamide as well as on the association of intake levels with biomarkers and internal dose has been published by Dybing et al. [13].

IARC classified acrylamide as probably carcinogenic to humans (group 2A) as early as 1994 [5]. However due to its discovery in food, several studies on toxicity and carcinogenicity of acrylamide were conducted and recently reviewed [14–16].

The main precursor identified so far is asparagine, which yields acrylamide by reacting with reducing sugars under low moisture conditions [17]. Other precursors were identified as well. Granvogl et al. [18, 19] identified 3-aminopropionamide (3-APA) as a potent precursor of acrylamide. 3-APA is a biogenic amine, which also seems to be formed during Maillard reactions [19, 20]. The influence of oil degradation products on the acrylamide content of food was studied too [21]. It was suggested that acrolein stemming from the degradation of edible oils might be oxidized to acrylic acid, which could react with ammonia to form acrylamide. However, the contribution of this formation pathway to the overall acrylamide content of fried food has not been completely clarified yet. Findings of Gertz and Klostermann [22] and Mestdagh et al. [21] are contradictory in that respect.

Processing of carbohydrate-rich food such as baking, frying or roasting may lead to acrylamide contents in the milligram per kilo range [23]. Data on the acrylamide content of various foodstuffs were collected in international databases [24]. An updated overview of the EU monitoring database on acrylamide levels in food was published by Wenzl and Anklam [25].

A large number of publications dealing with the analysis of acrylamide in food have been published since 2002, among them three review articles on analytical methods for the determination of acrylamide in food, which seems to be more than sufficient for such a short time period [26–28]. However, a review on analytical methods for the determination of heat-induced contaminants in food must not omit acrylamide due to the importance this substance has gained. Nevertheless the authors do not want to repeat previously published information. Therefore, this review will only devote a short section to the mainstream analytical procedures, but will present new approaches in sample extraction, sample cleanup and measurement that have not been reviewed yet. The main points of the analysis methods are outlined in Table 1.

Mainstream analytical methods

There is no doubt that most laboratories working in the field of acrylamide analysis in food apply one of the following three, briefly outlined methods. These are based on liquid chromatography–tandem mass spectroscopy (LC-MS/MS) or gas chromatography–mass spectroscopy (GC-MS) either with or without derivatization of acrylamide.

LC-MS/MS

The methods in this category are in principle based on a method that was published by Rosen and Hellenäs [29]. It consists of an aqueous extraction of acrylamide from the food matrix followed by cleanup employing single or multi-stage solid-phase extraction (SPE). Polymer-based sorption materials that show both reversed-phase and ion exchange properties are frequently applied. A defatting step or protein precipitation could be integral parts of the sample preparation. Finally, chromatographic separation is performed by

Table 1 Methods for the determination of acrylamide in food

Matrix	Internal standard (ISTD)	Extraction/pre-treatment	Cleanup	Derivatization	Detection	Column	LOD/LOQ	Ref.
Crispbread potato crisps	[$^2H_3$]acrylamide	Water, RT	SPE: Isolute Multimode (MM) 300 mg		LC-MS/MS, SRM	Hypercarb 50 mm× 2.1 mm, 5 μm	LOD 10–30 μg kg^{-1}	[29]
Bakery and potato products	[$^2H_3$]acrylamide	Water	SPE: (1) Isolute MM 500 mg; (2) ENV+ 500 mg		LC-MS/MS, SRM	Hypercarb, 50 mm× 2.1 mm, 5 μm	LOQ <20 μg kg^{-1}	[30]
Various foodstuff	Methacrylamide + [$^2H_3$]acrylamide butyramide	Water and 1-propanol	Solvent exchange to acetonitrile, extraction with *n*-hexane		GC-PCI-MS, SIM	Carbowax type, 10 m, 0.25-mm i.d., 0.4-μm film thickness	LOD 10–20 μg kg^{-1}	[31]
Tomatoes	Methacrylamide 2,3-dibromo-*N*,*N*-dimethylpropionamide	Water, RT	Ethyl acetate extraction, fractionation on silica columns	Bromination with bromine water, reaction overnight at 0 °C	GC-MS, SIM	DB 17, 30 m×0.25-mm i.d., 0.2-μm film thickness)	LOD 1 μg kg^{-1}, recovery 26–62%	[34]
Mushrooms	2,3-Dibromo-*N*,*N*-dimethylpropionamide	Water, RT	Ethyl acetate extraction, fractionation on silica columns	Bromination with bromine water, reaction overnight at 0 °C	GC-MS, SIM	DB 17, 30 m×0.25-mm i.d., 0.2-μm film thickness	LOD 1 μg kg^{-1}	[35]
Various foodstuff	[$^{13}C_1$]acrylamide	Defatting, water, RT	Ethyl acetate extraction, fractionation on Florisil cartridges	Bromination with KBr and $KBrO_3$, 90 min, refrigerated	GC-MS, SIM	DB-WAX 30 m× 0.25-mm i.d., 0.25-μm film thickness	LOD 9 μg kg^{-1}, LOQ 30 μg kg^{-1}	[36]
Coffee, crispbread, potato crisps, milk chocolate	[$^2H_3$]acrylamide [$^{13}C_1$] acrylamide	Different extraction methods	SPE: (1) Isolute MM 500 mg; (2) ENV+ 500 mg		LC-MS/MS, SRM	Hypercarb, 50 mm× 2.1 mm, 5-μm		[38]
Various food	[$^{13}C_3$]acrylamide	Alkaline extraction	SPE: (1) OASIS HLB 200 mg, (2) Isolute MM		LC-MS/MS, SRM	Hypercarb, 50 mm× 2.1 mm, 5 μm		[39]
Potato chips, various food	[$^{13}C_3$]acrylamide	Methanol	Carrez clarification, SPE: OASIS HLB 30 mg		LC-MS, SIM, APCI	Inertsil ODS-3, 250 mm×4.6 mm, 5 μm	LOD 2 μg kg^{-1}, LOQ 6 μg kg^{-1}	[43, 58]
Various food	[$^2H_3$]acrylamide	Water, *n*-hexane, acetonitrile	Phase separation, dispersive SPE with primary secondary amine (PSA)		LC-MS/MS	Aqua C18, 150 mm× 3 mm, 5 μm	LOQ <10 μg kg^{-1}	[44]
Various food	[$^2H_3$]acrylamide	Water, *n*-hexane, acetonitrile	Phase separation, dispersive SPE with primary secondary amine (PSA)		GC ion-trap MS, CI, DSI, SIM	Stabilwax DB, 20 m× 0.32-mm i.d., 1-μm film thickness	LOQ <25 μg kg^{-1}	[44]
Cereal-based food	[$^{13}C_3$]acrylamide	PLE extraction with acetonitrile, 35 °C	Carrez clarification, solvent exchange to water		LC-MS/MS, SRM	Hypercarb, 50 mm× 2.1 mm, 5 μm	LOQ 5 μg kg^{-1}	[45]
Baby food	[$^{13}C_3$]acrylamide	Defatting, NaCl solution, RT	L/L extraction with ethyl acetate, SPE: OASIS HLB 200 mg		LC-MS/MS, SRM	Atlantis dC_{18}, 250 mm× 4.6 mm, 5 μm	LOQ <5 μg kg^{-1}	[47]
Potato crisp, potato chips		Water, RT, CW/DVB SPME fibre			SPME-GC-PCI-MS/MS	DB-WAX 30 m× 0.25-mm i.d., 0.25-μm film thickness		[48]

Table 1 (continued)

Matrix	Internal standard (ISTD)	Extraction/pre-treatment	Cleanup	Derivatization	Detection	Column	LOD/LOQ	Ref.
Various food	[^{2}H]acrylamide	Defatting, water, 60 °C	Acetonitrile, Carrez clarification, ethyl acetate extraction		GC-MS/MS	DB-Wax, 30 m×0.25-mm i.d., 0.25-μm film thickness	LOQ 5 μg kg^{-1}	[49]
Various food	[$^2H_3$]acrylamide	*n*-Propanol	Solvent exchange to acetonitrile, dispersive SPE with primary secondary amine		GC-HRTOF-MS, SIM, mass resolution >7,000	INNOWax, 30 m× 0.25-mm i.d., 0.25-μm film thickness	LOQ 15–40 μg kg^{-1}	[50]
Cereal-based food	[$^{13}C_3$]acrylamide	Defatting, water	L/L extraction with ethyl acetate, SPE: OASIS HLB 200 mg		LC-MS/MS, SRM	Atlantis dC$_{18}$, 250 mm×4.6 mm, 5 μm	LOQ <5 μg kg^{-1}	[51]
Fried food		Defatting, NaCl solution, RT	L/L extraction with ethyl acetate	Bromination with KBr and $KBrO_3$, 30 min, refrigerated	GC-ECD	INNOWax, 30 m× 0.32-mm i.d., 0.25-μm film thickness	LOD 0.1 μg kg^{-1}, LOQ 3 μg kg^{-1}	[52]
Various food	[$^2H_3$]acrylamide	Water, RT	SPE: (1) Strata-X-C 200 mg; (2) ENV+ 200 mg		LC-ion-trap MS/MS, APCI	ODS-80-TS, 150 mm×2.1 mm, 5 μm	LOD 45 μg kg^{-1}	[53]
Various food	[$^{13}C_3$]acrylamide	Water, RT	Carrez clarification		LC-MS, SIM, APCI	Inertsil ODS-3, 250 mm×4.6 mm, 5 μm	LOD 6–10 μg kg^{-1}, LOQ 15–20 μg kg^{-1}	[54]
Potato chips	[$^{13}C_3$]acrylamide	NaCl solution, 60 °C	Carrez clarification, SPE: OASIS HLB + MCX (200 mg + 60 mg) or Isolute MM, 500 mg		LC-MS, SIM	Extrasyl ODS1, 200 mm×3.0 mm, 5 μm	LOQ 70 μg kg^{-1}	[55]
Various foodstuff	[$^{13}C_3$]acrylamide	Defatting, water, RT	SPE: Bond Elut Accucat 200 mg		LC-MS, SIM	Synergi polar-RP 80A, 150 mm×4.6 mm, 4 μm	LOQ 30 μg kg^{-1}	[56]
Potato chips		Methanol	Carrez clarification, SPE: OASIS HLB 30 mg		LC-DAD, 226 nm	Atlantis dC$_{18}$, 250 mm× 4.6 mm, 5 μm	LOQ 4.0 μg kg^{-1}	[58]
Coffee, cocoa	Methacrylamide	Water, 70 °C	Defatting	(Hydrolysis to acrylic acid/methacrylic acid)	NP, LC-UV, 200 nm	Aminex HPX 87H, 300 mm×7.8 mm	LOD 15 μg L^{-1}, LOQ 45 μg L^{-1}	[59]
Coffee, potato chips		Water, 60 °C	Carrez clarification,SPE on ion exchanger sorbent		LC-ECD	Synergi Hydro-RP, 250 mm, 4 μm		[60]
Potato chips, breakfast cereals, biscuits		Water, RT	Defatting, SPE: (1) Strata-X-C 200 mg; (2) ENV+ 200 mg	Alkaline 2-mercaptobenzoic acid	CZE, 210 nm	Uncoated fused silica capillary, 57 cm× 75-μm i.d.		[61]
Various food		Water, RT	Defatting, SPE: (1) Strata-X-C 200 mg; (2) ENV+ 200 mg	Alkaline 2-mercaptobenzoic acid, L/L extraction	FASI-CZE, 210 nm	Uncoated fused silica capillary, 57 cm× 75-μm i.d.		[62]
Potato chips	Methacrylamide	Methanol, RT	Solvent exchange to water, defatting		MEKC, 198 nm	Fused silica capillary, 76 cm×75-μm i.d.		[63]
Coffee		Water, boiling	SPE: Strata-X-C 200 mg		LC-MS, SIM	Synergi polar-RP 80A, 150 mm×4.6 mm, 4 μm	LOQ 120 μg kg^{-1}	[64]
Various food		0.2 M NaOH, 20–50 °C	Biosensor Kit: 2 enzymatic + 2 SPE sample preparation steps		Spectrophotometry			[64]

Potato crisps	Water, 60 °C	Defatting, Carrez clarification	Voltammetry	LOD 1.2×10^{-10} M [65]
Data on food samples not presented			QMB sensors	[66]

Abbreviations: ISTD internal standard, *GC-ECD* gas chromatography with electron capture detection, *GC-MS* gas chromatography–mass spectrometry, *CZE* capillary zone electrophoresis, *GC-MS/MS* gas chromatography–tandem mass spectrometry, *GC-HRMS* gas chromatography–high-resolution mass spectrometry, *LOQ* limit of quantification, *QMB* quartz microbalance, *GC-HRTOF-MS* gas chromatography–high-resolution time-of-flight-mass spectrometry, *LC-MS* liquid chromatography–mass spectrometry, *LC-MS/MS* liquid chromatography–tandem mass spectrometry, *LC-DAD* liquid chromatography with diode array detection, *LC-ECD* liquid chromatography with electrochemical detection, *LOD* limit of detection, *MEKC* micellar electrokinetic capillary chromatography, *FASI* field amplified sample injection, *SRM* selected reaction monitoring, *SIM* selected ion monitoring, *APCI* atmospheric pressure chemical ionization, *PCI* positive chemical ionization, *L/L* liquid/liquid, *NP* normal phase, *SPME* solid-phase microextraction, *SPE* solid-phase extraction, *DSI* direct sample introduction, *RT* room temperature, *PLE* pressurized liquid extraction

high-performance liquid chromatography (HPLC) on either carbon black, hydrophilic reversed phase, or ion exchange columns. Tandem mass spectroscopy operated in selected reaction monitoring mode is applied to analyte detection. Quantification is done by internal standardization with isotopically labelled acrylamide. The different variants of the methods in terms of food matrix to extraction solvent ratio, extraction temperatures, sizes and types of applied SPE cartridges as well as details on HPLC columns and operating conditions of the LC-MS/MS instruments were previously reviewed in detail [26–28]. The reproducibility of such an analysis method was recently evaluated by a collaborative trial for bakery and potato products [30].

GC-MS without derivatization

Biedermann and Grob [31] developed a GC-MS analysis method for the determination of native acrylamide in food. Several laboratories apply this or variants of this method [32]. The sample preparation is faster compared to the method including derivatization of acrylamide, and avoids handling of corrosive substances. It consists of analyte extraction employing organic solvents such as alcohols or ketones followed by sample cleanup by liquid/liquid extraction with or without using a sorbent. However, special attention has to be paid to the completeness of analyte extraction, which could require swelling of the matrix, and to the effectiveness of sample cleanup in order to avoid artefact formation during GC-MS analysis, which could occur in the injection port of the GC if acrylamide precursors are contained in the sample extract [33]. Polar columns of the Carbowax type are mainly applied to chromatographic separation and chemical ionization mass spectrometry in selected ion monitoring mode for analyte detection. Isotope-labelled acrylamide and/or methacrylamide is most often applied as internal standard. Details on methods for the determination of acrylamide by GC-MS without derivatization can be found elsewhere [27].

GC-MS with derivatization

Methods employing derivatization of acrylamide by bromination date back to the early 1990s [34, 35]. They consist of aqueous extraction of acrylamide from the matrix followed by derivatization of acrylamide to 2,3-dibromopropionamide. This could be done with an aqueous solution of elemental bromine or by using less hazardous potassium bromate [36]. The derivative is extracted into ethyl acetate, which could be directly injected into the GC-MS or further cleaned up to gain lower limits of detection. The advantage of this methodology is that the derivative is less polar than native acrylamide, which favours GC-MS analysis, enhances analyte extraction and analyte detection. Deliberate

dehydrobromination by adding triethylamine is applied in a variant of that method to avoid uncontrolled partial dehydrobromination in the injection port of the GC. GC-MS methods including derivatization of acrylamide were exhaustively reviewed by Castle and Eriksson [27].

Alternative approaches

Extraction and cleanup

Aqueous extraction is mostly applied to the extraction of acrylamide from different food matrices. However, extraction parameters such as temperature, time and sample/solvent ratio, the application of mechanical forces (e.g. stirring, shaking etc.) to support extraction as well as the particle size of the extracted food samples vary very much from method to method [26–28]. This is clearly demonstrated in proficiency test reports that contain brief summaries of the applied procedures [37]. Petersson et al. [38] systematically investigated the influences of extraction temperature, extraction time, extraction solvent composition, particle size of the food samples, defatting of the food matrix, and Ultra Turrax homogenization on the extraction yield of acrylamide from different foodstuffs (crispbread, potato crisps, coffee and milk chocolate). They concluded that plain water is the most suitable extraction solvent for their subsequent LC-MS/MS analysis procedure and that admixing of organic solvents did not show significant effects or even decreased extraction efficiency. The particle size of the samples had significant influence on the extraction. The authors recommend grinding of samples to particle sizes below 1,000 μm. Ultra Turrax homogenization and defatting did not have statistical significant influences on analyte extraction. Concerning extraction temperature and time, 25 °C and 30 min were found to be appropriate for a broad range of food. The authors confirmed the suitability of the optimized extraction parameters by analysis of different proficiency test samples.

Eriksson and Karlsson [39] investigated the influence of pH and digestive enzymes, such as amylases, on the extraction of acrylamide from food. While digestive enzymes did not show statistical significant influence on the amount of extracted acrylamide, extraction yield was drastically increased at high pH values. The authors postulated that the extractability of acrylamide changes under alkaline pH conditions due to alterations of the matrix. Goldmann et al. [20] followed up these findings and investigated the correlation of pH, extractability and formation of acrylamide in model systems and food. They concluded that the elevated acrylamide levels found at high pH conditions were a consequence of formation of acrylamide in the extract from water-soluble precursors and that extractability of native acrylamide was not changed by the pH.

As mentioned before, water is the dominant extraction solvent for the extraction of acrylamide in food; the extraction with organic solvents is less common. This was explained in several studies by potential artefact formation especially during extraction with methanol at elevated temperatures [40–42]. However, Gökmen and Şenyuva [43] presented a "generic method for the determination of acrylamide in thermally processed foods", which consists of extraction of the dried sample with methanol (at room temperature), followed by protein precipitation, removal of methanol, reconstitution in water and cleanup of the aqueous extract by solid-phase extraction (SPE) on OASIS HLB® cartridges prior to injection into the LC-MS system. The extraction method proposed by Mastovska and Lehotay [44] is analogous to the QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure developed for the extraction and cleanup of pesticides from plant material and consisting of defatting of the matrix and aqueous extraction with consecutive liquid/liquid partition of acrylamide into acetonitrile in a "one-pot" sequence. At first the sample is dispersed in *n*-hexane. Afterwards water and acetonitrile are added and acrylamide is extracted into the aqueous phase. Phase separation and thereby liquid/liquid partition is achieved by addition of magnesium sulfate and sodium chloride. Further sample treatment consists of pipetting an aliquot of the acetonitrile phase into a vial containing anhydrous potassium sulfate and primary secondary amine (PSA). Measurement of the acrylamide content was performed by LC-MS/MS as well as by GC-MS. The method performance was checked with different proficiency test materials and satisfactory agreement with the accepted values was stated. Acetonitrile was also employed for the extraction of cereal samples by pressurized liquid extraction (PLE) [45]. The authors tested other organic solvents (acetone, methanol and ethyl acetate) too. Using acetonitrile, the best sensitivity and the least matrix effects for a number of food matrices were obtained. Further sample cleanup consisted of Carrez clarification and solvent evaporation. Whereas methods applying aqueous extraction suffer frequently from low analyte concentration in the extract, due to a sample/extractant ratio of typically 1 g sample to 10 mL of water, PLE applying acetonitrile allowed analyte enrichment. The results obtained with the PLE method agreed well with results produced by an alternative method that was based on aqueous extraction and dual stage SPE [46]. Analyte enrichment on a hydroxylated polystyrene-divinylbenzene phase was described as well. A volume of 10 mL pre-cleaned extract was loaded on 1 g sorbent and after a rinsing step eluted with 2 mL of 60% methanol in water. The analyte concentration in the extract was further increased by evaporation of methanol. This analysis method was validated by collaborative trial [30]. Reproducibility rela-

tive standard deviations of less then 15% were achieved for bakery products and potato chips (French fries). Jiao et al. developed an LC-MS/MS analysis procedure for the determination of acrylamide in infant and baby food [47], which comprises extraction of the sample with sodium chloride solution followed by liquid/liquid extraction into ethyl acetate, solvent evaporation, and reconstitution of the residue in water. Solid-phase extraction on OASIS HLB® cartridges constitutes the final sample preparation step. The authors presented data showing high precision at acrylamide contents below 10 μg kg^{-1}.

Measurements based on gas chromatography

A novel method, applying solid-phase microextraction (SPME) and gas chromatography with positive chemical ionization tandem mass spectrometry (GC-PCI-MS/MS) was recently presented by Lee et al. [48]. A Carbowax/divinylbenzene-coated SPME fibre was immersed into the buffered, aqueous sample for 20 min and thereafter inserted into the hot injector. Chromatographic separation was performed on a capillary column of the Carbowax type. Acetonitrile was chosen as reagent gas. The most abundant ion was single protonated acrylamide. The limit of detection of the optimized SPME-GC-PCI-MS/MS method for aqueous acrylamide standard solutions was 0.1 μg L^{-1}, i.e. five times lower compared to splitless injection of a sample solution. Beside standard solutions, the method was also applied to the analysis of potato chips and potato crisps samples, but information on method performance characteristics for the analysis of food samples has not been reported [48]. The same instrumentation was applied to the analysis of potato chips, corn-based snacks and other food samples [44]. In contrast to Lee et al. who performed similar experiments [48], Mastovska and Lehotay did not find any improvement in signal-to-noise ratio when changing from single stage mass spectroscopy with methanol as chemical ionization agent to ion-trap tandem mass spectrometry [44]. Novelties in acrylamide analysis were the application of direct sample introduction (DSI), where a vial filled with sample is inserted into the large-volume injector of a low-pressure gas chromatograph [44]. Tandem mass spectroscopy on a triple quadrupole GC-MS/MS was presented by Hoenicke et al. [49]. They applied this technique to achieve low limits of quantification, as is required for baby food. An electron ionization high-resolution time-of-flight mass spectrometric method for the determination of native acrylamide was developed by Dunovská et al. [50]. The mass resolution was set to at least 7,000. Applying this method of analysis, the laboratory performed well in four proficiency tests.

Recent developments in gas chromatographic determination of acrylamide not only focussed on complex mass spectrometric detection methods, but also on the application of a much simpler approach based on electron capture detection (ECD) [51, 52]. Derivatization of acrylamide to 2,3-dibromopropionamide followed by dehydrobromination to 2-bromopropenamide was a prerequisite for analysis by ECD. Results obtained with the ECD method were in good agreement with results produced by GC-MS and LC-MS/MS [51, 52]. However, mass spectrometry should be preferred to ECD owing to its higher identification power.

Measurements based on liquid chromatography

The analysis of food extracts by liquid chromatography ion-trap tandem mass spectrometry was compared to LC-MS/MS applying a triple quadrupole mass spectrometer [53]. Atmospheric pressure chemical ionization (APCI) was applied with both techniques. The analysis results agreed well. However, it has to be noted that the limit of quantification for ion-trap mass spectrometry was about ten times higher than for the triple quadrupole measurements. APCI was also applied to the determination of acrylamide by single quadrupole LC-MS [54]. The reported method performance parameters were similar to those reported frequently for electrospray ionization (ESI) LC-MS/MS [26–28]. Single quadrupole LC-MS for the determination of acrylamide in food was also applied by other authors [55, 56]. Rufián-Henares and Morales published the determination of acrylamide in potato chips by ESI-LC-MS [55]. The limit of quantification of their analysis method was three to four times higher than the one determined by the former authors. It is not clear if this was a consequence of sample preparation, ionization technique, or a combination of both. Murkovic described the analysis of acrylamide in Austrian food by single stage LC-MS [56]. The method was applied to a variety of different food commodities. Quantification was reported to be possible above an acrylamide content of 30 μg kg^{-1}. However, the author did not indicate if this value was valid for all or only a part of the investigated food items.

When setting method performance specification, it is common practice in official food control to refer to Commission Decision 2002/657/EC, which sets provisions on the performance of analytical methods and interpretation of results for the determination of certain substances and residues in live animals and animal products [57]. According to this Decision, a number of characteristic fragment ions are required for confirmatory methods for single stage mass spectrometry (respectively precursor/daughter ion transitions in case of tandem mass spectrometry). The determination of acrylamide by liquid chromatography with single stage mass spectrometry does not comply with this requirement due to the lack of a sufficient number of fragment ions. Despite the fact that most acrylamide analyses fall outside the scope of the Decision, it should be regarded as a valuable guidance to

obtaining reliable results. This is especially important when it comes to official food control.

A different approach for the determination of acrylamide in food was chosen by Gökmen et al. [58], and Paleologos and Kontominas [59]. Both groups of researchers developed liquid chromatographic analysis methods with UV detection for that purpose. Whereas the former authors [58] applied reversed-phase chromatography for the analysis of potato products, ion-moderated partition chromatography was performed by the latter [59]. After hydrolysis of acrylamide and methacrylamide, acrylic acid and methacrylic acid, respectively, were separated from co-extractives on a column intended for the analysis of organic acids [59]. Detection of acrylamide was done at 200 nm, where acrylamide shows maximum absorption [59]. Gökmen et al. [58], however, selected 226 nm for analyte detection due to less interference compared to 200 nm.

Liquid chromatography with electrochemical detection was applied to the determination of acrylamide in coffee and fried potato products by an Italian group [60]. A drawback of this method is the complexity of the chromatograms, which complicates unambiguous peak identification.

Measurements based on capillary zone electrophoresis

Bermundo et al. [61, 62] published two articles on the determination of acrylamide in a variety of food items by capillary zone electrophoresis (CZE). The sample preparation for the CZE analysis includes two solid-phase extraction steps and final derivatization with 2-mercaptobenzoic acid amongst several other steps. A limit of detection of 3 μg kg^{-1} was achieved for crispbread samples by applying an additional liquid/liquid extraction step and in-line preconcentration by field amplified sample injection. Micellar electrokinetic capillary chromatography of acrylamide was described by Zhou et al. [63]. Sample preparation of that analysis method is much simpler compared to the two former methods. It encompasses methanolic extraction, solvent exchange, and defatting with *n*-hexane. Methacrylamide served as internal standard. Method characteristics were evaluated and the applicability of the method for the analysis of potato chip samples was demonstrated.

Sensor techniques

Sensor techniques for the determination of acrylamide in food have been developed recently. Sagratini et al. reported on the validation of a biosensor kit, which is based on the enzymatic hydrolysis of the amide group of acrylamide and spectrophotometric detection of the ammonium ions produced [64]. The described sample preparation procedure is quite simple. Nevertheless another enzymatic and two solid-phase extraction steps (consumables included in the kit) are required before hydrolysis of acrylamide can be performed, which is about equal to the amount of work needed to prepare samples for LC-MS/MS or GC-MS analysis. The limit of quantitation of that method was estimated to be 25 μg kg^{-1}. However, it should be noted that the detection method is not specific for acrylamide. Hence special attention needs to be paid to the quantitative elimination of interferents. A voltametric biosensor was presented by Polish researchers, which is based on the formation of acrylamide–haemoglobin adducts [65]. A haemoglobin-coated carbon paste electrode served as electrochemical sensor. Acrylamide was detected indirectly by monitoring the reduction of haemoglobin–Fe(III), which was altered by the acrylamide–haemoglobin adduct. Figure 1 shows the response curves of acrylamide at different concentrations in an aqueous extract of potato crisps. The limit of detection was evaluated to be 1.2×10^{-10} M.

A different approach for the determination of acrylamide makes use of an electronic nose, applying quartz microbalance (QMB) sensors coated with several tetralactame macrocycles of the Hunter–Vögtle type [66]. Binding of acrylamide to the macrocycles results in a changed oscillation frequency of the QMBs. However experiments were performed with pure acrylamide. The applicability of the technique to food samples has been mentioned, but data have not been presented yet.

Chloropropanols

Like acrylamide, chloropropanols are food-borne contaminants that can be formed during the processing of different foodstuffs. This class of food contaminants was first

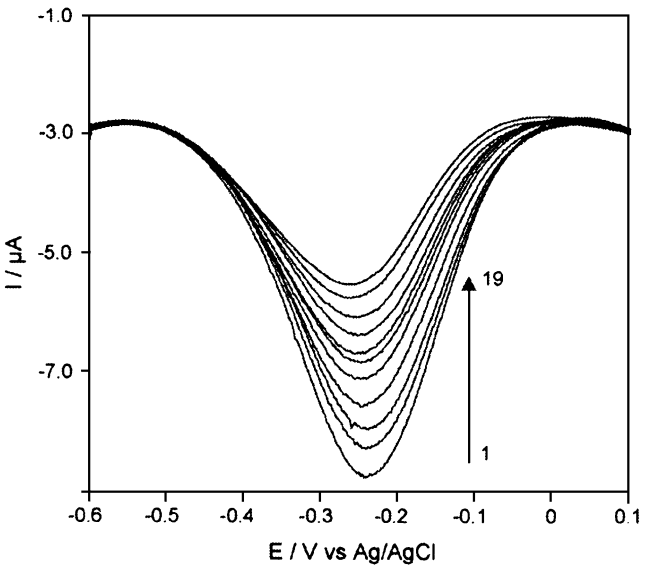

Fig. 1 Response of a haemoglobin-coated carbon paste electrode towards different acrylamide concentrations in an aqueous extract of potato crisps [65]. Acylamide concentrations increase from curve *1* to *19*

recognized in 1978 by the working group of Velíšek at the Institute of Chemical Technology in Prague [1] in acid-hydrolyzed vegetable protein (HVP), a seasoning ingredient widely used in a variety of processed and prepared foods such as soups, sauces, bouillon cubes and soy sauce [67]. The most abundant chloropropanols found in foodstuff are 3-monochloropropane-1,2-diol (3-MCPD) and to a lower level also 1,3-dichloropropan-2-ol (1,3-DCP) (Fig. 2) and they have been the centre of scientific, regulatory and media attention as they are considered carcinogens [68]. The isomers 2-MCPD and 2,3-DCP are usually found at much lower concentrations than 3-MCPD and 1,3-DCP.

3-MCPD is genotoxic in vitro, but there is no evidence of genotoxicity in vivo. The toxicological, metabolism and mechanistic data on 3-MCPD were reviewed by Lynch et al. [69]. Taking into account the lack of genotoxicity in vivo and the likely secondary mechanisms of the tumourigenic effects, the Scientific Committee on Food of the European Commission considered that a threshold-based approach for deriving a tolerable daily intake (TDI) would be appropriate. A TDI of 2 μg kg^{-1} body weight (bw) was derived [7]. The European Commission has set a regulatory limit of 0.02 mg kg^{-1} for 3-MCPD in HVP and soy sauce [8]. Since then, industry action reduced the level of contamination by chloropropanols of acid-HVP prepared in Europe [70].

Renewed interest in chloropropanols and the development of analytical methods in other food matrices was triggered by the detection of 3-MCPD in a wide range of foods and food ingredients, notably a range of thermally processed food ingredients such as malts, cereal products and meat [71–74]. In addition, domestic processing (e.g. grilling and toasting) can substantially increase the 3-MCPD content of bread or cheese [74, 75].

Several studies about the mechanism of 3-MCPD formation were performed [67, 76–85], showing that in heat-processed, fat-containing foodstuffs with low water activity, 3-MCPD is formed from glycerol or acylglycerols and chloride ions. Although the overall levels of 3-MCPD in bakery products as a whole are relatively low, the high level of consumption of, for example, bread, and additional formation due to toasting, indicates that this staple food alone can be a significant dietary source of 3-MCPD [74]. In malt products, 3-MCPD was only found in coloured malts with the highest levels in the most intensely coloured samples. The additional heat treatment including kilning or roasting was judged as a significant factor in the formation of 3-MCPD in these ingredients [73, 85]. Concentrations above 0.02 mg kg^{-1} were recently found in smoked fermented sausages and smoked ham. The smoking process was identified as a major source of 3-MCPD. As opposed to 3-MCPD formation in HVP, soy sauce, and bakery products, lipids are not precursors of 3-MCPD in smoked foods. A hypothetical mechanism with 3-hydroxyacetone as precursor was suggested for 3-MCPD formation in wood smoke [86].

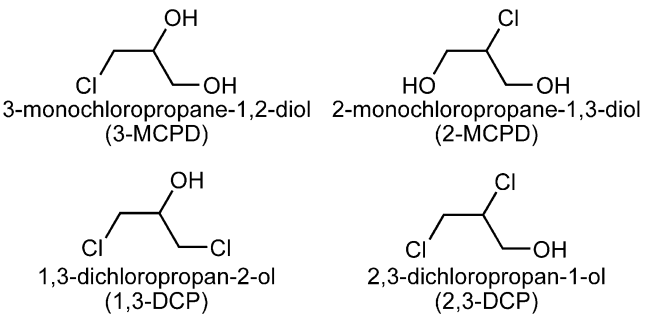

Fig. 2 Chemical structures of chloropropanols detected in foodstuffs

3-MCPD occurs in foodstuffs not only in its free form but also in the form of esters with higher fatty acids (so-called bound 3-MCPD). The working group of Velíšek recently provided evidence that the bound 3-MCPD contents exceeded the free 3-MCPD levels at least 5 and up to 396 times [87]. Hamlet et al. [88] found MCPD esters in baked cereal products and showed that 3-MCPD esters might be generated as stable intermediates or by-products of the formation reaction from mono- and diacylglycerol precursors. These esters must also be treated as food contaminants as 3-MCPD may be released in vivo by a lipase-catalyzed hydrolysis reaction.

Methods for determination of chloropropanols

The analysis of chloropropanols at the microgram per kilo level is complicated. The three main physical characteristics that contribute to this difficulty are the absence of a suitable chromophore, a high boiling point and a low molecular weight [72]. The initial methods developed for the determination of chloropropanols without derivatization showed a low sensitivity (Table 2).

Because of the missing chromophore, approaches based on HPLC with ultraviolet or fluorescence detection cannot be applied. So far, a single HPLC method with refractive index (RI) detection was proposed that was used to study the kinetics of 3-MCPD formation in model systems, but appears to be unfit to determine 3-MCPD at trace quantities in food matrices [76].

Direct analysis by GC without derivatization is also restricted. The low volatility and high polarity of 3-MCPD give rise to unfavourable interactions with components of the GC system that result in poor peak shape and low sensitivity. For example, 3-MCPD can react during GC with other components of the sample to form hydrochloric acid in the presence of water, as well as with active sites in the column and non-volatile residues in the column inlet [89]. Interferences may also derive from the reaction of 3-MCPD with ketones contained in the matrix to form ketals [89]. Peak broadening and ghost peaks were observed with GC-based methods for the analysis of underivatized 3-MCPD [90].

Table 2 Methods for the determination of chloropropanols

Matrix	Analytes	Internal standard	Extraction/pre-treatment	Cleanup	Derivatization	Detection	Column	LOD (μg kg^{-1})	Ref.	Adequate sensitivity to control EU max. level of 0.02 mg kg^{-1}
HVP	1,3-DCP	–	Micro-steam distillation, solvent extraction	–	None	GC-ECD	CP-Wax , 24 m× 0.32-mm i.d.× 0.19-μm film thickness	10	[93]	
Seasonings	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	–	Water, pH adjustment	Extrelut	None	GC-MS SIM	Stabilwax, 30 m× 0.32-mm i.d., 0.25-μm film thickness	50–100	[91]	No
HVP	3-MCPD	1-Chlorotetradecane	20% Aqueous sodium chloride	Extrelut	None	GC-ECD	Supelcowax-10, 60 m×0.75-mm i.d., 1-μm film thickness	250	[92]	No
Cereal products	MCPD esters	5-α-Cholestane	Ethyl acetate extraction	Preparative TLC	None	GC-MS scan	DB-1, 10 m×0.25-mm i.d., 0.1-μm film thickness	–	[88]	
Model systems	3-MCPD	–	–	–	None	HPLC-RI	Synergi RP80, 250 mm×4.60-mm, 4 μm	–	[76]	
Solvents	3-MCPD	*n*-Tetradecane	–	–	BSTFA	GC-FID	SPB-5, 30 m×0.75-mm i.d., 1.0-μm film thickness	5,000	[89]	
Paper	3-MCPD, 1,3-DCP	1-Fluoronaphthalene	Acetonitrile extraction	–	BSTFA	GC-MS SIM	CP-SIL-5, 25 m× 0.32-mm i.d., 1.2-μm film thickness	40	[96]	
Soy sauce	3-MCPD	–	Dilution with buffer	–	None	CE-ECD	Fused-silica, 50 cm× 25-μm i.d.	130	[95]	No
Soy sauce	1,3-DCP, 2,3-DCP	$[^2H_5]$-1,3-DCP	Ammonium sulfate	HS extraction	None	GC-MS	DB-Wax, 30 m× 0.25-mm i.d., 0.2-μm film thickness	3	[69]	
Standards	3-MCPD		–	–	PBA	GC-MI-FTIR	RSL-150, 10 m× 0.53-mm i.d., 1.2-μm film thickness	–	[90]	No
Aqueous solutions	3-MCPD	–	–	–	BBA	GC-ECD	10% SP1000, 20 ft.× 1/8 in. i.d., packed column	100	[98]	No
HVP	3-MCPD	*n*-Heptadecane	20% NaCl solution	–	PBA	GC-FID	CP-SIL-5, 50 m× 0.32-mm i.d., 0.12-μm film thickness	500–1,000	[99, 100]	No
Various foods	3-MCPD	$[^2H_5]$-3-MCPD	20% NaCl solution	–	PBA	GC-MS SIM	RTX-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	3–10	[5]	Yes
Various foods	3-MCPD	$[^2H_5]$-3-MCPD	20% NaCl solution	–	PBA	GC-MS/MS MRM (triple quadrupole)	HP-1, 60 m×0.25-mm i.d., 0.25 μm film thickness	5	[102]	Yes
Various foods	Free and bound 3-MCPD	$[^2H_5]$-3-MCPD	Fat extraction, interesterification	–	PBA	GC-MS SIM	SPB-1, 30 m×0.25-mm i.d., 1-μm film thickness	3	[103]	Yes
HVP, soy sauce	3-MCPD	–	Dilution 1:10	HS-SPME	PBA	GC-MS SIM	HP-1, 60 m×0.25-mm i.d., 25-μm film thickness	3.87	[104]	Yes
HVP	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	*p*-Dichlorobenzene	5 M NaCl solution	Extrelut, two-stage extraction	HFBI	GC-ECD, GC-MS	OV-1 25 m×0.2-mm i.d., 0.33-μm film thickness; DB-Wax, 25 m	10–100	[105]	No

HVP, seasonings	3-MCPD, 2-MCPD	[$^2H_7$]-3-MCPD	5 M NaCl solution	Extrelut	HFBI	GC-MS/MS MRM (ion trap)	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	5	[107]	Yes
Various foods	3-MCPD, 2-MCPD	[$^2H_5$]-3-MCPD	5 M NaCl solution	Extrelut	HFBI	GC-MS SIM or GC-MS/MS MRM (ion trap)	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness, or equivalent	5–10	[72, 106, 108] (AOAC method, EN 14573)	Yes
Water	3-MCPD, 1, 3-DCP (& bromopropanediols)	3-Fluoro-1, 2-propanediol, 1,4-dichloro-2-butanol	Ethyl acetate extraction	–	HFBA	GC-ECD	DB-5, 30 m×0.25-mm i.d., 1.0-μm film thickness	0.7–1.7	[117]	
Soy sauce	1,3-DCP, 3-MCPD	[$^2H_5$]-3-MCPD	5 M NaCl solution	Silica gel (60 mesh)	HFBA	GC-MS SIM	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	5	[111]	Yes
Cereal products	Free and bound 3-MCPD	[$^2H_5$]-3-MCPD	Enzyme hydrolysis (lipase)	Extrelut	HFBI	GC-MS	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	–	[88]	
Model systems	3-MCPD, 2-MCPD	[$^2H_5$]-3-MCPD	Hexane extraction	ASE	HFBI	GC-MS	DB-XLB ITD, 30 m×0.25-mm i.d., 0.25-μm film thickness or DB-5, 30 m×0.32-mm i.d., 0.25-μm film thickness	5	[82, 112]	
Soy sauce, flavouring	2-MCPD, 3-MCPD, 1, 3-DCP, 2,3-DCP	[$^2H_5$]-1,3-DCP, [$^2H_5$]-3-MCPD	5 M NaCl solution	Extrelut	HFBA-Et_3N	GC-MS EI SIM or NCI SIM	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	3 (EI), 0.6 (NCI)	[109]	Yes
Various foods	1,3-DCP, 3-MCPD	[$^2H_5$]-1,3-DCP, [$^2H_5$]-3-MCPD	Saturated NaCl solution	Aluminium oxide	HFBA	GC-MS SIM	DB-5, 30 m×0.25-mm i.d., 0.25-μm film thickness	1	[113]	Yes
Various foods	3-MCPD, 2-MCPD	–	Saturated NaCl solution	Extrelut	Acetone	GC-MS SIM	DB-1701, 30 m×0.32-mm i.d., 0.25-μm film thickness	10	[110]	Yes
Soy sauce	3-MCPD	[$^2H_5$]-3-MCPD	Saturated NaCl solution	Extrelut	4-Heptanone	GC-MS scan	DB-5, 25 m×0.25-mm i.d., 0.25-μm film thickness	1.2	[115]	Yes
Various foods	3-MCPD	[$^2H_5$]-3-MCPD	Pure water extraction	Extrelut	Acetone, filtration over aluminium oxide	GC-MS SIM	Innowax, 60 m×0.25-mm i.d., 0.25-μm film thickness	2–5	[114]	Yes

Abbreviations: GC-FID gas chromatography with flame ionization detection, *GC-ECD* gas chromatography with electron capture detection; *GC-MS* gas chromatography–mass spectrometry, *CE-ECD* capillary electrophoresis with electrochemical detection, *GC-MS/MS* gas chromatography–tandem mass spectrometry, *GC-MI-FTIR* gas chromatography–matrix isolation–Fourier transform infrared spectroscopy, *NCI* negative chemical ionization, *EI* electron ionization, *HPLC-RI* high-performance liquid chromatography with refractive index detection, *LOD* limit of detection, *SIM* selected ion monitoring, *MRM* multiple reaction monitoring, *TLC* thin-layer chromatography, *BSTFA* bis (trimethylsilyl)trifluoroacetamide, *PBA* phenylboronic acid, *BBA* *n*-butylboronic acid, *HFBI* heptafluorobutyrylimidazole, *HFBA* heptafluorobutyric anhydride

The low molecular weight of 3-MCPD aggravates mass spectrometric (MS) detection as diagnostic ions cannot be reliably distinguished from background chemical noise. Due to these apparent limitations, the methods based on direct GC (e.g. [91, 92]) are more or less obsolete. Due to their high limits of detection, these methods developed in the 1990s are unsuitable to control the European maximum levels of 3-MCPD.

During the analysis of 1,3-DCP further drawbacks arise from the volatility of 1,3-DCP, which complicate the concentration of solvent extracts without losses of analyte. The solvent extracts are likely to include a number of compounds which on gas chromatography will potentially co-elute with 1,3-DCP, and which might not be identified correctly when using electron-capture detection (ECD). The major problem of these approaches is the fact that they are time consuming and require a considerable degree of skill and experience in laboratory manipulations [70]. Steam distillation with extraction into co-distilled petroleum ether/ethyl acetate was therefore proposed to determine 1,3-DCP with subsequent gas chromatography with ECD of the underivatized analyte [93]. Crews et al. [70] developed an automated headspace (HS) sampling procedure for the analysis of 1,3-DCP. The advantages of the method are its rapidity, sensitivity and the requirement of only little sample preparation. The method provides accurate identification of 1,3-DCP using mass spectrometry, and precise quantification using a deuterium-labelled internal standard. It requires almost no sample preparation or reagents and a large batch of samples can be processed unattended overnight [70]. Nyman et al. [94] judged this HS-GC-MS method to be very fast and simple but with the disadvantage that a simultaneous analysis of 3-MCPD and 1,3-DCP is not possible because the analysis of the underivatized compounds requires different GC columns. In addition, the low molecular weight ion fragments of the underivatized compounds make this method susceptible to interferences and less reliable for confirmation of analyte identity.

Xing et al. [95] developed a simple and rapid method applying capillary electrophoresis (CE) with electrochemical detection. The advantage is that a diluted sample solution can be directly injected without any sample preparation and the method shows adequate sensitivity to control the regulatory limit of 1 mg kg^{-1} for 3-MCPD in HVP and soy sauces that has been set in China [95]. However, the sensitivity of CE appears to be insufficient for the control of the EU maximum level of 0.02 mg kg^{-1}, and not suitable for the determination of 3-MCPD at typical concentrations (in the lower microgram per kilo range) found in food groups other than those covered by EU legislation.

Recapitulating, none of these methods applying underivatized analytes is of sufficient sensitivity or selectivity for the determination of low microgram per kilo levels in foodstuffs. The same applies for derivatization using silylation with bis(trimethylsilyl)trifluoroacetamide (BSTFA) [89, 96], which showed detection limits above the maximum levels of 0.02 mg kg^{-1} even if MS is used (Table 2).

The three most common derivatization reactions that give adequate sensitivity and selectivity are shown in Fig. 3. The derivatization methods are summarized in Table 2 and are discussed in detail in the following sections.

Derivatization methods for determination of chloropropanols

Boronic acid derivatization

n-Butylboronic acid (BBA) was proposed by Schurig et al. in 1984 as a derivatization reagent in non-aqueous media to be used for gas chromatographic separation of 3-MCPD [97]. Pesselmann et al. [98] used this reagent to quantitatively measure 3-MCPD in aqueous solutions by GC and electron-capture detection after extraction of the derivative into *n*-hexane. Instead of *n*-butylboronic acid, Rodmann et al. [90] used phenylboronic acid (PBA), which was adapted by all of the subsequently developed methods.

A large advantage of PBA derivatization is the fact that no sample cleanup has to be carried out as PBA reacts specifically with diols forming non-polar cyclic derivatives extractable into *n*-hexane. The disadvantage is that other chloropropanols such as 1,3-DCP cannot be determined with this method.

The first method to determine 3-MCPD in HVP by gas chromatography using aqueous phenylboronic acid derivatization was reported by Plantinga et al. [99]. The organic extraction of the derivative was studied in detail. Although toluene showed best recovery, its extract produced more peaks with reduced resolution in the chromatogram, hindering accurate integration and reliable quantification at levels around 1 mg kg^{-1}. The *n*-hexane extract gave a

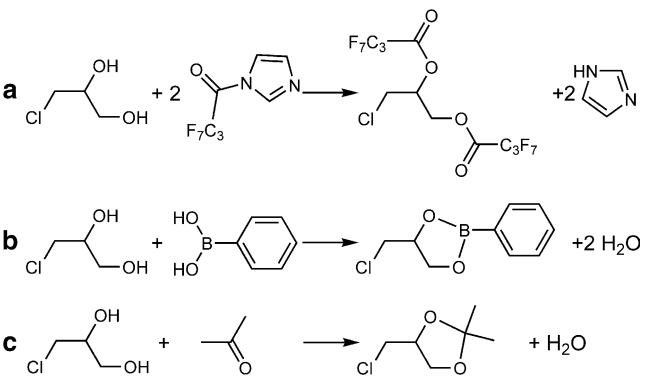

Fig. 3 Derivatization reactions of 3-MCPD with heptafluorobutyrylimidazole (**a**), phenylboronic acid (**b**) and acetone (**c**) for sensitive and selective determination with gas chromatography–mass spectrometry

rather clean chromatogram without interfering peaks. Therefore, *n*-hexane was selected as the extraction solvent [99]. The salt concentration also proved to be quite relevant as there is a clear desalting effect observable in the *n*-hexane extraction. A salt concentration in the range of 12–20% has been found to be essential. At increasing salt concentrations, the recovery of the phenylboronic derivative of 3-MCPD also increases, reaching a constant level at 12% NaCl. The sample preparation step therefore must include a dilution with sodium chloride solution resulting in a salt concentration higher than 12% [99]. In the case of phenylboronic acid derivatization, Breitling-Utzmann et al. [84] remarked that the injector temperature should not exceed 180 °C in order to prevent excessive derivatization reagent from getting onto the GC column, which leads to its rapid deterioration. Furthermore, it was advised to use a retention gap for extending column lifetime.

The method of Plantinga was fully validated and introduced in the collection of German official methods for food analysis [100]. As can be derived from the summary of PBA derivatization methods in Table 2, ECD or FID detectors do not have adequate sensitivity to control the EU maximum level of 0.02 mg kg^{-1}. Therefore, all further methods used GC-MS, which gave the required sensitivity. However, in the lower microgram per kilo range nowadays required to determine 3-MCPD in all kinds of foodstuff, conventional single quadrupole GC-MS was described as being problematic. To reach the required sensitivity, SIM mode has to be used and matrix contaminations might overlap with the selected ions leading to false-positive results [101]. Therefore, many laboratories have converted their methods from single stage MS to triple quadrupole MS. Recently, the introduction of low-cost benchtop triple quadrupole mass spectrometers made it possible to adopt these techniques in routine analysis of food contaminants. A triple quadrupole MS/MS method for the determination of 3-MCPD was proposed by Kuballa et al. [102].

An improved PBA procedure was reported by Divinová et al. [103]. In contrast to the previous PBA methods, a simple extraction of fat was employed for the sample purification prior to the derivatization of 3-MCPD.

So far, the most elegant approach for 3-MCPD analysis was performed by Huang et al. [104] who proposed a method comprising headspace solid-phase microextraction (HS-SPME), gas chromatography and mass spectrometry. The authors used PBA as derivatization reagent. For HS-SPME, 1 mL of sample and 9 mL of aqueous solution containing 4 mg of NaCl and excess derivatization reagent (about 0.25 mg) were placed in a headspace vial. After thermal equilibration at 90 °C for 5 min, the SPME fibre was exposed to the sample headspace for 30 min and immediately inserted into the injection port of the gas chromatograph for thermal desorption of the analytes. It is notable that all SPME steps and the subsequent GC-MS measurement can be fully automated. Therefore, the HS-SPME procedure is easier to perform than any other existing method for 3-MCPD analysis. However, it must be mentioned that the use of deuterated internal standards is mandatory for quantitative SPME and Huang et al. correctly noted that such internal standards must be used to ensure precision if the method is used in routine analysis.

HFBI/HFBA derivatization

Van Bergen et al. [105] reported the first procedure to determine chloropropanols in protein hydrolysates based on gas chromatography of heptafluorobutyrate derivatives. Heptafluorobutyrylimidazole (HFBI) was preferred by Hamlet as derivatization agent, as it reacts quantitatively with both 2-MCPD and 3-MCPD to give stable derivatives [106]. Although non-selective when compared to the use of boronic agents, HFBI was the reagent of choice: HFBI makes all co-extracted compounds with -OH or -NH groups volatile thereby minimizing contamination of the GC column and injector; the mass spectra of 3-MCPD-HFBI derivatives contained a greater number of diagnostic ions than the corresponding alkyl or phenyl boronic acids. Quantification by isotope dilution method applying stable isotope-labelled standards was considered the only reliable option. Deuterium-labelled d_7-3-MCPD was only available by customer-requested synthesis [106]. Nowadays, d_5-labelled 3-MCPD is commercially available.

Hamlet and Sutton [107] first reported a procedure for the determination of 3-MCPD at the low microgram per kilo level in HVP and seasonings. 3-MCPD was extracted into a saline solution and then partitioned into diethyl ether using a solid-phase extraction technique based on diatomaceous earth (Extrelut). Concentrated extracts were derivatized with HFBI to give the corresponding 3-MCPD di-esters, which were then analyzed by GC-MS. The procedure has been extended to cover other food matrices [106] and has been validated by a collaborative trial [71]. A range of 12 different food products was tested in 12 laboratories. Repeatability relative standard deviation (RSD_r) ranged from 4.9 to 11.6% and reproducibility relative standard deviation (RSD_R) from 12.8 to 38.6%. The method was considered fit for purpose and was adopted by AOAC International as AOAC Official Method 2000.01, as well as by the European standardisation body as European norm EN 14573 [108].

Nyman et al. [94] compared the HS-GC-MS method of Crews et al. [70] to the HFBI derivatization method of Hamlet and Sutton [107]. The HFBI method was found to be more labour intensive but had the advantage of covering both 1,3-DCP and 3-MCPD in the same GC-MS run. The HFBI derivative produced fragment ions with higher masses, which were less susceptible to interferences.

Nowadays, HFBI is one of the most widely used derivatization reagents for the determination of chloropropanols (Table 2). The same derivatives are produced when chloropropanols react with heptafluorobutyric anhydride (HFBA). Xu et al. [109] compared the two reagents and found that the peak areas of 2,3-DCP were about the same with both reagents; however, 1,3-DCP and 3-MCPD showed areas of approximately one third using HFBA. When HFBA was modified with triethylamine, the response was identical to the one using HFBI for all compounds due to triethylamine acting as a catalyst. HFBA modified with triethylamine was found to be about six times cheaper and more convenient to handle than HFBI.

The negative chemical ionization (NCI) mode was also evaluated by Xu et al. In comparison to EI mode, NCI showed a higher mass range of characteristic ions and only responded to electronegative compounds, which meant less matrix interferences, higher selectivity and higher sensitivity. The LOD of each chloropropanol was five times lower in NCI mode than in EI. The NCI mode was judged as especially suitable for samples with complex matrices like soy sauces or instant noodles owing to its lower detection limits and less matrix interference.

The summary of HFBI/HFBA methods in Table 2 shows that some methods include 2-MCPD as analyte. However, the determination of 2-MCPD is relatively problematic as a pure 2-MCPD standard is not commercially available. Usually, the determination of 2-MCPD is carried out using 3-MCPD as calibration standard [110, 111]. Using this method, Xu et al. found three times higher 2-MCPD levels in EI mode than in NCI mode and judged the method using 3-MCPD as standard as unsuitable to detect the real amount of 2-MCPD. The quantification of 2-MCPD in this way must be treated as a first approximation. Accurate quantification would necessitate a customary synthesis as presented in the study of Wittmann [91].

The Nestlé Research Center in Lausanne [82, 112] presented an interesting possibility to automate the procedure using accelerated solvent extraction (ASE) instead of manual extraction over diatomaceous earth columns. The extraction principle of ASE is similar to the use of diatomaceous earth (Extrelut), and the only difference is that the extraction time is dramatically reduced.

Abu-El-Haj et al. [113] also tried to improve the method for simultaneous determination of 3-MCPD and 1,3-DCP in soy-related products by using small portions of sample as well as small amounts of extracting solvents. The method is based on isotope dilution, and encompasses alumina column cleanup, dichloromethane extraction, derivatization, and GC-MS analysis. A typical chromatogram is shown in Fig. 4. The sample amount and particularly the volume of extracting solvent could be reduced by the use of aluminium oxide as column filling, instead of Extrelut or silica gel. Extrelut has a large volume and is loosely packed in the column, whereas silica gel does not adsorb water as effectively as aluminium oxide. By the use of small disposable columns, the volume of solvents was reduced by a factor of 10 in comparison between the method of Abu-El-Haj et al. [113] and previously published methods.

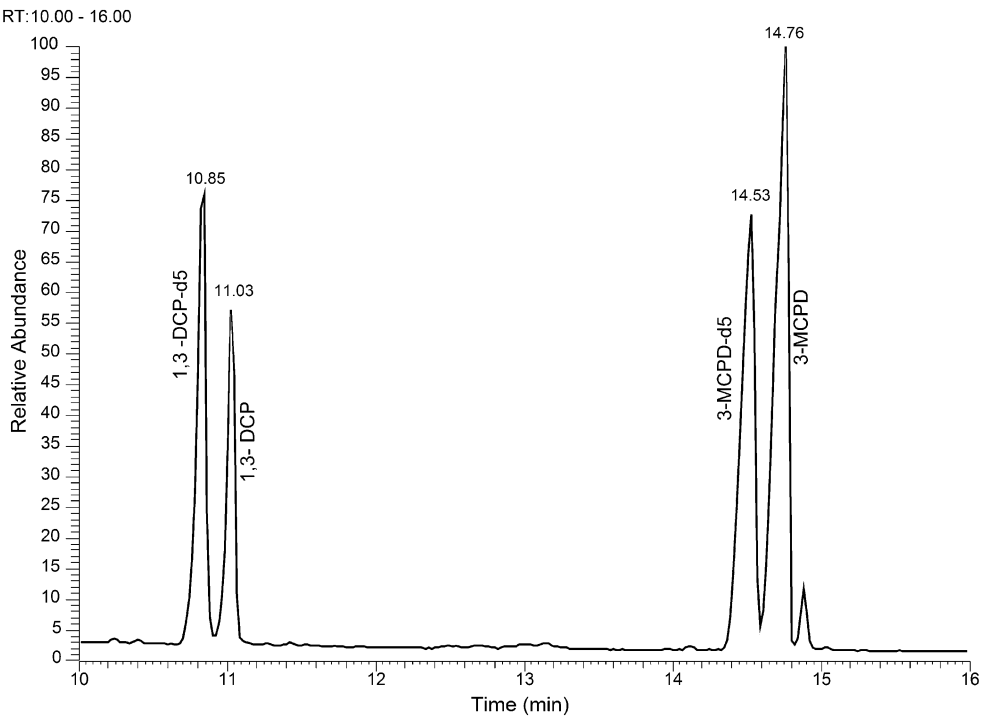

Fig. 4 Typical GC-MS chromatogram of the simultaneous determination of 1,3-DCP and 3-MCPD after derivatization with heptafluorobutyric anhydride (HFBA). Reprinted from [113] with permission from Elsevier

Derivatization with ketones

Rétho et al. [114] critically evaluated the HFBI derivatization procedure. They obtained chromatograms with many peaks corresponding to volatile derivatized molecules and found that HFBI or HFBA react with all nucleophilic molecules present in the extract. Moreover, the diagnostic ions used for MS quantification had a low abundance in the mass spectrum. Additionally, the reagents are very sensitive to moisture. Further limitations include the potential for incomplete derivatization, inefficient partitioning and short-term stability of the derivatives [110]. These problems may be prevented by derivatization of the hydroxyl groups with a suitable reagent to produce a more volatile derivative. After absorption of diluted aqueous sample on a Kieselguhr column, chloropropanediols can be extracted with diethyl ether, derivatized with acetone to the corresponding dioxolanes, which were described to be the optimal derivative, and measured by GC-MS [110]. Only diols are derivatized by ketones in acidic medium to form cyclic ketals; hence the derivatization is very specific. Moreover, the EI spectra of these cyclic ketal derivatives show intense and diagnostic isotope pattern [114]. Like PBA derivatization, the method is also suitable for the determination of 2-MCPD but not suitable for the determination of 1,3-DCP as this chloropropanol does not form a cyclic acetonide derivative.

Rétho et al. [114] adapted the method of Meierhans et al. [110] to a wide range of foods (Table 2). Most notable is the use of deuterated 3-MCPD as internal standard, and a further purification of the derivatized extract on a basic aluminium oxide cartridge.

Rétho et al. [114] remarked that although extraction was carried out with a saturated sodium chloride solution in most of the published protocols, the saturation of water with a salt does not promote the extraction of 3-MCPD into the aqueous phase. Therefore, they extracted all solid foods with pure water. However, the aqueous extract was

Table 3 Methods for the determination of furan in food

Matrix	Internal standard	Extraction/pre-treatment	Cleanup	Equilibration temperature (°C)	Detection	Column	LOD (μg kg^{-1})	Ref.
Various foods	[$^2H_4$] furan	Solid or semi-solid samples diluted with water or saturated NaCl solution	Static headspace sampling	80	GC-MS SIM	HP PLOT Q, 15 m× 0.32-mm i.d., 20-μm film thickness	–	[3][a]
Various foods	[$^2H_4$] furan	Solid or semi-solid samples diluted with water or saturated NaCl solution	Static headspace sampling	60	GC-MS SIM	HP PLOT Q, 15 m× 0.32-mm i.d., 20-μm film thickness	–	[3][b]
Various foods	[$^2H_4$] furan	Solid samples homogenized and diluted with water	Static headspace sampling	50	GC-MS SIM	HP PLOT Q, 15 m× 0.32-mm i.d., 20-μm film thickness	2.0	[126]
Coffee and fruit juices	[$^2H_4$] furan [$^2H_6$] acetone	Solid samples homogenized and diluted with water	Static headspace sampling	40–70	GC-MS SIM	PLOT HT-Q, 12.5 m× 0.32-mm i.d.	0.1	[125]
Jars, canned foods, coffee	[$^2H_4$]furan	Samples blended with water	Static headspace sampling	30	GC-MS SIM	CP-PoraBOND Q, 25 m×0.25-mm i.d., 3-μm film thickness	0.1	[123]
Various foods	[$^2H_4$]furan	Solid samples homogenized	Solid-phase microextraction	50	GC-MS SIM	HP-PLOT Q, 15 m× 0.32-mm i.d., 20-μm film thickness	0.2–0.6	[129]
Baby foods	[$^2H_4$]furan	Samples homogenized	Solid-phase microextraction	30	GC-MS SIM	HP-INNOWAX, 60 m×0.25-mm i.d., 0.5-μm film thickness	<0.1	[131]
Water	[$^2H_4$]furan		Solid-phase dynamic extraction (liquid and headspace)	30	GC-MS SIM	HP PLOT Q, 15 m× 0.32-mm i.d., 20-μm film thickness	1.5	[127]

GC-MS gas chromatography–mass spectrometry, *LOD* limit of detection, *SIM* selected ion monitoring
[a] Status May 2004
[b] Status October 2006

saturated afterwards with sodium chloride to enhance the effectiveness of solid-phase extraction.

The acetone derivatization was critically evaluated by Dayrit et al. [115] as erratic results were obtained in reproducing the procedure of Meierhans. The following problems were described: first, the derivatization step requires anhydrous conditions, which are difficult to maintain for acetone without special precautions. Second, the dioxolane formed by acetone is still relatively water soluble and losses may occur when the reaction mixture is partitioned between water and hexane. Third, the use of acetone limits the reaction temperature to the boiling point of acetone (56 °C). Dayrit et al. therefore investigated the use of 4-heptanone as an alternative ketone. The 4-heptanone derivatization method was judged to be an accurate, simple and inexpensive alternative for the determination of 3-MCPD.

Determination of MCPD esters and bromopropanediols

Only a single method was found in the literature for the direct analysis of unhydrolyzed MCPD esters. Hamlet et al. [88] analyzed the esters by extraction into an organic solvent, followed by cleanup with preparative thin-layer chromatography as described by Davídek [116], and analysis by GC-MS. A faster method was presented by Hamlet [88] who used a commercial lipase from *Aspergillus oryzae* to hydrolyse bound 3-MCPD, followed by HFBI derivatization and GC-MS. In a similar way, Divinová [103] studied 3-MCPD bound in esters with higher fatty acids. The determination of bound 3-MCPD was possible after transesterification of the sample with sulfuric acid at 40 °C for 16 h. Levels of bound 3-MCPD varied in 20 samples between LOD and 2.4 mg kg^{-1}.

Other studies included the analysis of bromopropanediols. Matthew et al. [117] were able to analyze 3-bromo-1,2-propanediol (3-MBPD) in water samples simultaneously with 3-MCPD after HFBA derivatization. Rétho et al. [114] reported the presence of monobromopropanediols in a grape seed oil, a rape seed oil and a sesame oil in amounts of 35 μg kg^{-1}, 45 μg kg^{-1} and 7 μg kg^{-1} of 3-MBPD, respectively. In all cases a mixture of the 3-bromo and 2-bromo isomers was identified.

Furan

Furan is a colourless liquid having a low molecular weight of 68 g mol^{-1} and a high volatility with a boiling point of 31 °C [118]. Furan and its derivatives have been associated with the flavour of many foodstuffs. Their existence in many types of processed foods has long been known [119]. Furan is considered a hazardous chemical, which has been classified as a possible human carcinogen by the IARC [6].

Researchers at the US Food and Drug Administration (FDA) have identified furan in a number of thermally treated foods, especially canned and jarred foods [3]. Recent studies have shown that there are several distinct pathways responsible for the formation of furan. These are based on the decomposition of ascorbic acid and related compounds, the oxidation of polyunsaturated fatty acids, the Maillard reaction, and the pyrolysis of sugars at extreme temperatures [120–122].

The kinetics of furan formation are quite sensitive to changes of the reaction conditions and precursor compositions. Therefore, not only the concentration of potential precursors is important, but also the composition of the complete food system. Accordingly, almost every single component present in a particular foodstuff may directly or indirectly affect the formation of furan during thermal processing.

Analytical methodology

Sample pre-treatment

Sample preparation is often the bottleneck in food analysis and therefore minimizing the number of sample preparation steps to reduce both analysis time and sources of error is advantageous. For the analysis of volatile analytes the use of extraction solvents can be avoided by analyzing the headspace over a sample. The headspace sampling technique also limits the accumulation of non-volatile compounds in the GC system.

Since the foods subjected to furan analysis are diverse, their physical properties (liquid, wet or dry) need to be taken into account in sample preparation. Due to the high volatility of furan, samples should be stored, if necessary, refrigerated, and analysed as fast as possible after opening packages and/or preparation. Liquid samples or reconstituted powdered samples can be transferred directly to headspace vials (10 or 20 mL) for equilibrium HS analysis of furan. Solid samples (inhomogeneous and with variable amounts of fat) should be homogenized prior to HS extraction. To prevent the loss of furan, extended blending times (>1 min) should be avoided [123].

Equilibrium headspace analysis

The direct and accurate analysis of furan as well as other volatiles in foods by headspace GC requires careful standardization of parameters such as equilibration temperature and time, headspace volumes (phase ratio), matrix components and instrumental conditions required for the separation of volatile compounds in food matrices [124, 125].

Recently published analytical methods using HS-GC-MS utilize very distinct equilibration temperatures ranging from room temperature to 80 °C [3, 121, 123]. However, temperatures must be carefully controlled not only to establish equilibrium between the analyte contents in the sample and headspace, which will be subsequently determined by GC-MS, but also to prevent artefact formation during the equilibration process. Experiments applying a range of equilibration temperatures for the determination of furan in unprocessed foods (green coffee and freshly squeezed juices) showed that furan could be formed during equilibration even at 40 °C [125]. Similar results were reported by Hasnip et al. [126] indicating formation of furan in processed foods at temperatures of 60 °C or higher. It was shown for certain food matrices that the furan content may increase by 10% to up to 300% when the equilibration temperature was increased to 80 °C [126]. Consequently, the FDA [3] decreased the equilibration temperature from 80 °C to 60 °C in its web-published analysis method. Other researchers adopted a temperature of 30 °C for the equilibrium HS-GC-MS determination of furan in food [127]. Details on analytical methods are listed in Table 3.

Solid-phase micro-extraction (SPME) is an alternative solvent-free sampling technique widely used for the analysis of volatile compounds. It can provide effective enrichment with high enrichment factors, and can be used for headspace sampling prior to GC-MS analysis [127]. Some researchers recently developed and validated in-house SPME-GC-MS methods for the determination of furan in heated foodstuffs [128–131]. Proper selection of the SPME fibre is important to increase the extraction yield; Carboxen–polydimethylsiloxane (CAR-PDMS) fibres are preferred by many authors owing to their superior performance for furan analysis [129–131]. A commercial in-tube sorptive extraction device, known as solid-phase dynamic extraction (SPDE), has also been evaluated for the extraction of furan from aqueous solutions in both headspace and liquid injection modes [127].

Usually, a portion of homogenized sample is mixed with water to facilitate phase equilibrium in the sealed HS vial. Certain amounts of sodium chloride or sodium sulfate may be added to the vial to increase the concentration of furan in the HS as the solubility of furan in salt-saturated aqueous phase decreases [123, 129, 130]. Preferably d_4-furan is applied as internal standard to allow quantification of furan by HS-GC-MS. Due to the high volatility of furan, furan standard solutions must be stored in completely filled vials to prevent any partition of furan into the HS, which could cause bias [131].

Porous layer open tubular (PLOT) capillary columns are the preferred option for chromatographic separating of furan from co-extractives, applying a variety of instrument parameters [3, 120, 121, 123, 125–127, 129]. Splitless injection, with or without cryogenically refocusing the injected HS gas, is the natural choice to obtain sufficient sensitivity of the method.

For the detection and quantification of furan, mass spectrometers are usually operated in the SIM mode. Electron ionization and source temperatures between 200 and 280 °C have been shown adequate for furan analysis.

Quantification can be performed by means of internal standardisation [121, 123, 125, 130, 131], standard addition [3, 126] or external calibration [121, 129]. However care must be taken to ensure suitable equilibration of the internal standard with the food sample [121].

Conclusion

There is no doubt that most analyses of heat-induced contaminants in carbohydrate-rich foodstuffs are performed by applying one or other well-established method based on LC-MS/MS or GC-MS either with or without derivatization of the target analytes. Internal standardization with isotopically labelled standards became routine owing to their commercial availability, and the application of mass spectrometry for analyte detection. Recent developments focussed very much on further development of sample preparation in order to (1) cover a broad range of food matrices with one analysis protocol, (2) to decrease limits of detection and quantification, (3) to improve sample cleanup, (4) to speed up sample preparation, and (5) to limit solvent consumption. A variety of new approaches for analyte detection was presented. Special attention in this respect should be given to sensor techniques, which could provide the possibility to analyse e.g. acrylamide with low expense, outside sophisticated laboratories.

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