Simultaneous Determination and Differentiation of Glycidyl Esters and 3-Monochloropropane-1,2-diol (MCPD) Esters in Different Foodstuffs by GC-MS

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ABSTRACT: The aim of this study was the development of a method for the simultaneous determination and differentiation of fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD esters) and glycidol (glycidyl esters) in different foodstuffs. The esters were isolated from fat-rich food samples using a single extraction step and separated from interfering substances. For differentiation of 3-MCPD esters and glycidyl esters the glycidol moiety was converted into 3-methoxypropane-1,2-diol (3-MPD) by acidic alcoholysis. Subsequent determination was achieved by isotope dilution GC-MS after transesterification using an isotope-labeled 3-MCPD ester as internal standard. During optimization of the procedure, critical parameters affecting simultaneous determination and differentiation of these analytes were investigated. Rapid ester cleavage and derivatization at ambient temperature proved to be essential for the simultaneous determination of these analytes. The method was validated for various fat-rich foodstuffs such as bakery products, sweets, gravy, and soup powders as well as edible fats and oils. LODs of 8 and 15 μg/kg (fat-rich foodstuffs) as well as 50 and 65 μg/kg (edible oils and fats) were obtained for 3-MCPD esters and glycidyl esters, respectively. Recoveries for 3-MCPD esters and glycidyl esters ranged within 98 ± 4 and 88 ± 2% in all tested foodstuffs (0.05−2.5 mg/kg) and within 99 ± 16 and 93 ± 13% for edible oils and fats (0.15−3 mg/kg) over a wide concentration range. These results proved an accurate and differentiated determination of 3-MCPD esters and glycidyl esters with successful application to the fast screening of samples, avoiding tedious and laborious sample preparation.

KEYWORDS: 3-MCPD esters, glycidyl esters, GC-MS, foodstuff, oil

■ INTRODUCTION

3-Monochloropropane-1,2-diol (3-MCPD), known as a food-processing contaminant with a maximum tolerable daily intake of 2 μg/kg of body weight as recommended by the European Scientific Committee on food, is detected in various types of food, such as acid-hydrolyzed vegetable proteins, soy sauces, and bakery and meat products.[1] In 2006, the first report about the occurrence of fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD esters) in fats and oils was published.[2]

Several analytical methods using ester cleavage by transesterification with sodium methoxide and derivatization with phenylboronic acid (PBA) have been presented, and many samples of edible fats and oils as well as fat-containing foodstuffs have been analyzed.[3−5] 3-MCPD esters are formed during the refining process of fats and oils because of deodorization as an identified critical step.[6]

Dependent on the used analytical procedure, different values of 3-MCPD esters were found and confirmed by several studies. Kuhlmann[7] reported significantly lower values of 3-MCPD esters using aqueous solutions of NaBr or $(NH_4)_2SO_4$ instead of NaCl as solvent for derivatization. Further examinations demonstrated fatty acid esters of glycidol (oxirane-2-methanol, 2,3-epoxy-1-propanol) as a reason for the higher values of 3-MCPD esters, which were almost completely transformed into the cyclic 1,3,2-dioxaboralane derivative of 3-MCPD by derivatization with phenylboronic acid in NaCl solution.[8]

All of these observations indicated a possible occurrence of significant amounts of glycidyl esters besides 3-MCPD esters in some foodstuffs.

The International Agency for Research on Cancer (IARC) as part of the World Health Organization (WHO) classified glycidol as probably carcinogenic to humans (group 2A). Hence, amounts of this contaminant should be investigated and determined accurately in foodstuffs. Weisshaar has verified the presence of fatty acid esters of glycidol in refined oils.[8] However, that paper describes only qualitative results for the glycidyl esters. The approach proposed by Weisshaar[5] calculates the amount of glycidyl esters using the difference of determined 3-MCPD before and after acid hydrolysis. Masukawa et al. have reported on an analytical procedure in edible oils using LC-MS after a two-step solid phase extraction and a multitude of standards for quantitative calculation.[9]

Due to the conversion of the glycidyl moiety into 3-MCPD phenyl boronate during the derivatization procedure with PBA in NaCl solution, a differentiation of 3-MCPD esters and glycidyl esters is prevented. Furthermore, 3-MCPD esters are transformed into glycidyl esters during transesterification with sodium methoxide.[5] Kuhlmann[7] reported on minimizing the conversion of glycidyl esters into 3-MCPD phenyl boronate using sodium

bromide for derivatization and on preventing the formation of glycidyl esters by careful ester cleavage. Nevertheless, a sample preparation with different steps and various conditions for considering the transformation rates was necessary.

At present, a robust and validated method for the simultaneous determination of glycidyl esters and 3-MCPD esters is not available.

This study presents an analytical procedure for the simultaneous determination and differentiation of fatty acid esters of 3-MCPD and glycidol in different foodstuffs such as edible oils and fats as well fat-rich products such as soup and gravy powders, cookies, bread, and mayonnaise. The described method uses a simple and rapid extraction step separating the esters followed by conversion (ring-opening) of the glycidyl moiety into esters of 3-methoxypropane-1,2-diol (3-MPD) and subsequent ester cleavage by sodium methoxide. Within the scope of method development critical parameters such as ester cleavage and derivatization conditions as well as conversion of the glycidyl moiety were investigated and optimized. Amounts of glycidyl esters in foodstuffs other than edible oils are presented for the first time.

■ MATERIALS AND METHODS

Reagents and Materials. Sodium chloride (p.A.), phenylboronic acid (≥98%, PBA), acetone, hexane, methyl *tert*-butyl ether (MTBE), methanol, and ethyl acetate (all suprasolv for GC) as well as glacial acetic acid and sulfuric acid 96% (suprapur) were bought from VWR (Darmstadt, Germany). Sodium methoxide (25% w/v in methanol), 3-monochlorpropane-1,2-diol (98%), and 3-methoxypropane-1,2-diol (98%) were obtained from Sigma-Aldrich (Weinheim, Germany). A solution of 50 μL of sulfuric acid in 5 mL of methanol was prepared subsequently for conversion of glycidyl esters (methanol/sulfuric acid). 1,2-Dipalmitoyl-3-chloropropane and 1,2-dipalmitoyl-3-chloropropane-d_5 as well as glycidyl palmitate were purchased from Campro Scientific (Berlin, Germany).

A sodium chloride solution (NaCl solution 20%) of 200 g/L was prepared in deionized water. The derivatization reagent PBA was prepared by dissolving 5 g of PBA in 19 mL of acetone and 1 mL of deionized water.

Preparation of Standard Solutions. Stock solutions with concentrations of 50 mg/mL of 1,2-dipalmitoyl-3-chloropropane and 1,2-dipalmitoyl-3-chloropropane-d_5 as well as glycidyl palmitate were prepared in MTBE. These solutions were further diluted to 50 μg/mL (working solution for foodstuffs) as well as 5 μg/mL (working solution for fats and oils) with MTBE, expressed as 3-MCPD and glycidol. From these solutions the calibration standards for the determination of 3-MCPD esters and glycidyl esters in fats and oils (0.01–0.3 μg/mL) as well as in other matrices (0.05–0.5 μg/mL) were prepared by dilution with MTBE. Concentrations of the internal standard 1,2-dipalmitoyl-3-chloropropane-d_5 were 0.15 and 0.25 μg/mL in the respective calibration standards, expressed as 3-MCPD-d_5. In relation to sample preparation, standard solutions were prepared in 2 mL (liquid samples) as well as 10 mL (other samples) MTBE followed by the same treatment as samples. All solutions were stored at 4 °C.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis. GC-MS was carried out on a 6890 Hewlett-Packard (HP) gas chromatograph equipped with a 5973 HP mass selective detector (positive electron impact ionization) and a split/splitless injector including a MPS II auto sampler (Gerstel, Mülheim a.d. Ruhr, Germany). Two microliters of the derivatized sample solution and of each derivatized calibration solution were injected in splitless injection mode and detected in selected ion monitoring mode.

A HP 5 MS capillary column (Agilent, Waldbronn, Germany; 5% phenyl–95% dimethylpolysiloxane; 30 m × 0.25 mm, 0.25 μm film thickness) was used for separation.
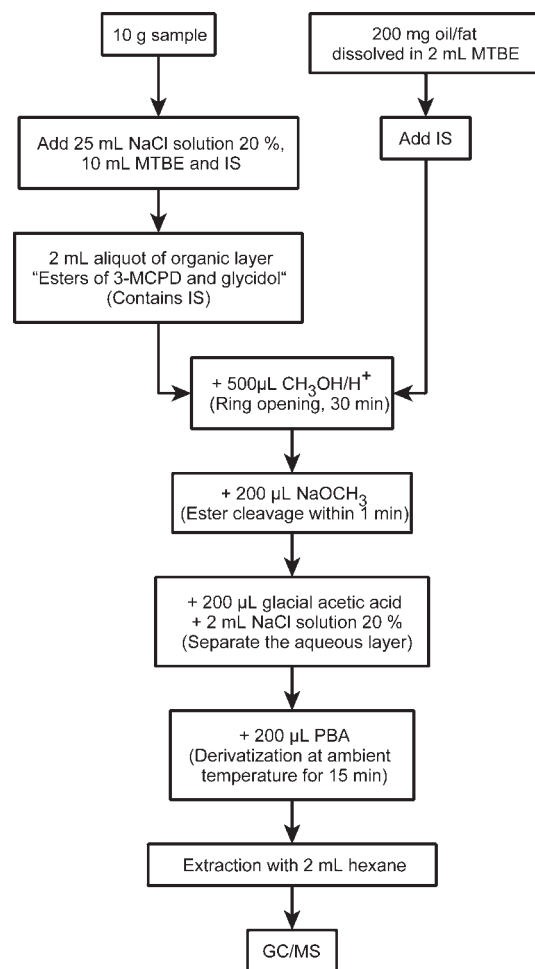

Figure 1. Scheme of analysis for the simultaneous determination and differentiation of 3-MCPD esters and glycidyl esters in foodstuffs and oils or fats. IS = 1,2-Dipalmitoyl-3-chloropropane-d_5.

The injector temperature was kept at 250 °C, and ultrapure grade helium was used as carrier gas with a flow of 0.7 mL/min. GC oven temperature was programmed from an initial temperature of 50 °C (1 min hold), ramped at 10 °C/min to 210 °C, and finally ramped at 30 °C/min to 300 °C with holding for 5 min. This program resulted in a total run time of 25 min. The following temperatures were selected for transfer line, MS source, and MS quad: 280, 230, and 150 °C, respectively. EM voltage was 1800 V, and the dwell time for each measured ion was set to 25 ms. A solvent delay of 8 min was used. The software MSD ChemStation used to control the GC-MS was obtained from Agilent Technologies 2004 (version D.01.02.16).

Quantitative analysis was carried out by monitoring characteristic ions (quantifier) at m/z 147 (3-MCPD and 3-MPD) and m/z 150 (3-MCPD-d_5). Ions at m/z 196 (3-MCPD), m/z 192 (3-MPD), and m/z 201 (3-MCPD-d_5) were used as qualifiers.

Contents of 3-MCPD esters and glycidyl esters were calculated as the ratio of the peak area responses for 3-MCPD and 3-MPD (m/z 147) and internal standard (m/z 150) for calibration solutions as well as blank and fortified samples.

Sample Preparation (See Figure 1). Procedures for extraction and derivatization were selected from previous studies with slight modifications.[3] The scheme of analysis is shown in Figure 1. Homogenization of the samples was accomplished by thorough pulverizing or mixing using a laboratory mill. All further procedures of mixing, shaking,

and homogenizing during the sample preparation were accomplished using a vortex mixer (2500 rpm, 30 s).

Extraction of Glycidyl Esters and 3-MCPD Esters from Foodstuffs. About 10 g of the homogenized sample was weighed into a screw-capped centrifuge tube, and 2.5 μg of the internal standard (50 μL of 50 μg/mL working solution of 1,2-dipalmitoyl-3-chloropropane-d_5 for foodstuffs) was added. Afterward, 25 mL of NaCl solution 20% and 10 mL of MTBE were added. The tube was closed tightly, shaken vigorously for 30 s, and centrifuged for phase separation at 3000 rpm (5 min).

Extraction of Glycidyl Esters and 3-MCPD Esters from Oil and Fat. About 200 mg of the homogenized sample was weighed into a screw-capped centrifuge tube. Two milliliters of MTBE and 0.3 μg of the internal standard solution (60 μL of 5 μg/mL working solution of 1,2-dipalmitoyl-3-chloropropane-d_5 for fats and oils) were added and homogenized thoroughly for 30 s. No further sample preparation was required.

Conversion of the Glycidol Moiety. To 2 mL of separated organic layer or solution of oil or fat was added 500 μL of methanol/sulfuric acid; the mixture was shaken well and heated for 30 min at 50 °C. Afterward, the esters were cleaved in the way described below without further preparation.

Ester Cleavage. Esters of 3-MCPD and converted glycidol were cleaved by adding 200 μL of sodium methoxide. The tube was closed tightly and the solution mixed well on a vortex mixer for 30 s. After 1 min, the reaction was stopped with 200 μL of glacial acetic acid and 2 mL of NaCl solution 20%. Extraction of the analytes was accomplished by vigorous shaking for 30 s. After phase separation, the upper layer was discarded. The aqueous layer was derivatized as described below.

General Derivatization Procedure. Two hundred microliters of the derivatization reagent PBA were added to the aqueous layer, and the tube was shaken well. After a reaction time of 15 min at ambient temperature, the cyclic phenylboronate derivatives were extracted with 2 mL of hexane. The organic layer was separated and analyzed by GC-MS.

Method Validation. For the validation study and method development, food products of the categories soup and gravy powders, bakery products, and sweets as well as oils and fats (including fat-rich foodstuffs such as mayonnaise and margarine) were selected. Because high amounts of glycidol were found in refined oils, fat-rich products with fat and/or oil as an ingredient were used for validation within all product groups. Recoveries for 3-MCPD esters were determined at spiking levels according to the actually found contents of 3-MCPD esters in foodstuffs.[10,11] Similar concentrations were selected for spiking samples with glycidyl ester.

For testing the recovery, blank samples (original samples) were spiked with the above-mentioned solutions (1,2-dipalmitoyl-3-chloropropane as well as glycidyl palmitate) at five levels of about 150–3200 μg/kg for fats and oils and 50–2500 μg/kg for other matrices. If present, amounts of 3-MCPD esters or glycidyl esters in blank samples were considered for correction of recoveries. All samples were investigated at least in duplicate per day, and three replicates of these series were performed for each on three different days. Recoveries and precision as well as repeatability were determined from this data set.

The linearity of the method was established by analysis of six standard solutions in the above-mentioned ranges. Detection and quantification limits (LOD and LOQ, respectively) were determined by standard solutions based on signal-to-noise ratios (S/N) of 3:1 for LOD and 10:1 for LOQ. The LOQ was verified by analyzing spiked samples at the respective level. The reproducibility of the derivatization reaction was investigated by comparing the calibration curve slopes.

■ RESULTS AND DISCUSSION

Extraction of the Analytes. Most methods for the determination of 3-MCPD esters or glycidyl esters described in the literature use only edible oils and fats. To analyze these contaminants in foodstuffs, a sample preparation is necessary to obtain the fat from

sample and reduce interfering substances. As previously described,[3] a rapid and simple sample preparation is possible using isotope-labeled 1,2-dipalmitoyl-3-chloropropane-d_5 as internal standard and simultaneous double liquid extraction. The respective ester-bound concentrations of 3-MCPD and glycidol are determined after transesterification.

The use of isotope-labeled 3-MCPD ester as an internal standard compensates for both the degradation of the analytes by sodium methoxide as a strong nucleophilic agent affecting the molecules by nucleophilic substitution during transesterification and the increase of volume with respect to the extracted fat.

Thus, the analytes were extracted in one step and interfering matrix was reduced simultaneously without further tedious purification steps.

Conversion of Glycidyl Esters and Glycidol. As already mentioned, the differentiation of glycidol esters and 3-MCPD esters is very important.

A general scheme of reactions in the determination and differentiation of 3-MCPD esters and glycidyl esters is shown in Figure 2. As presented there, glycidol itself is difficult to determine due to its epoxide structure (low stability) and associated reactions. A conversion is required; otherwise, glycidol is determined as 3-MCPD phenyl boronate by nucleophilic ring-opening with chloride ions during derivatization.

Additionally, as a side reaction, sodium methoxide forms high amounts of glycidol from α-halohydrins such as 3-MCPD by intramolecular nucleophilic substitution.[12] This formed glycidol from 3-MCPD during transesterification with sodium methoxide is transformed to 3-MCPD phenyl boronate by derivatization with PBA in sodium chloride solution as well.

Therefore, minimization of glycidol formation during transesterification is required. In addition, existing glycidyl esters have to be transformed into stable derivatives without reverse reaction of the formed derivatives into glycidol during ester cleavage as observed in 3-MCPD.

To avoid these interactions, only one approach is feasible for the differentiation, conversion of the glycidol moiety before transesterification together with minimization of glycidol formation from 3-MCPD esters as well as formed derivatives of glycidyl esters during transesterification.

Ring-Opening Reaction. Several options of ring-opening reactions for the epoxide moiety were investigated. Especially, nucleophilic ring-opening by halides[13] and mercaptans[14] as well as amines[15] is most suitable. In the case of acidic catalysis alcohols can react as well.[16] Direct conversion of glycidol by sodium methoxide[17] was performed at ambient temperature and under mild conditions (40 °C). All experiments were carried out using 1,2-dipalmitoyl-3-chloropropane and glycidyl palmitate as well as 3-MCPD and glycidol to investigate each reaction step (e.g., ring-opening, ester cleavage, extraction into aqueous solvent, and derivatization).

As a result, reaction products of halides were transformed into glycidol during transesterification similar to 3-MCPD, and no differentiation was obtained in the further derivatization procedure with PBA in sodium chloride solution (cf. Figure 2).

Using benzyl mercaptan or piperidine as nucleophiles, very lipophilic compounds were formed, which could not be extracted into aqueous solution for derivatization after transesterification. During direct conversion by sodium methoxide both 3-MCPD esters and glycidol esters were already converted into 3-methoxypropane-1,2-diol by nucleophilic substitution at ambient temperature, and no differentiation was obtained.
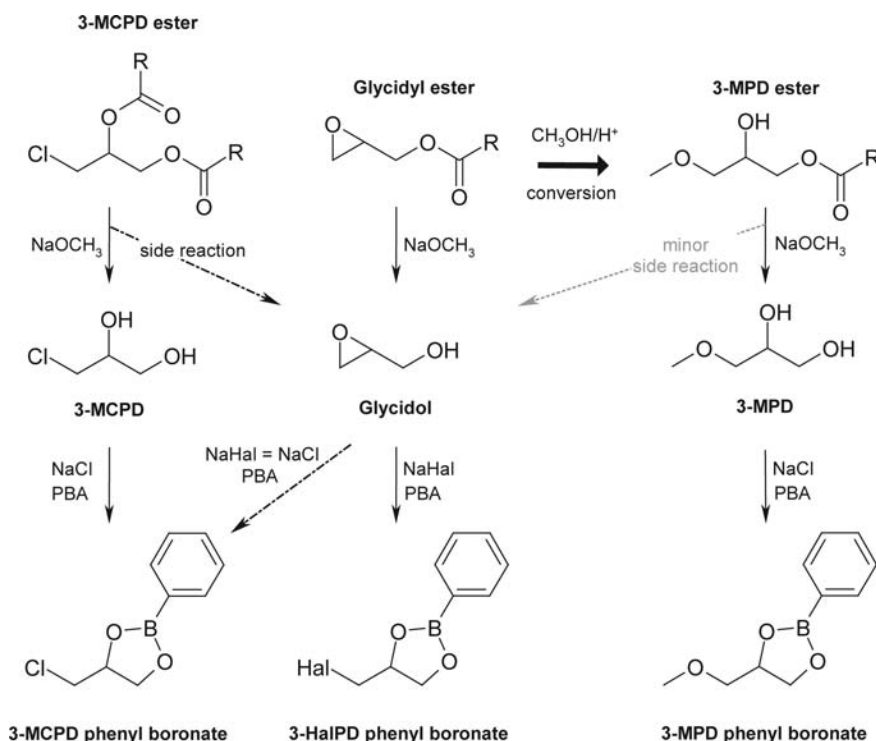
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Figure 2. Scheme of reactions in determination and differentiation of 3-MCPD esters and glycidyl esters. As a side reaction during transesterification, additional glycidol is formed from 3-MCPD and converted into 3-halidepropane-1,2-diol (3-HalPD) by the derivatization procedure at high temperatures in the presence of halides (NaHal; Hal = Cl⁻, Br⁻, I⁻). Ring-opening (conversion) with acidic methanol before transesterification with sodium methoxide transforms glycidyl esters into 3-MPD esters, which form significantly minor yields of glycidol (minor side reaction) compared to 3-MCPD by ester cleavage. Additionally, using mild derivatization conditions neither glycidol formed from 3-MCPD nor original glycidol is transformed into the respective 3-HalPD.
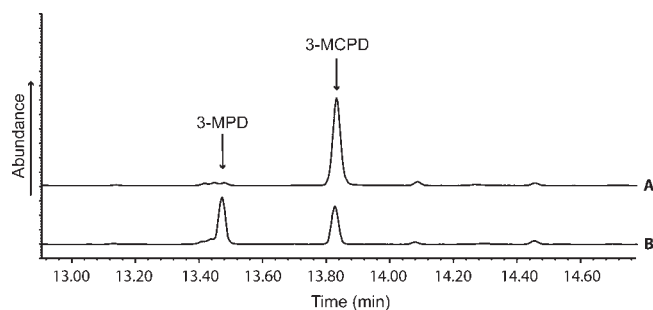

Figure 3. GC-MS chromatograms (extracted ion m/z 147) of a sample analyzed without glycidol conversion (A) and with the optimized procedure (B). In the case of A, the glycidol moiety is converted completely into 3-MCPD in contrast to B, differentiating both fatty acid esters of 3-MCPD and glycidol (as 3-MPD).

Figure 4. Full scan EI mass spectrum (background subtracted) of 3-MPD as PBA derivative. The ion m/z 192 corresponds to $[M]^+$.

In summary, regardless of the ring-opening reactions, interfering amounts of glycidol were formed in most cases due to the transesterification as observed in 3-MCPD, and no differentiation was obtained.

However, the acidic alcoholysis by methanol and sulfuric acid led to satisfying results. A clear differentiation of 3-MCPD esters and glycidyl ester was achieved, and this procedure was selected for further optimization.

In addition, the product of conversion, namely, 3-MPD, is available as a reference compound. All further experimental data (e.g., mass spec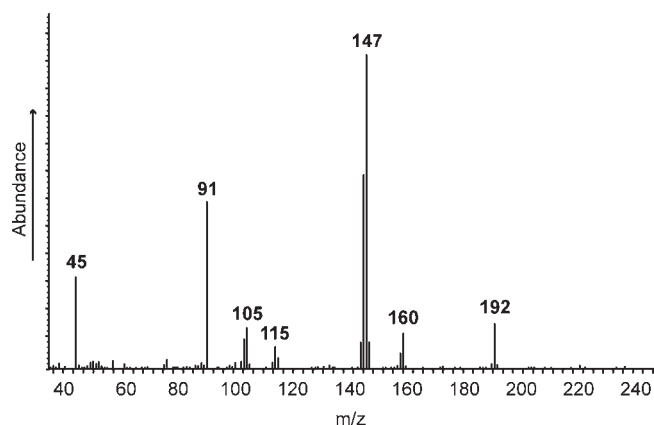tra, conversion rate, extraction yield, and degradation characteristics during transesterification) were obtained in comparison to 3-MPD reference compound. Therefore, both the reaction time and temperature of the ring-opening reaction were optimized. For time as well as temperature an optimum was identified with 30 min and 50 °C. Higher temperatures resulted in poor yields and large interferences.

The ring-opening reaction with methanol and sulfuric acid was found to be highly regioselective, and 3-MPD was formed predominantly with excellent reproducibility. These results could be confirmed with the reference compound 3-MPD.

Table 1. Results for 3-MCPD Esters and Glycidyl Esters in Validation Study for Edible Oils and Fats[a]

3-MCPD Esters					
spiking level (μg/kg)	160	395	790	1580	3160
measured (μg/kg) (RSD[b] (%))	160 ± 47 (29)	400 ± 61 (15)	750 ± 63 (8)	1625 ± 209 (13)	3083 ± 157 (5)
recovery (%)	100 ± 29	101 ± 16	95 ± 8	103 ± 13	98 ± 5
correl coeff of recovery function (R^2)	0.999				
LOD[c] (μg/kg)	50				
Glycidyl Esters					
spiking level (μg/kg)	150	350	700	1400	2750
measured (μg/kg) (RSD[b] (%))	142 ± 39 (28)	300 ± 35 (12)	642 ± 74 (11)	1333 ± 41 (3)	2730 ± 45 (2)
recovery (%)	94 ± 28	86 ± 12	92 ± 11	95 ± 3	99 ± 2
correl coeff of recovery function (R^2)	0.999				
LOD[c] [μg/kg]	65				

[a] Recovery values and concentrations at different spiking levels are shown as the average of all analyzed samples (n = 5; olive oil, sunflower oil, rapeseed oil, and sesame oil as well as margarine). Each product at each spiking level was investigated at least in duplicate per day, and three replicates of these series were performed for each on three different days. LOD was calculated by standard solutions based on signal-to-noise ratio (S/N) of 3:1. [b] Relative standard deviation of overall determinations (interday variation). [c] Corresponding to a sample weight of 200 mg. The first spiking level corresponds to limit of quantification.

Table 2. Results for 3-MCPD Esters and Glycidyl Esters in Validation Study for Fat-Rich Foodstuffs[a]

3-MCPD Esters					
spiking level (μg/kg)	50	125	250	501	1252	2505
measured (μg/kg) (RSD[b] (%))	44 ± 3 (7)	105 ± 9 (9)	260 ± 41 (16)	492 ± 29 (6)	1331 ± 132 (10)	2701 ± 153 (6)
recovery (%)	87 ± 6	84 ± 7	104 ± 16	98 ± 6	106 ± 11	108 ± 6
correl coeff of recovery function (R^2)	0.999					
LOD[c] (μg/kg)	8					
Glycidyl Esters						
spiking level (μg/kg)	55	138	276	551	1378	2756
measured (μg/kg) (RSD[b] (%))	54 ± 8 (15)	96 ± 12 (13)	253 ± 45 (18)	464 ± 90 (19)	1278 ± 182 (14)	2448 ± 304 (12)
recovery (%)	98 ± 15	70 ± 9	92 ± 16	84 ± 16	93 ± 13	89 ± 11
correl coeff of recovery function (R^2)	0.999					
LOD[c] (μg/kg)	15					

[a] Recovery values and concentrations at different spiking levels are shown as the average of all analyzed samples (n = 9; soup and gravy powders, bread, cookies/candy bar, and mayonnaise). Each product at each spiking level was investigated at least in duplicate per day, and three replicates of these series were performed for each on three different days. LOD was calculated by standard solutions based on signal-to-noise ratio (S/N) of 3:1. [b] Relative standard deviation of overall determinations (interday variation). [c] Corresponding to a sample weight of 10 g. The first spiking level corresponds to limit of quantification.

Minimization of Glycidol Formation. The ring-opening of glycidyl esters by methanol and sulfuric acid is an effective and reproducible procedure for differentiation of both 3-MCPD esters and glycidyl esters. Yields of glycidol formed by transesterification with sodium methoxide are observable in 3-MCPD esters as well as in 3-MPD esters anyway. To decrease these yields of glycidol the reaction time should be as rapid as possible. To prove the efficiency of ester cleavage with regard to the reaction time as well as the added volume of sodium methoxide, 1,2-dipalmitoyl-3-chloropropane, 1,2-dipalmitoyl-3-chloropropane-d_5, and glycidyl palmitate, each separated at five levels (0.025, 0.05, 0.1, 0.15, and 0.3 μg/mL MTBE, expressed as 3-MCPD and glycidol), were used as reference compounds to determine the rate of ester cleavage in olive oil with the described procedure in triplicate. As the result of the optimized procedure, a reaction time of 1 min proved to be the minimum required time for complete ester cleavage and the least glycidol formation.

Nevertheless, our results revealed the generation of considerable yields of glycidol even though very small volumes of sodium methoxide were used. Further investigations demonstrated a clear dependency of glycidol formation on 3-MCPD concentration. An exponential increase of glycidol formation and a decrease of 3-MCPD concentration could be observed as a function of time during transesterification by determination in triplicate of both 1,2-dipalmitoyl-3-chloropropane-d_5 and 1,2-dipalmitoyl-3-chloropropane as well as the resulting yields of 3-MPD. After 1 min, approximately 6 ± 1% of 3-MCPD was transformed into glycidol. In addition, our results implied a high influence of the derivatization procedure on differentiation of formed and original glycidol as described in the following.

Influence of Derivatization Procedure. Because a moderate temperature is crucial during the ring-opening of glycidyl esters with methanol and sulfuric acid, the glycidol moiety appears to be more stable at ambient than at higher temperature. This suggested
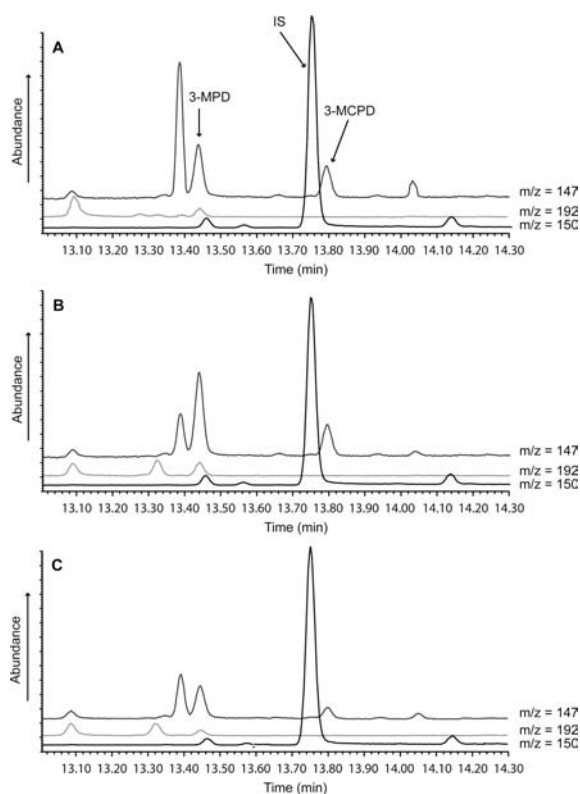
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Figure 5. GC-MS chromatograms (extracted ion) of a standard solution (A; 690 μg/kg) compared to a representative spiked (B; 690 μg/kg) and blank (C) sunflower oil sample. IS = 3-MCPD-d_5.

Figure 6. GC-MS chromatograms (extracted ion) of a standard solution (A; 550 μg/kg) compared to a representative spiked (B; 550 μg/kg) and blank (C) sample of gravy powder. IS = 3-MCPD-d_5.

a major influence of the derivatization procedure (especially derivatization temperature) on the determination and differentiation of formed and original glycidol.

All derivatization procedures using PBA described in the literature are performed at 80 °C or even higher.[18] Therefore, the derivatization procedure was carried out at ambient temperature in addition to 80 °C. During derivatization at 80 °C glycidol formed from 3-MCPD by transesterification was derivatized and detected, whereas none occurred during derivatization at ambient temperature. These results provided clear evidence of the influence of derivatization temperature. At ambient temperature PBA in NaCl solution rapidly derivatized diols, whereas ring-opening of the glycidol moiety did not proceed. The epoxide was not transformed into 3-MCPD at ambient temperature during derivatization, but immediate ring-opening occurred at higher temperatures. Hence, a differentiation between 3-MCPD esters and glycidol esters was successful. Glycidol formed during transesterification was not derivatized by PBA at ambient temperature, in contrast to 3-MPD and 3-MCPD. Furthermore, underivatized glycidol remains in the aqueous layer during subsequent hexane extraction.

Additionally, derivatization at ambient temperature compared to 80 °C demonstrated differences neither in sensitivity nor in precision determined by comparison of the peak area ratios for 1,2-dipalmitoyl-3-chloropropane or glycidyl palmitate as well as 1,2-dipalmitoyl-3-chloropropane-d_5 (n = 3 for each temperature with relative standard deviations of <1% overall). This result also proved excellent transformation of glycidyl esters into 3-MPD as well as small formation of glycidol during transesterification.
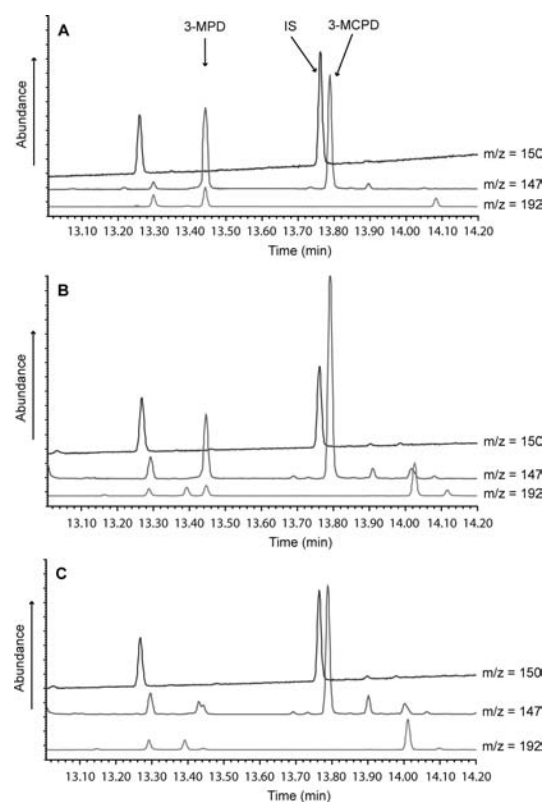
Hence, the derivatization was accomplished at ambient temperature together with the optimized procedures of glycidol conversion into 3-MPD as well as transesterification.

The optimized derivatization reaction was highly reproducible, showing a relative standard deviation of <5% by comparison of the slope of the calibration curves within the validation period. A derivatization time of 15 min was sufficient (determined as the maximum achievable intensity of analyte signals after derivatization with regard to the derivatization time), and no influence of formed glycidol was observed. Neither for 3-MPD esters (converted glycidyl esters) nor for 3-MCPD esters were any interfering side products observed at different tested concentrations after transesterification, underscoring the performance of the optimized procedure.

Chromatograms of a soup powder analyzed with and without conversion of the glycidol moiety by alcoholysis are shown exemplarily in Figure 3.

Additionally, the used aliquots of the organic extract (fat-rich foodstuffs) as well as fat or oil should not exceed a total fat content of 250 mg in order to derivatize both the analytes and the glycerol originated from triglycerides with an excess of PBA.

Results of the Validation. *Stability of Derivatives.* The formed phenyl boronates (1,3,2-dioxaborolane derivatives) of 3-MCPD and 3-MPD by derivatization with PBA were stable over a period of at least 5 days at ambient temperature as already shown in a previous study.[3]

Specificity. The characterizations of 3-MCPD, 3-MCPD-d_5, and 3-MPD were based on their retention times. Besides, peak area ratios of the m/z 147 and 196 ions (for 3-MCPD) and m/z 150 and 201 ions (for 3-MCPD-d_5) as well as m/z 147 and 192 ions

(for 3-MPD) were checked systematically. The differences between ratios m/z 147/196, 150/201, and 147/192 for the sample and the mean of the same ratios for the standards should not exceed 10%. Mostly, no interferences were observed on these characteristic ions due to the efficiency of purification as well as the selectivity of derivatization. In the case of interferences, the characteristic ions m/z 192, 196, and 201 could be used for calculation. The ions m/z 91 (3-MCPD, 3-MPD) and 93 (3-MCPD-d_5) as well as 104 (3-MCPD), 105, and 160 (3-MPD) were used only as qualifiers because interferences were observed in many cases. A full scan EI mass spectrum (background subtracted) of 3-MPD as PBA derivative is shown in Figure 4.

Analytical Characteristics of the Method. For the analysis of 3-MCPD esters or glycidyl esters, no performance criteria have been established so far. A series of experiments with regard to the linearity, LOD, LOQ, repeatability, and recoveries were performed to validate the proposed method with the optimized conditions. The results obtained are listed in Tables 1 and 2. Five miscellaneous oils and fats as well as nine foodstuffs of the mentioned categories were used for validation study.

Calibration curves were linear in the range studied, showing correlation coefficients of 0.999 or better. The achieved LOD permitted a sensitive determination of 3-MCPD esters and glycidol esters in foodstuffs as well as edible oils and fats after rapid transesterification and liquid extraction with NaCl solution 20%. Hence, an accurate and simultaneous determination of 3-MCPD esters and glycidyl esters was possible with the presented method in various foodstuffs. The observed lower recovery rates for glycidyl esters compared to 3-MCPD esters in foodstuffs (cf. Table 2) could be caused by matrix interferences on determination of 3-MPD. Similar results were not observed for fats and oils. During the chromatography or the mass spectrometric detection no influence was detected. This implies an evident influence of the foodstuff matrix on the determination of glycidyl esters. Nevertheless, the occurrence of these interferences was verified to be reproducible without any effects on the performance of the method as shown by the validation study.

Improvement of the LOD might be obtained using large-volume injection techniques.

As a result, the described method could be applied to a wide range of foodstuffs avoiding long sample preparation and several drying or cleaning steps as well as additional fat separation using 1,2-dipalmitoyl-3-chloropropane-d_5 as internal standard. No further sample purification was required as PBA reacts specifically with diols, forming nonpolar cyclic 1,3,2-dioxaboralane derivatives extractable into hexane. Because only one isotope-labeled internal standard is required, the described procedure is less expensive than others. Typical chromatograms of an oil sample as well as a gravy sauce sample are given in Figures 5 and 6.

The accuracy was confirmed with an excellent result by a proficiency test on the determination of 3-MCPD esters in edible oil organized by the European Commission Joint Research Centre IRMM. The assigned values of a contaminated palm oil test sample as well as a spiked extra virgin olive oil test sample (spiked with 3-MCPD-1,2-dioleate) were specified as 8.77 and 4.58 mg/kg, respectively.

With the optimized procedure, the determination resulted in 8.36 and 4.75 mg/kg for the contaminated palm oil test sample and the spiked extra virgin olive oil test sample, respectively. Without acidic alcoholysis the determination showed very high yields of 3-MCPD esters resulting from the formation of 3-MCPD from present glycidyl esters.

Table 3. Results of the Determination of 3-MCPD Esters and Glycidyl Esters in Conventional Samples Obtained with the Described Method

	glycidyl esters[a] (μg/kg)	3-MCPD esters[b] (μg/kg)
oxtail soup (powder)	307 ± 6	386 ± 15
gravy (powder)	72 ± 5	660 ± 44
sauce for chicken (powder)	67 ± 10	372 ± 6
vegetable soup (powder)	140 ± 1	231 ± 1
bread	<LOD	72 ± 1
cookies	939 ± 73	865 ± 4
cornflakes	<LOQ	36 ± 4
chocolate−hazelnut bar	74 ± 4	71 ± 1
mayonnaise	<LOD	<LOD
margarine	3625 ± 318	1417 ± 58
sunflower oil	850 ± 91	325 ± 35
olive oil (extra virgin)	1221 ± 214	<LOD
sesame oil	666 ± 80	345 ± 28
rapeseed oil	1175 ± 165	414 ± 37

[a] Calculated as 3-MPD with standard deviations of replicate determinations ($n = 3$). [b] Calculated as 3-MCPD with standard deviations of replicate determinations ($n = 3$).

Application to Real Samples. The developed method was applied to the analysis of different fat-rich foodstuffs as well as edible oils and fats. Results of the determination of 3-MCPD esters and glycidol esters in these samples are given in Table 3. Very high concentrations of glycidol esters were found in highly processed foodstuffs such as margarine and bakery products (e.g., cookies). Edible oils were contaminated in most cases as well, particularly one olive oil declared as extra virgin. Especially oil and fat samples contained considerably higher concentrations of glycidol esters than of 3-MCPD esters. As a result of deodorization, in oils and fats such amounts could be expected. Yields of glycidyl esters detected in foodstuffs could be caused by addition of fat (as an ingredient) as well as manufacturing and high temperatures. Further investigations are required to clarify the formation mechanisms and precursors of glycidyl esters in food products.

In summary, the described method for the simultaneous determination and differentiation of 3-MCPD esters and glycidyl esters is both simple and rapid in sample preparation, using a conversion step of the glycidyl moiety before derivatization. The use of a rapid ester cleavage and derivatization at ambient temperature were essential for the simultaneous determination of the analytes. Glycidyl esters were converted completely into 3-MPD, preventing the formation of additional 3-MCPD. Furthermore, only one isotope-labeled internal standard was required. This avoids the above-mentioned difficulties and allows the simultaneous determination and differentiation of 3-MCPD esters and glycidyl esters without the necessity of various standard substances or tedious and expensive multistage procedures.

Due to its simplicity, rapidity, and economy as well as the simultaneous extraction and determination of both 3-MCPD esters and glycidyl esters, the presented method can be used as a micromethod for routine analysis of various foodstuffs. The optimized method exhibits minimal sample preparation and low LOD including high precision for the analysis of these trace-level contaminants.

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■ ABBREVIATIONS USED

3-MCPD, 3-monochloropropane-1,2-diol; 3-MPD, 3-methoxy-propane-1,2-diol; GC-MS, gas chromatography—mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; PBA, phenylboronic acid; MTBE, methyl *tert*-butyl ether; NaCl solution 20%, sodium chloride solution 20%.

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