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ARTICLE

Rapid and Simple Micromethod for the Simultaneous Determination of 3-MCPD and 3-MCPD Esters in Different Foodstuffs

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This paper describes for the first time a micromethod for the simultaneous determination of 3-monochloropropane-1,2-diol (3-MCPD) and fatty acid esters of 3-MCPD (3-MCPD esters) in different foodstuffs. 3-MCPD and 3-MCPD esters were isolated from food products using a single extraction step separating hydrophilic and lipophilic compounds. An aliquot of the aqueous layer was analyzed for the content of 3-MCPD while a part of the organic layer was analyzed for 3-MCPD esters after cleavage with sodium methoxide. After a simple derivatization procedure with phenylboronic acid (PBA), the determination was achieved by isotope dilution GC-MS using isotope-labeled 3-MCPD and 3-MCPD ester as internal standards. The method was validated for various foodstuffs like bakery products, meat and fish products, and soups as well as seasonings with LOD of 1−2 μg/kg (3-MCPD) and 6 μg/kg (3-MCPD esters), respectively. Recoveries ranged within 95 ± 9% and 96 ± 10% at spiking levels of 15 and 25 μg/kg in all matrices for 3-MCPD and 84 ± 9% and 85 ± 7% at spiking levels of 0.05 mg/kg and 2 mg/kg for 3-MCPD esters. The method avoids tedious and laborious sample preparation and was successfully applied to the rapid screening of samples conforming to the EU performance criteria for methods of analysis for 3-MCPD.

KEYWORDS: 3-MCPD; 3-MCPD esters; micromethod; simultaneous determination; GC-MS; foodstuffs

INTRODUCTION

3-Chloropropane-1,2-diol (3-MCPD) is known as food processing contaminant since 1978 (*1*). After the first detection in acid-hydrolyzed vegetable proteins (HVP) and in soy sauces, 3-MCPD was detected in many other types of food, like bakery products, meat and fish products, and soups (*2*,*3*). Several studies about the mechanism of 3-MCPD formation have been carried out, showing that in heat-processed fat-containing foodstuffs with low water activity, 3-MCPD is formed from glycerol or acylglycerols and chloride ions (*4*−*8*). An alternative formation route is described for smoked foodstuffs (*9*). 3-MCPD is carcinogenic in rat and possibly has genotoxic activity. UK Committees on Mutagenicity and Carcinogenicity, JEFCA Joint FAO/WHO Expert Committee on Food Additives and European Communities Scientific Committee on Food (SCF) reviewed toxicological studies. Literature about the toxicological evaluations of 3-MCPD are summarized by the European Commission (*10*). On the basis of these studies, the European Commission has adopted a regulatory limit of 20 μg/kg for 3-MCPD in soy sauce and hydrolyzed vegetable protein (HVP) (*11*). A provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bodyweight per day was recommended for 3-MCPD by JEFCA and SCF (*10*).

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In many foodstuffs, a part of 3-MCPD is ester-bound with fatty acids (*12*). The toxicological properties of these 3-MCPD esters are so far unknown, yet it might be plausible that in the human intestinal tract 3-MCPD esters may be hydrolyzed by lipases. Recently, the occurrence of high levels of 3-MCPD-esters (monoesters and diesters with higher fatty acids) in some edible oils has been reported (*13*). A versatile further reading to toxicological evaluations and investigations about 3-MCPD esters can be found in ref (*14*).

With regard to the toxicological evaluation, minimization of MCPD esters is recommended (*15*).

Several analytical techniques have been developed to determine 3-MCPD in foods including gas chromatography (GC) with electron capture detection (ECD), GC combined with mass spectrometry (MS), and molecular imprinting. Mostly, GC-MS is used as a sensitive approach for analyzing 3-MCPD. The conventional pretreatment method for 3-MCPD in soy sauce or other matrices consists of purification with a glass chromatography column loaded with diatomaceous earth or an Extrelut column, followed by concentration and derivatization with, e.g., heptafluorobutyrylimidazole (HFBI) or heptafluorobutyric acid anhydride (HFBA). The pretreatment procedures are not only tedious and troublesome but also require large volumes of organic solvents with influence on the detection limit of trace analysis. Solid-phase microextraction (SPME) is a rapid and solvent-free technique but does not exist in every laboratory.

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Baer et al. (*16*) give a detailed review of methods to determine 3-MCPD.

Determination of 3-MCPD esters usually uses detection of free 3-MCPD by GC-MS after acidic hydrolysis using sulfuric acid/methanol or transesterification with sodium methoxide and derivatization with phenylboronic acid (*17*, *18*).

For the determination of free and ester-bound 3-MCPD in foodstuffs, only very limited information is available in literature (*18*). In summary, two different methods are necessary for the analysis of 3-MCPD and 3-MCPD esters.

A method for the simultaneous determination of free and ester-bound 3-MCPD is not described so far in the literature; however, it is necessary for the rapid investigation of foodstuffs.

In the following, a fast and simple method for the simultaneous determination of free and ester-bound 3-MCPD using a single extraction step combined with isotope dilution GC-MS is presented for the first time. This micromethod needs only a small volume of organic solvent. The quick and easy procedure allows the determination of the analytes in various foodstuffs with only one extraction step followed by derivatization in aqueous phase for 3-MCPD avoiding the above-mentioned difficulties.

MATERIALS AND METHODS

Reagents and Materials. Sodium chloride (pA), phenylboronic acid (≥98%), acetone, hexane, methyl *tert*-butyl ether (MTBE), methanol, and ethyl acetate (all suprasolv for GC) as well as glacial acetic acid (suprapur) were bought from Merck (Darmstadt, Germany). Sodium methoxide (25% w/V) and 3-monochlorpropane-1,2-diol (>98%, 3-MCPD) were obtained from Sigma Aldrich (Weinheim, Germany). Sodium methoxide was diluted subsequently with methanol (1 + 1 v/v) for ester cleavage (sodium methoxide solution). 3-Monochlorpropane-1,2-diol-d_5 (99 atom %, 3-MCPD-d_5) was purchased from Campro Scientific (Berlin, Germany).

1,2-Dipalmitoyl-3-chloropropane and 1,2-dipalmitoyl-3-chloropropane-d_5 (both approximately 95%) were synthesized according to Kraft et al. (*19*).

A sodium chloride solution (NaCl solution 20%) of 200 g/L was prepared in deionized water.

The derivatization reagent phenylboronic acid (PBA) was prepared by dissolving 5 g PBA in 19 mL of acetone and 1 mL of deionized water.

Preparation of Standard Solutions. Stock solutions with concentrations of 10 μg/mL 3-MCPD were prepared in deionized water as well as ethyl acetate. Additionally, a stock solution of 10 μg/mL 3-MCPD-d_5 in deionized water was used for the determination of 3-MCPD. From these solutions, the calibration standards of 3-MCPD for the determination of free 3-MCPD in liquid samples (0.01−0.1 μg/mL; e.g., for soy sauce) as well as other matrices (0.004−0.04 μg/mL) were obtained. The concentration range of 0.02−3.2 μg/mL were chosen for the determination of 3-MCPD esters. The calibration solutions were prepared by serial dilution with deionized water (3-MCPD) and MTBE (3-MCPD esters), respectively. For the determination and recovery study of 3-MCPD esters stock solutions of 1,2-dipalmitoyl-3-chloropropane and 1,2-dipalmitoyl-3-chloropropane-d_5 with a concentration of 5 mg/mL were prepared in ethyl acetate. These solutions were further diluted to 0.5 mg/mL with ethyl acetate (working solution). The concentrations of the internal standards 3-MCPD-d_5 and 1,2-dipalmitoyl-3-chloropropane-d_5 were 0.01 and 0.1 μg/mL in the respective calibration standards. In relation to sample preparation, the standard solutions were prepared in 10 and 25 mL NaCl solution 20% for 3-MCPD as well as 10 mL of MTBE for 3-MCPD esters, followed by the same treatment as samples. All solutions were stored at a temperature of 4 °C.

Selection of Matrices. For the validation study and the method development, a total of 30 food products of the categories seasonings, soup and gravy powders, and meat and fish products as well as bakery products were selected. Products of different fat contents were used within all product groups. These were examined with the new procedure and spiked with five as well as six concentrations of 3-MCPD and 1,2-dipalmitoyl-3-chloropropane for the determination of recoveries and precision (CV) as described below. Homogenization of the samples was accomplished by thorough stirring (liquid samples) and pulverizing (other samples) using a laboratory mill.

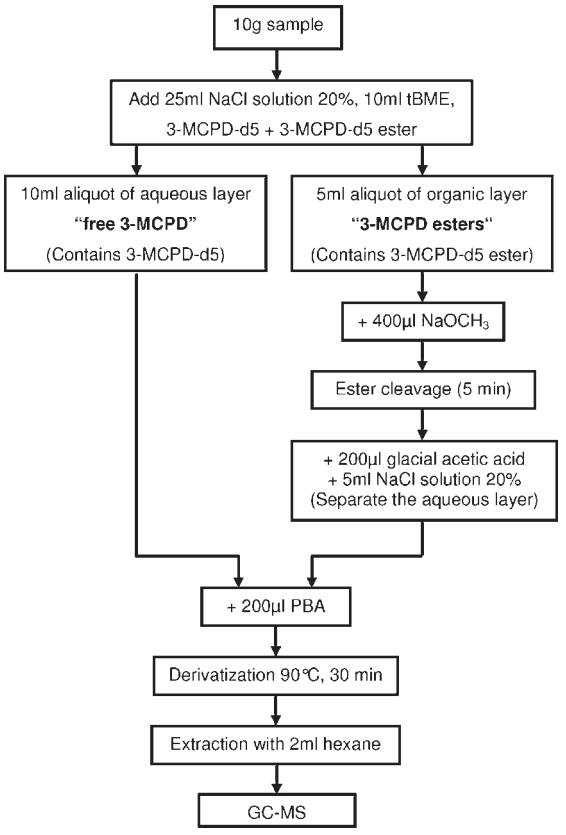

Figure 1. Scheme of analysis for the simultaneous determination of 3-MCPD and 3-MCPD esters in foodstuffs. Further information is provided in the text.

Sample Preparation (cf. Figure 1). *Extraction of Free 3-MCPD from Liquid Samples (e.g., Soy Sauce).* About 10 g of the homogenized sample were weighed into a screw-capped centrifuge tube, 0.25 μg of internal standard (stock solution of 3-MCPD-d_5 in deionized water) was added, and the tube was tightly closed and vigorously shaken on a vortex mixer before immediate derivatization.

Extraction of Free 3-MCPD and 3-MCPD Esters from Foodstuffs. About 10 g of the homogenized sample were weighed into a screw-capped centrifuge tube, 0.25 μg of 3-MCPD-d_5 (stock solution of 3-MCPD-d_5 in deionized water) and 1 μg of 1,2-dipalmitoyl-3-MCPD-d_5 (working solution of 1,2-dipalmitoyl-3-chloropropane-d_5 in ethyl acetate) were added and homogenized thoroughly. Afterward, 25 mL of NaCl solution 20% and 10 mL of MTBE were added, the tube was tightly closed, vigorously shaken, and centrifuged for phase separation at 3000 rpm. From the upper organic layer, an aliquot was transferred to another tube and the MCPD esters were cleaved in the same way as oils and fats without further preparations. The used aliquot was calculated with respect to the fat content of the sample. Only a maximum amount up to 250 mg of fat could be analyzed. From the lower aqueous layer 10 mL were transferred to another tube and derivatized as described below.

Ester Cleavage of Oils and Fats. 3-MCPD esters were cleaved according to the procedure of Weisshaar (*17*) with slight modifications. About 250 mg of the homogenized sample was weighed into a screw-capped centrifuge tube, 5 mL of MTBE, 1 μg of the internal standard 1,2-dipalmitoyl-3-chloropropane-d_5 (working solution of 1,2-dipalmitoyl-3-chloropropane-d_5 in ethyl acetate), and 400 μL of sodium methoxide solution were added. The tube was tightly closed and the solution mixed on vortex mixer for 1 min. After 5 min, 200 μL of glacial acetic acid and 5 mL of NaCl solution 20% were added and vigorously shaken for extraction of the analytes. After phase separation, the upper layer was discarded. The aqueous layer was derivatized as described below.

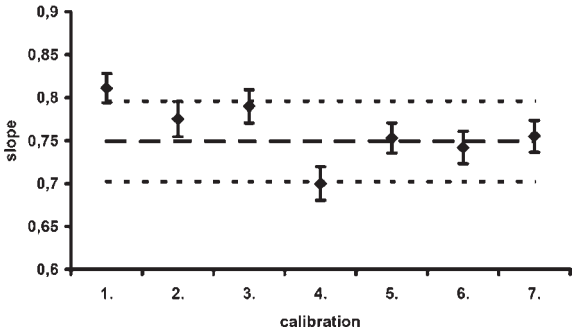

Figure 2. Stability of the derivatization reaction during the validation study. Dashed line demonstrates the mean of all results with standard deviation as dotted lines ($n = 3$).

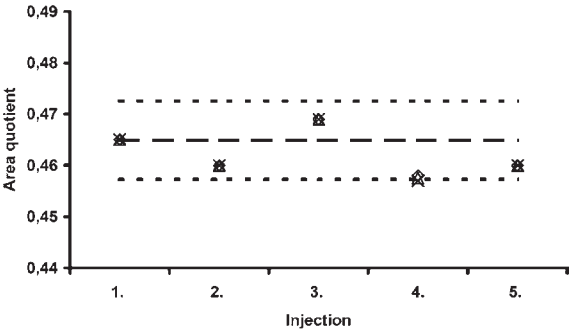

Figure 3. Stability of derivatives during 5 days at ambient temperature (injection 1−5). Dashed line demonstrates the mean of all results with standard deviation as dotted lines ($n = 3$).

General Derivatization Procedure. Two hundred μL of the derivatization reagent PBA were added to the aqueous layer. The tube was closed and heated at 90 °C for 30 min. After cooling to room temperature, the cyclic phenylboronate derivative of 3-MCPD was extracted by shaking with 2 mL of hexane. The hexane layer was separated and analyzed by GC-MS.

Method Validation. In accordance with the European Commission Regulation (*9*), the maximum levels in foodstuffs (in particular HVP and soy sauce) for 3-MCPD are set to 20 μg/kg and our spiking levels were adapted. The recoveries for 3-MCPD esters were determined at five spiking levels according to the really found contents of 3-MCPD esters in foodstuffs (*20*, *21*).

For testing the recovery, blank samples (original samples) were spiked with the above-mentioned solutions (stock as well as working solutions of 3-MCPD and 1,2-dipalmitoyl-3-chloropropane) at levels of LOQ, 15, 25, 50, and 100 μg/kg for 3-MCPD and LOQ, 0.05, 0.25, 0.75, 1.5, and 1.88 mg/kg for 3-MCPD esters. Olive oil spiked with 1,2-dipalmitoyl-3-chloropropane was used to verify the complete ester cleavage. If present, amounts of 3-MCPD or 3-MCPD esters in blank samples were considered for correction of recoveries.

Afterward, blank and fortified samples were analyzed with the described method. 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, precision (CV) as well as repeatability, were determined from this data set. Accuracy for the determination of free 3-MCPD was verified using soy sauce offered as a certified reference material by FAPAS (Food and Environment Research Agency, York/UK) containing 3-MCPD 47.9 μg/kg (assigned value). This material was analyzed in triplicate.

Linearity of the method was established by the analysis of six standard solutions in the above-mentioned ranges. Detection and quantification limits (LOD and LOQ, respectively) were determined by standard solutions (for free 3-MCPD and 3-MCPD esters) 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 and stability of the obtained PBA derivatives were investigated by comparing the calibration curve slopes as well as replicate measurements of a standard solution (25 μg/kg 3-MCPD) within approximately five days.

GC-MS Analysis. Gas chromatography−mass spectrometry 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). Then 2 μL of the derivatized sample solution and of each derivatized calibration solution were injected in the splitless injection mode and detected in the selected ions 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 analysis.

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. The GC oven temperature was programmed from an initial temperature of 50 °C (1 min hold), ramped at 10 °C/min up to 210 °C, and then ramped finally at 30 °C/min up to 300 °C with holding for 5 min. This program resulted in a total run time of 25 min.

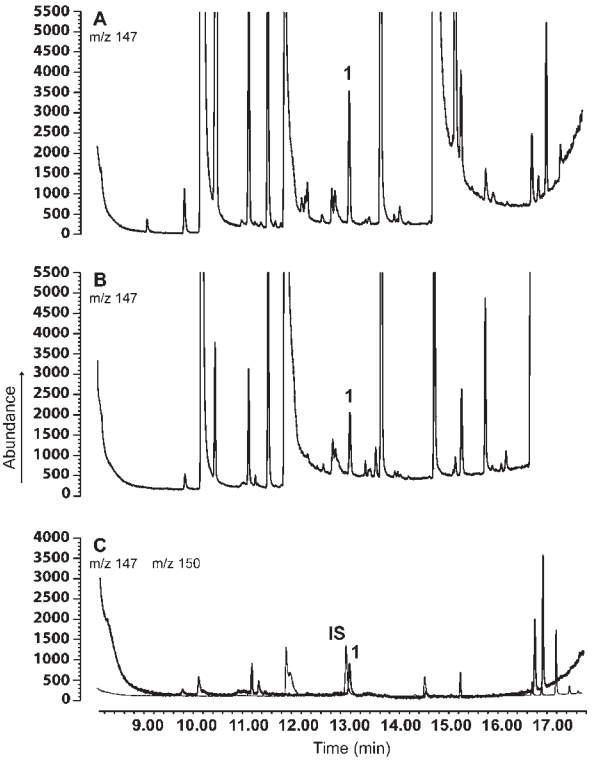

Figure 4. GC-MS chromatograms (extracted ion) of different soup and gravy powders (**A**,**B**) compared to a calibration standard (**C**, 25 μg/kg 3-MCPD). IS = 3-MCPD-d_5; 1 = 3-MCPD.

The following temperatures were selected for transfer line, MS source, and MS quad: 280, 230, and 150 °C. The EM voltage was 1800, 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 and 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 at m/z 150 (3-MCPD-d_5). Ions at m/z 196 (3-MCPD) and at m/z 201 (3-MCPD-d_5) were used as qualifiers.

The contents of 3-MCPD and 3-MCPD esters were calculated as ratio of the peak area responses for 3-MCPD (m/z 147) and internal standard (m/z 150) for calibration standards as well as blank and fortified samples. The respective content was determined from a calibration graph constructed by plotting the peak area ratios for the calibration standards (y-axis) against the amount of 3-MCPD (x-axis).

RESULTS AND DISCUSSION

Sample Preparation. *Determination of Free 3-MCPD*. Until now, 3-MCPD was determined by the frequently used procedure

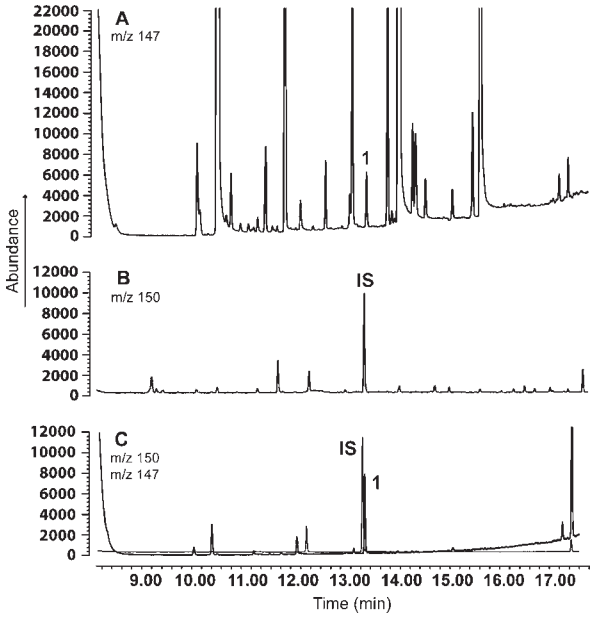

Figure 5. GC-MS chromatograms (extracted ion) of a representative seasoning (**A**,**B**) compared to a calibration standard (**C**, 25 μg/kg 3-MCPD). IS = 3-MCPD-d_5; 1 = 3-MCPD.

of column chromatography loaded with diatomaceous earth and ethyl acetate extraction in our laboratory. Although fat and other lipophilic substances were extracted with hexane or similar solvents in accordance to the literature (*22*, *23*), concomitant lipophilic compounds remained with the analytes disturbing derivatization.

Because of the storage of the sample extract in a refrigerator, some parts of these mentioned contaminations were frozen out. Nevertheless, the same interferences appeared in the afterward clear solvent. Hence, an elimination of these distracting compounds was obligatory. Our investigations of other extraction procedures (e.g., with hexane/acetone as described by Divinova et al. (*16*)) were laborious and time-consuming due to the necessity of evaporation the obtained water−acetone mixture. For this reason, we tried direct extraction with different kinds of solvents (hexane/acetone, MTBE, acetone, ethyl acetate) in combination with evaporation of the extracts to dryness. The final derivatization step with, e.g., heptafluorobutyric acid anhydride of the residue assumes an obligatory anhydrous medium. The evaporation proved to be fast but the residue remained moist, which inhibited the final derivatization.

To achieve a purification, the re-extraction of 3-MCPD from the organic layer with water was verified as a successful procedure which admitted only the derivatization with PBA in the aqueous layer without other tedious steps such as evaporation. Therefore, the obtained residue was dissolved with NaCl solution 20%. The insoluble lipophilic compounds were extracted with hexane and discarded. The aqueous layer was derivatized with PBA and analyzed by GC-MS. Because of the obtained unsatisfying results and the missing applicability for simultaneous determination of 3-MCPD esters using this procedure, another sample preparation technique was required.

For this reason, the test material was extracted directly with NaCl solution 20% for prevention the described interferences. A volume of 25 mL of NaCl solution 20% was adequate for extraction. Merely with very expanding products (e.g., some kinds of bread), the volume of NaCl solution 20% was increased by steps of 5 mL until an aliquot of 10 mL was obtained for analysis. However, in all cases, a maximum of 35 mL was sufficient.

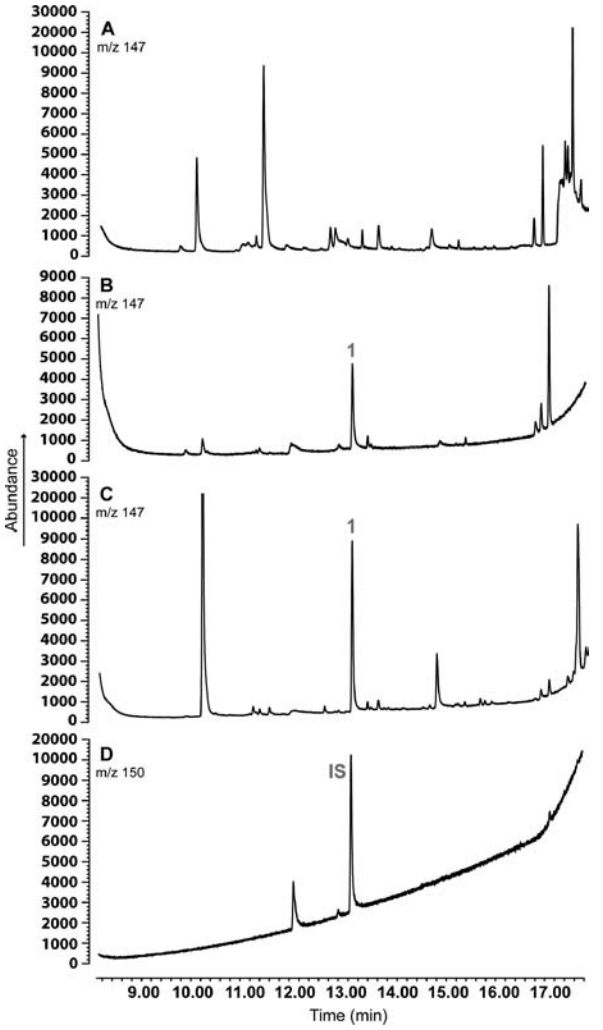

Figure 6. GC-MS chromatograms (extracted ion) of representative samples after preparation of the 3-MCPD esters with the described method (**A**,**B**) and a 3-MCPD calibration standard (**C**,**D**; 1.2 mg/kg). IS = 3-MCPD-d_5; 1 = 3-MCPD.

By the simultaneous extraction with MTBE, any fat present in the samples was disposed and a separation of disturbing substances was accomplished.

Liquid foodstuffs like soy sauce or Worchester sauce were taken directly for derivatization with PBA. With the following extraction of the phenylboronic acid derivatives by hexane, no colorings or comparable substances were extracted. A preceding treatment of the samples like extraction with hexane or another organic solvent was not necessary. Example chromatograms are shown in **Figures 4** and **5**. The use of isotope-labeled 3-MCPD-d_5 as internal standard allowed a simple extraction procedure and correction of analyte losses.

In addition, the used volume of derivatization reagent (PBA) could be reduced to 200 μL compared to procedures previously cited. This prevented a crystallization of excess reagent, and no filtration step was necessary for the removal.

Determination of 3-MCPD Esters. The analysis of 3-MCPD esters usually needs isolation of the fat from the sample (*18*, *20*). The fat is separated, and afterward the esters are cleaved. On the one hand, the ester cleavage can be carried out in a tedious procedure by hydrolysis with sulfuric acid that forms measurable contents of 3-MCPD esters according to Weisshaar (*17*). On the other hand, the ester cleavage is possible with sodium methoxide

Table 1. Results for 3-MCPD in Validation Study[d]

	Spiking Level µg/kg	LOD µg/kg	LOQ µg/kg	Recovery%	Interday coefficient of variation (CV)	n [a]
Seasonings (e.g. Worchester sauce, soy sauce)	LOQ (4)	1 [b]	4 [b]	108 ± 14	0.13	4
	15			87 ± 3	0.02	4
	25			93 ± 2	0.03	6
	50			101± 1	0.02	4
	100			100 ± 1	0.004	4
Soup and gravy powders	LOQ (7)	2 [c]	7 [c]	102 ± 11	0.11	4
	15			98 ± 7	0.07	4
	25			98 ± 8	0.08	8
	50			106 ± 5	0.05	4
	100			100 ± 1	0.005	4
Meat and fish products (e.g. smoked salami and trout)	LOQ(7)			88 ± 7	0.1	6
	15			96 ± 13	0.07	3
	25			100 ± 7	0.07	7
	50			98 ±1	0.001	6
	100			98 ±1	0.014	6
Bakery and potato products (e.g. bread, crispbread, hamburger bun, candy bar, French fries)	LOQ (7)			102 ±12	0.11	4
	15			92 ± 7	0.1	3
	25			100 ± 3	0.03	10
	50			103 ±1	0.002	4
	100			100 ±2	0.02	4
Mean	**LOQ (4)**			**108 ± 14**	**0.13**	**4**
	LOQ (7)			**97 ± 10**	**0,11**	**14**
	15			**96 ± 9**	**0.09**	**14**
	25			**97 ± 10**	**0.1**	**31**
	50			**102 ± 6**	**0.06**	**18**
	100			**99 ± 3**	**0.03**	**18**

[a] *n* = number of products analyzed in triplicate. [b] Corresponding to 10 g sample (direct derivatization and extraction). [c] Corresponding to an aliquot of 10 mL (taken from 25 mL sample extract of 10 g weighted sample). [d] Recovery values at different spiking levels are shown as the average of all analyzed samples for the respective product groups. Additionally, the mean of all product groups are shown as well. LOD and LOQ were calculated by standard solutions based on signal-to-noise ratios (S/N) of 3:1 for LOD and 10:1 for LOQ and checked by analyzing spiked samples at this level. Different LOD and LOQ result from various extraction volumes depending on several product groups.

Table 2. Results of 3-MCPD Esters in Validation Study[e]

	Spiking Level [mg/kg]	LOD [µg/kg] [a]	LOQ [µg/kg] [a]	Recovery[%]	Interday coefficient of variation (CV)	n [b]
Soup and gravy powders	LOQ (0.020)	6 [c]	19 [c]	105 ± 16	0.15	2
	0.049			88 ± 8	0.09	3
	0.25			88 ± 8	0.09	2
	0.75			99 ± 2	0.01	2
	1.5			99 ± 1	0.01	2
	1.88			79 ± 3	0.04	3
Meat and fish products	LOQ (0.020)			93 ± 6	0.06	2
	0.049			86 ± 7	0.07	2
	0.25			94 ± 3	0.05	2
	0.75			96 ± 1	0.01	2
	1.5			98 ± 1	0.01	2
	1.88			93 ± 3	0.03	2
Bakery products	LOQ (0.020)			92 ± 4	0.04	2
	0.049			90 ± 1	0.02	3
	0.25			98 ± 2	0.05	2
	0.75			95 ± 3	0.03	2
	1.5			95 ± 1	0.01	2
	1.88			90 ± 7	0.08	2
Olive oil	0.96	120 [d]	400 [d]	96 ± 1	0.01	3
	1.96			101 ± 5	0.05	3
Mean (without olive oil)	**LOQ (0.020)**			**96 ± 10**	**0,1**	**6**
	0.049			**84 ± 9**	**0.05**	**8**
	0.25			**94 ± 7**	**0.08**	**6**
	0.75			**97 ± 4**	**0.04**	**6**
	1.5			**98 ± 2**	**0.03**	**6**
	1.88			**85 ± 7**	**0.09**	**7**

[a] µg 3-MCPD/kg foodstuff. [b] *n* = number of products analyzed in triplicate. [c] Corresponding to 10 g weighted sample. [d] Corresponding to 250 mg weighted sample. [e] Recovery values at different spiking levels are shown as the average of all analyzed samples of the respective product groups. Additionally, the mean of all product groups are shown as well. LOD and LOQ were calculated by standard solutions based on signal-to-noise ratios (S/N) of 3:1 for LOD and 10:1 for LOQ and checked by analyzing spiked samples at this level.

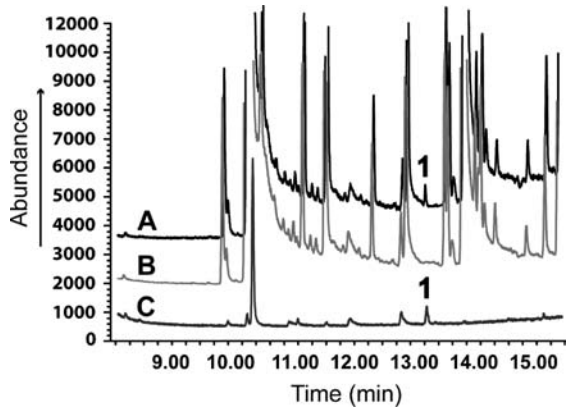

Figure 7. GC-MS chromatograms (extracted ion *m*/*z* 147) of a representative soy sauce sample spiked at LOQ (A, 4 μg/kg) compared to blank sample (B) and standard solution (C, 4 μg/kg). 1 = 3-MCPD.

as a transesterification, which is accomplished fast without any other laborious steps.

Maybe methoxide ions are strong nucleophilic agents affecting the 3-MCPD molecule by nucleophilic substitution as discussed in literature (*16*, *17*). Nevertheless, the use of isotope-labeled 3-MCPD as internal standard compensates the degradation. Within the scope of our method development, the procedure of ester cleavage using sodium methoxide was used.

The extraction was achieved in one step beside free 3-MCPD. Additionally, because free 3-MCPD remained in the aqueous layer, a separate treatment of the sample was avoided as recommended for other methods. Briefly, further extraction to the elimination of the free 3-MCPD from the organic layer was prevented, as necessary in the case of other extraction methods, e.g., fat extraction by diethyl ether followed by extensive washing of the organic layer with water for removal of free 3-MCPD (*18*, *21*).

An aliquot of 5 mL or respective to 250 mg of fat of the organic phase was used. After transesterification by sodium methoxide, the released 3-MCPD was extracted by NaCl solution 20% and derivatized directly while reaction byproduct (e.g., fatty acid methyl esters) remained in the upper organic layer. In comparison to hexane used for fat extraction, MTBE indicated a homogeneous solution after addition of sodium methoxide solution as well as a clear phase separation after the reaction.

The increase of volume with respect to the extracted fat was corrected by using isotope-labeled 1,2-dipalmitoyl-3-chloropropane-d_5 as an internal standard. In this manner, both the aqueous and the organic layer contained internal standard with the advantage of the simultaneous determination of 3-MCPD and 3-MCPD esters in one step (cf. **Figure 1**).

The used aliquot should not exceed a fat content of 250 mg in order to derivatize both the analytes and the glycerol originated from triglycerides with an excess of PBA. Further, the additional extraction with hexane of the aqueous layer after ester cleavage as described by Weisshaar (*17*) showed neither cleaning nor advantages. Typical chromatograms of samples analyzed for 3-MCPD esters are given in **Figure 6**.

Results of the Validation. *Stability of Derivatization and Derivatives*. The derivatization reaction was reproducible with the applied conditions and showed a relative standard deviation of 6% by comparing the slope of seven calibration curves within the validation period (**Figure 2**). The derivatives were stable over a period of at least 5 days at ambient temperature with a relative standard deviation $<2\%$ as studied by replicate measuring a standard solution (**Figure 3**). The conditions of the derivatization procedure were chosen as described by Divinova et al. (*18*). Only

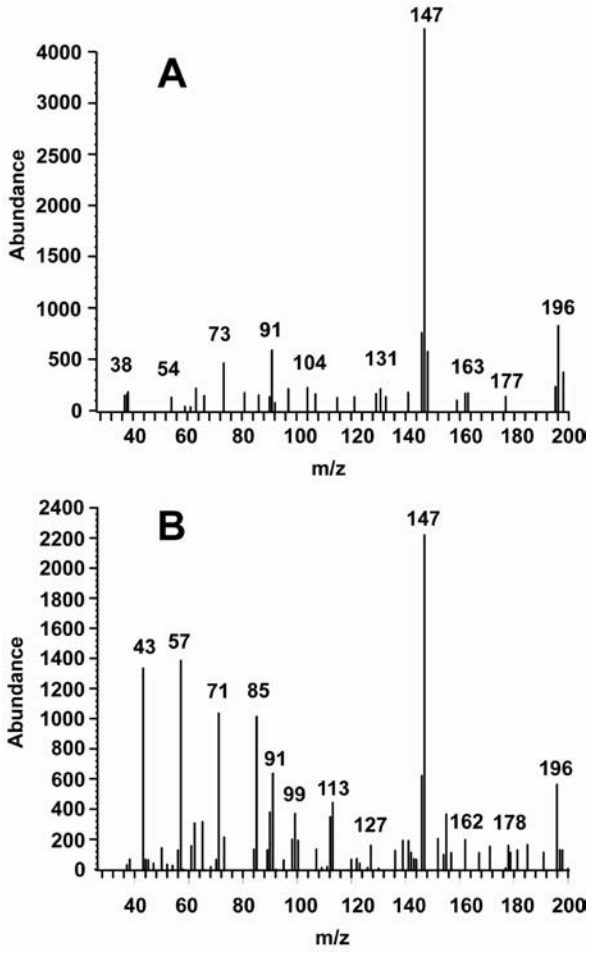

Figure 8. Full scan EI mass spectra (background subtracted) for PBA derivatives of (**A**) 3-MCPD (standard solution, 20 μg/kg) and (**B**) 3-MCPD in a soy sauce sample (spiked at 20 μg/kg). The ion *m*/*z* = 196 corresponds to $[M]^+$.

the derivatization time was set to 30 min regarding the required heating-up period using a drying oven instead of a water bath.

Specificity. The characterizations of 3-MCPD and 3-MCPD-d_5 based on their retention times. Besides, peak area ratios of the *m*/*z* 147 and 196 ions (for 3-MCPD) as well as *m*/*z* 150 and 201 ions (for 3-MCPD-d_5) were checked systematically. The differences between ratios *m*/*z* 147/196 and 150/201 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 the purification as well as the selectivity of the derivatization (cf. Figures **4**−**6**).

In case of interferences, the characteristic ions *m*/*z* 196 and 201 could be used for calculation (*17*) and was applied successfully to a soy sauce in one case. The ions *m*/*z* 91 (3-MCPD) and 93 (3-MCPD-d_5) were used only as a qualifier because interferences were observed in many cases. Therefore, positive results were confirmed by full scan EI mass spectra as shown in **Figure 8**.

Performance of the Method. In accordance to "Performance criteria for methods of analysis for 3-MCPD" by the European Commission (*24*), the recoveries must be achieved in the range of 75−110%. The precision has to be reached as a function of the 3-MCPD content. Therefore, a precision of <4 and <6 μg/kg is necessary at spiking levels of 15 and 25 μg/kg. The applied method has to achieve a LOD of ≤ 5 μg/kg and a LOQ of ≤ 10 μg/kg. For the analysis of 3-MCPD esters, no performance criteria were established so far.

Table 3. Results of the Determination of Free and Ester-Bound 3-MCPD Contents in Conventional Samples Obtained with the Described Method

	free 3-MCPD [μg/kg]	3-MCPD (ester bound) [mg/kg]
FAPAS proficiency test 2625 3-MCPD in soy sauce (47.9 μg/kg)	47.0 ± 1.4	*a*
seasonings	19.5 ± 0.7	*a*
soup and gravy powders	11.5 ± 0.5	0.09 ± 0.01
	68.2 ± 0.98	0.47 ± 0.02
	22.5 ± 2.1	0.10 ± 0.01
	33.0 ± 4.0	1.53 ± 0.09
	44.0 ± 7.1	0.57 ± 0.02
meat and fish products	59.0 ± 4.2	<LOD[b]
	5.3 ± 0.6	<LOD[b]
bakery products	8.5 ± 2.1	<LOQ[c]
	<LOD[b]	0.33 ± 0.03
	40.5 ± 5.5	1.52 ± 0.05

[a] Not analyzed. [b] LOD = 2 μg/kg for free 3-MCPD and 6 μg/kg for 3-MCPD esters. [c] LOQ = 19 μg/kg for 3-MCPD esters.

As shown in **Table 1**, all performance criteria were fulfilled with excellent results. 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 in liquid foodstuffs by direct derivatization and extraction as well as in solid foodstuffs after economy liquid extraction with NaCl solution 20%. The LOQ was confirmed by determining spiked sample matrices at the respective level, resulting in satisfying recoveries and precisions (cf. Tables **1** and **2** as well as the chromatogram in **Figures 7** as an example).

3-MCPD esters were extracted exactly and cleaved as shown by fortified olive oil in **Table 2**.

Hence, an accurate and simultaneous determination of 3-MCPD and 3-MCPD esters was possible with the presented method in various foodstuffs. The accuracy was confirmed by a proficiency test of soy sauce with an excellent result (**Table 3**).

As a result, the described method could be applied to a wide range of foodstuffs without 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. Some results of the determination of free and ester-bound 3-MCPD in foodstuffs are given as an example in **Table 3**. No further sample purification was required as PBA reacts specifically with diols, forming nonpolar cyclic 1,3,2-dioxaborolane derivatives extractable into hexane.

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

ABBREVIATIONS USED

3-MCPD, 3-monochloropropane-1,2-diol; CV, coefficient of variation; EU, European Commission; GC-MS, gas chromatography−mass spectrometry; HVP, hydrolyzed vegetable proteins; LOD, limit of detection; LOQ, limit of quantitation; MTBE, methyl *tert*-butyl ether; NaCl solution 20%, sodium chloride solution 20%; PBA, phenylboronic acid.

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