Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils

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A new method for the parallel determination of bound 2,3-epoxy-1-propanol (glycidol) and 2- and 3-chloropropanediol (2- and 3-MCPD) in oil matrices is presented. It is based on an improved alkaline catalysed release of MCPD and glycidol, followed by a transformation of glycidol to monobromopropanediol (MBPD), derivatisation with phenylboronic acid (PBA) and analysis by GC–MS. Quantification was performed using isotopic labelled standards. Method validation was carried out for the complete method procedure using glycidyl stearate and 3-chloropropanediol-1,2-bis-palmitoyl ester as reference compounds. Linearity was verified ($r^2>0.999$) within the concentration range from 0.1 to 5.7 mg/kg. The limit of detection (LOD) for bound glycidol was 0.025 mg/kg in absence of 3-MCPD. Due to a lack of certified reference material a validation of bound 2-MCPD analysis was not feasible, but an indirect approach yielded a semi-quantitative estimation. The method was applied to the analysis of several different oils. None of the analytes was detected in virgin or crude oils. In contrast bound MCPD and bound glycidol was detected ubiquitously in refined oils. The determined concentrations of 3-MCPD and glycidol in the tested samples varied in 2 orders and 4 orders of magnitude, respectively. Bound 2-MCPD was estimated to occur commonly in refined oils.

Practical application: The presented application is advantageous to other methods that are either not suitable for the parallel determination of bound glycidol and bound 2- and 3-MCPD within on step or are based on an estimation of bound glycidol contents by calculation. In comparison to those methods based on the direct determination of the single mother compounds this approach to convert the eventually unclear assembly of different MCPD- and glycidyl derivatives into the basic analytes glycidol and 2- and 3-MCPD simplifies identification and quantification, enhances the analytical sensitivity and lowers the risk of underestimation. Furthermore, the investigation of basic transformations that may occur during sample preparation can be helpful for a better understanding of the complex chemistry in MCPD and glycidol analysis. Results from analysis of different oils and fats can be used for an estimation of the occurrence and distribution of bound MCPD and bound glycidol.

Keywords: Bound glycidol / Bound 3-MCPD / Induced 3-MCPD / Bound 2-MCPD / Refined oil

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1 Introduction

Since 2006 the occurrence of 3-MCPD esters in refined oils was reported [1], and different determination methods have been published. Meanwhile most common is either an acidic or alkaline catalysed transesterification, followed by derivatisation of the released 3-MCPD and determination via GC–MS [2, 3]. Another approach is the determination of single 3-MCPD esters and glycidyl esters using liquid chromatography–MS (LC–MS) [4] or LC with time-of-flight MS (LC–MS–TOF) [5]. Overestimations of 3-MCPD that were observed after alkaline treatment when the sample preparation was carried out in presence of inorganic chloride led to the hypothesis that...
reactive compounds like glycidyl derivatives appearing in refined oils and fats may cause this effect [6]. The presence of different glycidyl fatty acid esters in refined oils and fats was reported by Weißhaar and Perz in 2009 [7]. Furthermore, the authors presented an indirect determination to estimate the overall amount of glycidyl esters. This approach is based on the effect that glycidol and glycidyl derivatives can be eliminated from samples by an acidic pre-treatment, whereas the amount of 3-MCPD remains unchanged and can be determined as the ‘true’ 3-MCPD value. The elimination of glycidyl derivatives as well takes place using methods which include an acidic catalysed transesterification. If the acidic pre-treatment is not applied, alkaline catalysed transesterification or saponification will release 3-MCPD as well as glycidol. The latter can be converted into additionally induced 2- and 3-MCPD by reaction with chloride in acidic solution. The amount of glycidol that originates from glycidyl derivatives can be calculated by subtracting the true 3-MCPD value from that one that is representing the sum of true and induced 3-MCPD. This difference has to be corrected by multiplication with a stoichiometric factor of 0.67 which results from the assumption that glycidol can be converted completely into 3-MCPD during sample preparation. The method C III 18 (09) of the German Society of Fat Science is based on this procedure [8]. Beside many advantages of this indirect determination some restrictions have to be taken into account. Firstly, the calculative combination of two differently achieved 3-MCPD values may cause a relatively high uncertainty of measurement. This excludes the accurate determination of glycidol in the presence of dominant amounts of 3-MCPD. Furthermore, the combination of acidic sample pre-treatment with the use of chloride during sample preparation for analysis on true 3-MCPD theoretically contains the risk to overestimate this analyte in the case either that glycidyl derivatives are not eliminated completely or that 3-MCPD derivatives are generated during acidic pre-treatment from glycerol derivatives and sample related chloride. A general disadvantage of all methods based on a conventional alkaline treatment is the partial conversion of 3-MCPD to glycidol. In dependence of the way the further sample preparation is performed, this undesired reaction may change the original distribution between glycidol and 2- and 3-MCPD.

Due to the fact that 3-MCPD- and glycidyl esters are undesired compounds which possibly occur widespread in refined or heated oils, fats and oil and fat containing food, it is desirable to reduce the generation of these contaminants during the refining steps or to eliminate them at a later stage of product processing. Data received from investigations concerning the influence of different physical or chemical treatment of oils and fats indicate that the generation as well as the decrease in 3-MCPD- and glycidyl derivatives is not correlated to each other as closely as one might have estimated [7]. Therefore, a sensitive and accurate analytical method is desirable which allows the direct determination of both 3-MCPD derivatives as well as glycidyl derivatives independent of each other.

This paper reports a novel method for the sensitive and accurate measurement of glycidyl derivatives (determined as glycidol) and 3-MCPD derivatives (determined as 3-MCPD) in oils and fats, using 3-bromopropane-1,2-diol-d$_5$ (3-MBPD-d$_5$) as surrogate standard for glycidol and 3-chloropropanediol-1,2-bis-palmitoyl ester-d$_3$ as surrogate standard for 3-MCPD. Validation data indicate good linearity, repeatability and low limits of quantification. The determination of 2-MCPD derivatives is part of the method, but has to stay semi-quantitative until an isotopic labelled internal standard compound is available. The application to several different oils and fats showed a heterogeneous distribution of the analytes.

# 2 Materials and methods

## 2.1 Chemicals and solvents

3-Chloropropane-1,2-diol (99%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany), 3-chloropropane-1,2-diol-d$_5$ (90%, isotopic purity 99%) from CIL (Andover, USA) 3-chloropropanediol-1,2-bis-palmitoyl ester (98%) and 3-chloropropanediol-1,2-bis-palmitoyl ester-d$_3$ (97%, isotopic purity 99%) from TRC (North York, Canada), glycidol (96%) and 3-bromopropane-1,2-diol (97%) from Sigma-Aldrich (Taufkirchen, Germany), glycidyl stearate (>95%) from TCI Europe (Zwijndrecht, Belgium). All other supplied reagents and solvents were of analytical purity.

## 2.2 Solutions of chemicals

Methanolic sodium hydroxide solution I: 250 mg sodium hydroxide dissolved in 100 mL methanol (MeOH).

Acidified sodium bromide solution I: 3.33 mL orthophosphoric acid (85%) added to 1 L of aqueous sodium bromide solution (500–600 g/L). 600 µL of this solution have to neutralise 350 µL of the before mentioned sodium hydroxide solution 2.2.1 (2.5 mg/mL in MeOH) and adjust the pH-value to the acidic range.

Phenyboronic acid (PBA) saturated in diethyl ether: approximately 200 mg PBA is added to 10 mL of diethyl ether in a screw cap vial. After efficient shaking of the mixture non-dissolved PBA is allowed to settle and may remain as precipitation. For derivatisation purposes the clear supernatant is used.

Methanolic sodium hydroxide solution II: 2 g sodium hydroxide dissolved in 100 mL MeOH.

Acidified sodium bromide solution II: 35 mL sulphuric acid (25%) is added to 965 mL of aqueous sodium bromide solution (500–600 g/L). 600 µL of this solution have to neutralise 200 µL of the before mentioned sodium hydroxide solution 2.2.4 (20 mg/mL in MeOH) and adjust the pH-value to the acidic range.

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2.3 Solutions of standard and spiking compounds

Standard solution AI: 3-MCPD-d₅: 10 µg/mL in MeOH.
Standard solution AII: 3-MBPd-d₅: 10 µg/mL in MeOH.
Standard solution BI: 3-MCPD-1,2-bis-palmitoyl ester-d₅: 26.9 µg/mL in toluene (proportional 5.0 µg/mL free 3-MCPD).

Spiking solutions were prepared by diluting stock solutions of 3-MCPD-1,2-bis-palmitoyl ester (5.33 mg/mL in toluene) and glycidyl stearate (4.60 mg/mL in toluene), respectively, to the desired concentration using toluene as solvent.

2.4 Samples

Samples were purchased in 2010 from various local and international suppliers.

2.5 Synthesis

3-Bromopropane-1,2-diol-d₅ was synthesised in microscale by conversion of 3-MCPD-d₅ to glycidol-d₅ and reaction of the latter with an acidified sodium bromide solution: 4 mL of a solution of 3-MCPD-d₅ (1 mg/mL in MeOH) was placed in a screw cap vial and the solvent carefully evaporated just to dryness under a gentle stream of nitrogen. The residue was redissolved in 400 µL of a methanolic solution of sodium hydroxide (2.2.4) and allowed to stay at RT for 16–24 h. The thereby generated glycidol-d₅ reacted under acidic conditions to 3-MBPd-d₅ and a smaller amount of 2-MBPd-d₅ by addition of 1.2 mL sulphuric sodium bromide solution (2.2.5). After a reaction time of 15 min the mixture was extracted three times with 600 µL of ethyl acetate each. The combined extracts were dried over anhydrous sodium sulfate and the solvent carefully evaporated just to dryness under a gentle stream of nitrogen. The residue was redissolved in 2 mL MeOH. To determine the completeness of the reaction, an aliquot of the analyte solution was spiked with not deuterated 3-monobromopropanediol (3-MBPd) and 3-MCPD, followed by derivatisation with PBA and GC–MS analysis. Quantification via 3-MBPd yielded the concentration of 3-MBPd-d₅, whereas quantification of the not reacted 3-MCPD-d₅ via 3-MCPD showed, if undesired remains of the educt were present. A removal of 2-bromopropane-1,3-diol-d₅ was not necessary. The corresponding mass spectra of all monobromopropanediol (MBPd) isomers are displayed in Fig. 1.

2.6 Alkaline catalysed transformation of 3-MCPD

A series of blank sesame oil (100 mg per sample) was spiked with 3-MCPD at the 10 mg/kg level and treated with 200 µL of 2% sodium hydroxide in MeOH at RT by increasing reaction time. Matrix removal, extraction, derivatisation and detection of the analytes were performed as described in Section 2.7 but using acidified sodium bromide solution II (2.2.5) instead of acidified sodium bromide solution I (2.2.2).

2.7 Real sample preparation and derivatisation

2.7.1 Aliquotation

Liquid oil or fat samples were processed directly. Solid material was melted smoothly until homogenisation had taken place at approximately 80°C in a drying chamber or using a water bath. For high-melting fats or waxes the applied temperature was increased carefully in 10°C steps until the melting process starts. Solid samples which contain higher amounts of water, resulting in a phase separation after melting, were aliquoted in solid state. From each sample two 100 mg (±0.5 mg) aliquots were weighed each into a 1.5 mL screw cap vial.

2.7.2 Procedure (spiking, ester cleavage, matrix clean up, derivatisation)

Assay A was spiked with 50 µL of surrogate standard solution A1 and A2 each, Assay B was spiked with 100 µL of surrogate standard solution B I. After adding 600 µL of diethyl ether to each assay both reaction vessels were shaken until the sample material was completely dissolved. To dissolve high melting sample material it was tempered lukewarm.

The mixtures were stored for minimum 30 min in a freezer to cool down to −22 to −25°C (a precipitation of sample material at this stage of sample preparation was unproblematic). Three hundred and fifty microlitres of methanolic sodium hydroxide solution (2.2.1) that had been cooled down to −22 to −25°C was added to each assay. To complete the ester cleavage the vials were sealed, shaken shortly and allowed to stay at minimum for another 16 h at −22 to −25°C. The reactions were stopped by addition of 600 µL of acidified sodium bromide solution (2.2.2) that had been cooled down to −22 to −25°C C to each of both assays. The mixtures were shaken shortly and placed under a gentle stream of nitrogen for a few minutes to reduce the volume of the organic phase to approximately 100 µL. Six hundred microlitres of iso-hexane was added to both assays, the vials were sealed and shaken vigorously. The mixtures were allowed to remain at RT for approximately 5 min, shaken again vigorously, followed by quantitative separation and dischargement of the organic phases using Pasteur pipettes. This step was repeated with another 600 µL of iso-hexane for both assays.

Using new Pasteur pipettes, the remaining aqueous phases of assay A and B were extracted three times with 600 µL of a mixture of diethyl ether/ethyl acetate each (e.g. 600 µL/mL diethyl ether; 400 µL/mL ethyl acetate) each. The composition and amount of extraction solvent can be varied. For each assay the organic extracts of every assay without fractions of the aqueous phases were combined in
Figure 1. Mass spectra of phenylboronic acid derivatives of 2- and 3-MBPD isomers (mass range: $m/z = 93–300$).
a new screw cap vial containing a small amount of anhydrous sodium sulphate resulting in two organic extracts (A and B). If the drying agent got sticky, the solution was transferred into a new screw cap vial with fresh sodium sulphate.

2.7.3 Derivatisation

Derivatisation was achieved by addition of 10–100 μL of the derivatisation reagent (2.2.3) to both extracts resulting (A and B) from 2.7.2. The amount of derivatisation agent can be adapted to the capability of the chromatographic system. To complete the derivatisation reaction and to remove excess reagent both assays were evaporated to dryness using a gentle stream of nitrogen. Soluble fractions were redissolved subsequently in approximately 500 μL iso-octane. For GC/MS measurement one part of each solution was transferred in a 200 μL microinsert.

2.7.4 GC–MS

For this work, a HP 7890A GC system with 5975C mass selective detector and a Gerstel split/splitless PTV injection system were used. Separation of the analytes was performed either on a Restek Rxi\textsuperscript{18}-17 GC column (30 m, id: 0.25 mm, film thickness: 0.25 μm with 2.4 m pre-column: Agilent J&W GC column, HP-5MS, id: 0.32mm, film thickness: 0.25 μm) or a Agilent J&W GC column (30 m, id: 0.25mm, film thickness: 0.25 μm with 2.4 m pre-column: Agilent J&W, GC column, HP-5MS, id: 0.32mm, film thickness: 0.25 μm). Carrier gas was Helium 5.0 with a constant flow of 1.2 mL/min. 2 μL sample was injected in pulsed splitless mode (injection pulse pressure: 25 psi), purge flow: 3 mL/min; 50 mL/min after 0.75 min. PTV temperature program: 80 °C, with 300 °C/min to 165 °C, 10 min isothermal, with 300 °C/min to 320 °C, 8 min isothermal. GC oven temperature program: 90 °C, isothermal 0.1 min, with 7 °C/min to 175 °C, isothermal 1.0 min with 20 °C/min to 290 °C, isothermal 8.3 min. Two hundred and eighty degree celsius was the temperature of the transfer-line, 230 °C the temperature of the ion source, 150 °C the temperature of the quadrupole. The mass selective detector was used for selected ion monitoring, focussing on the ions with a mass to charge ratio (m/z) of 147 (target) \( m/z = 146, 196, 198 \) (qualifiers) for 3-MCPD,

\[
m/z = 150 \text{ (target) } m/z = 149, 201, 203 \text{ (qualifiers) for 3-MCPD-d}_3,
\]

\[
m/z = 196 \text{ (target) } m/z = 198 \text{ (qualifier) for 2-MCPD},
\]

\[
m/z = 240 \text{ (target) } m/z = 242 \text{ (qualifier) for 3-MBPD},
\]

\[
m/z = 245 \text{ (target) } m/z = 247 \text{ (qualifier) for 3-MBPD-d}_3.
\]

2.7.5 Quantification

Quantification of bound 3-MCPD was carried out by multiplying the ratio of signal areas of 3-MCPD and 3-MCPD-d\textsubscript{3} based on corresponding ion traces with the spiking amount of 3-MCPD-d\textsubscript{3} in assay B. Quantification of bound glycidol was carried out by the analogue determination of glycidol induced 3-MBPD via 3-MBPD-d\textsubscript{3} in assay A and applying a transformation factor representing the amount of 3-MBPD that is generated from glycidol under the conditions of sample preparation. Due to the circumstance that no esterified 3-MBPD-d\textsubscript{3} was available as internal standard, the rate of 1,2-bis-palmitoyl ester cleavage was determined as the ratio of 3-MCPD measured in assay A and assay B. The ratio of responses of 3-MCPD-d\textsubscript{3} in assay A and 3-MBPD-d\textsubscript{3} in assay B served to determine the undesired transformation of released 3-MCPD to glycidol.

3 Results and discussion

3.1 Method adaption for the determination of bound glycidol

With the intent to determine the content of bound glycidol in oily sample matrices, two general approaches seem to be promising. One is the determination of single glycidyl fatty acid esters using GC–MS or LC–MS techniques, the other way is to determine free glycidol that has been liberated from its derivatives analogue to the analytical way that is usually applied to the determination of bound 3-MCPD. The first approach has the advantage to be more direct, needs presumably a minor degree of sample preparation and thereby bears a lesser risk of overestimation. A disadvantage can be seen in the instance that, in purpose of proper quantification, several reference and standard compounds have to be available, making quantification more complex. Even more important is the point of principle that unknown glycidyl derivatives may occur in differently treated real samples. Without knowledge about their analytical behaviour these compounds would possibly be not detected and thereby underestimations or false negative results could result.

The analysis of released glycidol as the second approach includes chemical reactions making this analysis more indirect and encloses more sources of error during sample preparation. Furthermore, free glycidol is difficult to determine in its original shape due to its non-specific atomic composition, small molecular size and high instability. On the other hand, it has to be considered that the determination of released glycidol allows the acquisition of all glycidyl derivatives that undergo alkaline catalysed ester cleavage independently if the single compounds are known or not. The accumulation of a whole group of compounds to one analyte should furthermore have the benefit of lower detection limits in comparison to the single mother compounds.

We decided to follow the second approach, because in respect to the potentially harmful nature of glycidyl esters the determination of released glycidol seemed to minimise the risk of underestimating this compound. Since it seemed to be reasonable to go an analytical way that includes MCPD...
derivatives as well as glycidyl derivatives in one method, some challenges appeared. The first question was, how to detect free glycidol that tends to degrade or polymerise and, furthermore, would be difficult to separate from organic solvents due to its low boiling point (62–65°C). Here we took benefit from its extraordinary reactivity in acidic aqueous solutions by converting it into bromopropanediol using concentrated aqueous solutions of sodium bromide. This reaction turned out to be reproducible, rugged and almost quantitative. It yielded 3-MBPD as major product whereas 2-monobromo-propanediol (2-MBPD) was detected in minor intensity. 3-MBPD is highly stable, shows excellent GC–MS behaviour and exhibits a homologue structure to 3-MCPD. This allows applying the same sample preparation, derivatisation and analytical conditions for both analytes. Furthermore the synthesis of deuterated MBPD was easy to realise. In Fig. 2 is displayed a chromatogram of derivatised 3- and 2-MBPD resulting from the conversion of glycidol (≥1 μg/mL) by reaction with inorganic bromide in acidified aqueous solution followed by extraction and derivatisation of the analytes and GC–MS analysis. Detection of the molecular ions showed properly shaped intense signals. Due to the presence of naturally occurring Br79 and Br81 both MBPD homologues are represented by two molecular ions of nearly the same intensity. The signal ratio 3- to 2-MBPD seemed to be constant under same reaction conditions and varied slightly from 1: 0.14–0.20 under various reaction conditions. These results indicated an excellent suitability of the glycidol induced 3-MBPD for sensitive and specific GC–MS detection of glycidol.

A major challenge was to avoid the transformation of parts of the original 3-MCPD content to additional glycidol during alkaline treatment. This effect was investigated by treating 3-MCPD in a blank oil matrix with a low concentrated solution of sodium hydroxide in MeOH (solution 2.2.4). In Fig. 3 is exhibited that the transformation of 3-MCPD reached 25% after 3 min of reaction time and nearly 50% after 9 min of alkaline treatment. One hour of reaction time caused a 3-MCPD transformation of >99%. Vice versa, the amount of glycidol generated 3-MBPD increased in adequate correlation. Triple replication of the analyses showed relative SD of 0.2–2.2% for the determined 3-MCPD values and 0.8–2.4% for the determined glycidol values. Taking into account a stoichiometric factor of 1.4 that represents the higher molecular mass of MBPD in comparison to MCPD the determined values exhibited reaction yields of 87–93% for 3-MBPD. The occurrence of 2-MBPD in the same signal ratio as it is shown in Fig. 2 lead to the conclusion that the transformation of the intermediate glycidol into MBPD can be assumed as almost quantitatively.

The transformation of 3-MCPD to glycidol during alkaline treatment excluded the exact determination of the original content of glycidol in oil and fat samples under common reaction conditions. Assuming that the
transformation of 3-MCPD to glycidol exhibits a higher activation energy than the alkaline catalysed cleavage that releases MCPD and glycidol from its derivatives, it seemed to be promising to lower the alkaline strength of the reagent and the reaction temperature with the aim to prevent generation of glycidol out of the liberated MCPD. This was achieved successfully by carrying out a smooth alkaline catalysed ester cleavage at −25°C using a more diluted solution of sodium hydroxide in MeOH. Under these conditions the degradation of 3-MCPD became negligible even though the reaction time had to be extended significantly to guarantee a sufficient reaction rate. To prove the efficiency of ester cleavage, 3-MCPD-1,2-bis-palmitoyl ester-d5 was taken as model compound to determine the rate of ester cleavage in sesame oil in dependence from the reaction time. The results presented in Table 1 indicate within the range of measurement uncertainty an almost quantitative ester cleavage when the reaction was carried out for 15 h or longer. In parallel the generation of glycidol-d5 (determined as 3-MBPD-d5) increased with the reaction time but was estimated to be negligible for reaction times <25 h.

### 3.2 Validation

Validation data were achieved by analysing spiked blank sesame oil samples in triple replication. Eight equidistant spiking levels ranged from 0.46 to 26 mg/kg glycidyl stearate (equal to 0.1–5.7 mg/kg glycidol) and from 0.56 to 31 mg/kg 3-MCPD-1,2-bis-palmitoyl ester (equal to 0.1–5.7 mg/kg 3-MCPD). To take into account the influence of 3-MCPD on the determination of bound glycidol, the procedure was repeated for the determination of glycidyl stearate in the presence of 26.6 mg/kg 3-MCPD-1,2-bis-palmitoyl ester (equal to 5.0 mg/kg free 3-MCPD). The linearity of the whole analytical procedure was determined with correlation coefficients >0.999 for bound glycidol, bound 3-MCPD and bound glycidol in the presence of 5 mg/kg bound 3-MCPD. One example is presented in Fig. 4. Matrix matched limit of detection (LOD) values were taken as analyte concentrations related to a ratio of signal to noise ≥3, whereas matrix matched LOQ values were taken as analyte concentrations related to a ratio of signal to noise ≥10. Precision and repeatability was determined by 10-fold analysis of refined safflower oil containing 0.7 mg/kg of bound glycidol and 1.0 mg/kg of bound 3-MCPD. A summary of the received validation data is displayed in Table 2.

The method was applied to the determination of bound 3-MCPD in five edible oils and fats within a recent proficiency test arrange by the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung/BfR) in Berlin. The resulting z-scores for the presented method were in the range from −0.62 to +0.35. Bound glycidol was not included in this test.

### 3.3 Determination of bound 3-MCPD and bound glycidol in oils and fats

Since the method validation was successfully completed, several different edible oils and fats have been analysed. With regard to the limits of detection nearly all non-heated and non-refined oils and fats were free of bound glycidol.

#### Table 1. Rate of 3-MCPD-1,2-bis-palmitoyl ester-d5 cleavage and glycidol-d5 generation in sesame oil under smooth reaction conditions

<table>
<thead>
<tr>
<th>Reaction time (h)</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>40</th>
<th>50</th>
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<tr>
<td>Induced glycidol-d5 (µg/kg)</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
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<tr>
<td>Ester cleavage (%)</td>
<td>89</td>
<td>97</td>
<td>95</td>
<td>99</td>
<td>99</td>
<td>101</td>
<td>101</td>
<td>100</td>
<td>98</td>
</tr>
</tbody>
</table>

**Figure 4.** Calibration graph for bound glycidol in the presence of 3-MCPD (Triple replication exhibited relative SD of 1.0–3.0% for the determined values).
(<0.025 mg/kg) and bound 3-MCPD (<0.05 mg/kg). This was tested for the following non-refined oils and fats: avocado, butter, cacao, castor plant, caraway, fish (caplin, salmon), manila, olive, palm, paprika, para nut, pumpkinseed, rapeseed, sesame, sunflower, walnut.

In refined oils and fats bound MCPD and bound glycidol was detected almost ubiquitously but with widely varying concentrations. Certain refined oils like soy bean oil, rapeseed oil, and palm kernel oil for example contained bound 3-MCPD as well as bound glycidol commonly in a relatively low concentration range between <LOQ and 1 mg/kg. Other refined oil products (corn oil, sunflower oil, coconut oil, safflower oil, olive oil) more often carried one or the other analyte in a concentration range >1 mg/kg. Refined palm oil inhabited bound 3-MCPD frequently in higher concentrations between 2 mg/kg and 10 mg/kg and bound glycidol in a broader range of concentration between 0.5 and 20 mg/kg. Some other kinds of oil were available only in a few numbers of samples so that analysis could give just hints for the occurrence of MCPD and glycidol but no conclusions in regard to the comparability of the results could be made. However, some of the analysed samples, namely dietary supplement salmon oil capsules, refined walnut oil and refined hazelnut oil carried in single cases unexpected high loads of bound 3-MCPD >10 mg/kg. Notably was the finding that in none of the tested salmon oil, walnut oil and hazelnut oil samples comparably high amounts of bound glycidol were detected. An overview about some of the test results is presented in Tables 3 and 4.

In general no clear dependency between the detected content of bound 3-MCPD and bound glycidol was evident. The matrix dependant level of bound 3-MCPD appears to be less varying than the content of bound glycidol. Furthermore, a significant lower maximum level for the occurrence of bound 3-MCPD seems to exist in comparison to that one of bound glycidol. Within the samples that were investigated the determined 3-MCPD values never exceeded significantly 20 mg/kg, excluding one exception where a value of 55 mg/kg was found. In comparison to these findings bound glycidol was determined several times in the range of some 100 mg/kg or even above in the low per mill area. The extraordinary high amounts of bound glycidol seemed to appear mostly in palm oils being processed under experimental conditions.

### 3.4 Estimation of bound 2-MCPD in edible oils and fats

Seefelder [9] reported that 2-MCPD was found frequently in refined oils. In seed oils, the detected levels were mostly <150 μg/kg, in refined palm oil >1 mg/kg, respectively. Furthermore, it was shown that by raise of deodorisation temperature in palm oil the amount of 2-MCPD esters as well as the ratio of 2- and 3-MCPD esters was enhanced. The occurrence of bound 2-MCPD was investigated within the previously reported analysis of different oils and fats using the method that is presented in this work. Each of the tested samples that contained 3-MCPD above the limit of quantification showed 2-MCPD in detectable amounts as well. Quantification of bound 2-MCPD was not feasible due to the lack of commercially available pure reference material. However, a rough estimation seemed to be possible based on the unproved assumption that 2-MCPD shows largely similar

### Table 2. Summary of validation data

<table>
<thead>
<tr>
<th></th>
<th>Linearity ($r^2$)</th>
<th>LOD (mg/kg)</th>
<th>LOQ (mg/kg)</th>
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<td>0.9996</td>
<td>&lt;0.025</td>
<td>&lt;0.100</td>
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<td>Bound glycidol + 5 mg/kg bound 3-MCPD</td>
<td>0.9994</td>
<td>0.050</td>
<td>&lt;0.100</td>
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<td>Bound 3-MCPD</td>
<td>0.9996</td>
<td>0.050</td>
<td>0.100</td>
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</table>

<table>
<thead>
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<th>Precision (n=10) rsd (%)</th>
<th>Repeatability (n=10) rsd (%)</th>
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</thead>
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<td>Bound glycidol (0.7 mg/kg in safflower oil)</td>
<td>1.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Bound 3-MCPD (1.0 mg/kg in safflower oil)</td>
<td>0.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

### Table 3. Range of levels of bound glycidol and bound 3-MCPD in different refined oils (n ≥ 20 each), estimated range of levels of bound 2-MCPD in different refined oils (n ≥ 5 each; palm oil: n ≥ 20)

<table>
<thead>
<tr>
<th>Refined</th>
<th>Bound glycidol (mg/kg)</th>
<th>Bound 3-MCPD (mg/kg)</th>
<th>Bound 2-MCPD estimated (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy bean oil</td>
<td>&lt;0.1–0.6</td>
<td>&lt;0.1–0.5</td>
<td>&lt;LOQ–0.1</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>&lt;0.1–0.3</td>
<td>&lt;0.1–1.0</td>
<td>&lt;LOQ–0.3</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>&lt;0.1–0.4</td>
<td>0.1–2.1</td>
<td>&lt;LOQ–0.3</td>
</tr>
<tr>
<td>Palm oil</td>
<td>0.3–18</td>
<td>1.1–10</td>
<td>0.2–5.9</td>
</tr>
</tbody>
</table>
To consider different molecular ion signal responses of 

derivatised 3- and 2-MCPD in mass spectrometric 
detection, the true concentration proportional signal 

ratio between the PBA derivates of 2- and 3-MCPD was 
determined by GC analysis using a flame ionisation 
detector. This indicated that under the applied GC–MS 

conditions 2-MCPD as PBA derivative showed nearly the 
double mass to signal ratio than 3-MCPD as PBA derivative.

The estimation of bound 2-MCPD contingents in differ-

ent refined oils supported the results of Seefelder [9]. The 
determined contingents of bound 2-MCPD were in the range 
of 10–60% of the 3-MCPD levels. Especially in those samples 

containing higher levels of bound 3-MCPD the overall 
amount of MCPD increased significantly by the presence 
of bound 2-MCPD. An overview of the estimation of bound 

2-MCPD is given in Tables 3 and 4.

4 Conclusions

The combination of a smooth alkaline catalysed ester 

cleavage, the transformation of released glycidol into 

MBPD and the usage of isotopic labelled MBPD as surrogate 

standard compound approved the sensitive and accurate 

measurement of bound glycidol (determined as glycidol) 

and bound 3-MCPD derivatives (determined as 3-MCPD) 

in oils and fats. Further optimisation of the method may be 

achieved by replacement of MBPD-d5 by an isotopic labelled 

glycidyl ester and the use of an isotopic labelled 2-MCPD 

derivative. The analysis of different oils and fats showed in 

line with former investigations [2] that bound glycidol 

and bound MCPD seems not to occur in crude or native 

oils and fats. In this context the applied method reached for 

bound glycidol notably low detection limits of 25

m g/kg. In refined oils and fats all investigated analytes were 
detected widespread. A maximum concentration range of 

approximately up to 30 mg/kg bound MCPD was deter-

mined casually in commercially available pure walnut oil 

and in the one tested hazelnut oil. In general, the appearance 
of bound glycidol and bound MCPD seemed to correlate but 

not the corresponding concentrations. In real samples, the 
determined contents of bound 3-MCPD varied over two 

orders of magnitude and normally seemed not to exceed 
a certain level whereas bound glycidol was found in signifi-

cant higher maximum amounts. These findings suggest 

the requirement of analogue reaction conditions for the

\begin{table}

<table>
<thead>
<tr>
<th>Table 4. Single levels of bound glycidol, bound 3-MCPD and estimated bound 2-MCPD in different oils and fats; <strong>bold</strong>: values &gt;1 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refined/processed</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Apricot kernel oil</td>
</tr>
<tr>
<td>Borage oil</td>
</tr>
<tr>
<td>Coconut oil</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Salmon oil in dietary supplement capsules</td>
</tr>
<tr>
<td>Evening primrose oil</td>
</tr>
<tr>
<td>Hazelnut oil</td>
</tr>
<tr>
<td>Grape kernel oil</td>
</tr>
<tr>
<td>Olive oil</td>
</tr>
<tr>
<td>Palm kernel oil</td>
</tr>
<tr>
<td>Palm oil degummed and bleached</td>
</tr>
<tr>
<td>Peanut oil</td>
</tr>
<tr>
<td>Rose hip oil</td>
</tr>
<tr>
<td>Safflower oil</td>
</tr>
<tr>
<td>Sheabutter</td>
</tr>
<tr>
<td>Walnut oil</td>
</tr>
<tr>
<td>Wheat germ oil</td>
</tr>
<tr>
<td>Crude/pristine Oil or fat: avocado, butter, cacao, castor plant, caraway, fish (caplin, salmon), lard, linseed, manila, olive, palm, paprika, para nut, pomegranate kernel, pumpkin eed, rapeseed, sesame, sun lower, walnut</td>
</tr>
</tbody>
</table>

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generation of both groups of analytes but a different pathway of generation. It is considerable that the formation of bound glycidol needs only common MAG as precursors whereas the generation of MCPD derivatives requires certain precursors or co-reactants that are matrix dependently available in stricter limited amounts. Bound 2-MCPD was estimated to exceed frequently the mg/kg level in refined oils containing higher amounts of bound 3-MCPD. These results indicate that bound 2-MCPD may occur in refined oils and fats in amounts that suggest an assessment about the potential effects of this compound in the human metabolism. It seems to be that the ratio 2-MCPD/3-MCPD can vary significantly so that bound 2-MCPD should be detected as a separate analyte. Further investigations to prove the above assumptions should be made.

In regard to common analysis of 3-MCPD esters it was shown how fast alkaline treatment at RT converts 3-MCPD and 3-MCPD-d₅ into glycidol. Applying to long reaction times this effect may cause significant losses of analytes during sample preparation in absence of chloride. If chloride is present during sample preparation, the induced glycidol will re-react to MCPD but the original distribution of 2- and 3-MCPD is lost. This information is important for a better understanding of the complex chemistry in MCPD and glycidol analysis.

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References
