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Sensitive method for the determination of 3-chloropropane-1,2-diol and 2-chloropropane-1,3-diol by capillary gas chromatography with mass spectrometric detection

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Abstract

An improved routine method for the determination of 3-chloropropane-1,2-diol (3-MCPD) and 2-chloropropane-1,3-diol (2-MCPD) in different savoury foods using capillary gas chromatography with mass spectrometric detection is presented. Monochloropropanediols were extracted from Extrelut columns with diethyl ether and derivatised with acetone to the corresponding dioxolanes. Detection was performed on two different mass spectrometers using single ion monitoring, to reach ultimate sensitivity in the low μg/kg range. A limit of detection of 1 μg kg^{-1} was achieved using Extrelut 20 for extraction. The method proved to be fast and reliable and showed good recoveries and good accuracy, confirmed by successful participation in two ring test studies. Results of analyses of finished goods, such as seasonings and soy sauces, often containing MCPDs only in the sub-ppb level, are presented. © 1998 Elsevier Science B.V.

Keywords: Food analysis; Extraction methods; Chloropropanediols

1. Introduction

Monochloropropanediols (MCPDs) and dichloropropanols (DCPs) are toxic compounds, often present in different foods containing protein hydrolysates, like seasonings and savoury food products [1–4]. 3-Chloropropane-1,2-diol (3-MCPD) is known to have a post-testicular anti-fertility effect in male rats [5] and has been shown to be mutagenic in bacterial assays [6]. Studies of its carcinogenicity have led to controversial results and there is no direct evidence for toxic effects in humans. Nevertheless, all efforts should be made to minimise its occurrence in the human diet and efficient monitoring is required.

Because of the potential hazards to human health, a tolerance limit of 10 μg kg^{-1} is currently under discussion.

MCPDs and DCPs are frequently formed during manufacturing of protein hydrolysates. The protein sources typically contain residual lipids that have the potential to react under unfavourable conditions to produce MCPDs and DCPs, especially if hydrolysis is carried out using hydrochloric acid. Through improved manufacturing processes, e.g. enzymatic fermentation instead of hydrochloric acid treatment, levels of chloropropanols could be lowered considerably. MCPD levels have been reduced within

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the last decade from up to 700 mg kg^{-1} to less than 1 mg kg^{-1} and DCPs are no longer detectable.

Several analytical methods for the quantitative determination of MCPDs have been published. Gas chromatography with different detectors was shown to be the method of choice.

Rodman and Ross [7] utilised phenylboronic acid to derivatise 3-MCPD in non-aqueous media for subsequent determination by gas chromatography. The same derivative was used by Plantinga et al. [8] and Ushijima et al. [9] to determine 3-MCPD in hydrolysed vegetable proteins (HVPs) and seasonings. Quantitative determination of 3-MCPD in water using the butaneboronic acid derivative was reported by Pesselman and Feit [10]. van Bergen et al. [11] described a procedure for the determination of chloropropanols in protein hydrolysates based on gas chromatography with electron-capture detection of heptafluorobutyrate derivatives. Determination of 3-MCPD and related dioxolanes by gas chromatography was reported by Kissa [12] using *N,O*-bis-(trimethylsilyl)trifluoroacetamide as the derivatising agent, and the reaction of 3-MCPD with acetone and its kinetics are also discussed. Methods to determine underivatised MCPDs have been published by Wittmann [13], using mass spectrometric detection and by Spyres [14], who used gas chromatography with electrolytic conductivity detection.

Limitations of these methods include the potential for incomplete derivatisation, inefficient partitioning and short term stability of the derivatives, whereas the direct determination of MCPDs has proven difficult, because of reactions with other components of the sample or with active sites on the column or in the inlet. As a result, peak shape is often rather poor and deteriorates with time. Special capillary columns are needed for good separation of the isomers 3-MCPD and 2-chloropropane-1,3-diol (2-MCPD). These problems can be prevented by derivatisation of the hydroxyl groups with a suitable reagent to produce a more volatile derivative, although some of the derivatives still need a rather time-consuming gas chromatographic procedure.

This paper reports an improved method (and its performance) to quantitatively determine the two isomers of monochloropropanediol. It has been applied to different matrices, since 1989, for monitoring manufacturing processes, levels of occurrence in hydrolysed proteins and foodstuffs for sale to the consumer. After absorption of diluted aqueous sample on a Kieselguhr column, MCPDs are extracted with diethyl ether, derivatised with acetone to the corresponding dioxolanes, which were shown to be the optimal derivative [15], and measured by gas chromatography using mass spectrometric detection. Improvements in the, often unsatisfactory, limit of detection achievable with other procedures were achieved by using the most sensitive single ion monitoring detection, which facilitated the fast determination of MCPDs in the low μg kg^{-1} range.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade unless otherwise stated. Organic solvents, such as acetone, diethyl ether, *n*-hexane and toluene, were of organic residue analysis grade and were obtained from Merck (Dietikon, Switzerland). Water was of high purity quality, obtained from a Waters Millipore (Volketswil, Switzerland) Milli-Q-plus system. Sodium chloride and anhydrous sodium sulphate were from Merck (Dietikon, Switzerland), as were Extrelut 20 columns, Extrelut 3 columns and refill material. Toluene-4-sulfonic acid monohydrate and sodium hydroxide (purum) were obtained from Fluka (Buchs, Switzerland).

2.2. Standards

3-MCPD was obtained from Fluka. 2-MCPD was a gift provided by Dr. F. Ruf (CPC Europe Research and Development, Heilbronn, Germany). 4-(Chloromethyl)-2,2-dimethyl-1,3-dioxolane was prepared by reaction of 3-MCPD with acetone, as described in Section 2.3.

2.3. Synthesis of 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane [16]

A 13.3-g amount of 3-MCPD, 5.8 g of acetone and 10 mg of toluene-4-sulfonic acid monohydrate were dissolved in 20 ml of toluene and refluxed for approx. 2 h, while collecting the reaction water in an

appropriate separator. After cooling to ambient temperature, the reaction mixture was washed three times with diluted sodium hydroxide solution (1 mol l^{-1}) and with water until the pH was neutral. The organic layer was dried over anhydrous sodium sulphate and toluene was evaporated on a rotary evaporator. After distillation under vacuum, a purified fraction of 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane was obtained with a yield of 54%, which was characterised by gas chromatography–mass spectrometry (GC–MS) as described below and was found to be >98% pure.

2.4. Preparation of calibration solutions and spiking solutions

A stock solution of 4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane was prepared by weighing 100.0 mg into a 100-ml volumetric flask and making up to the required volume with *n*-hexane. This stock solution was diluted with *n*-hexane twice (1:10, v/v) to obtain a stock solution with a concentration of 10 mg l^{-1}. Calibration solutions covering the range 0.01–5 mg l^{-1} were prepared by serial dilution of the stock solution with *n*-hexane, as appropriate.

A stock solution of 3-MCPD and 2-MCPD was prepared by weighing 400.0 mg of each compound into a 100-ml volumetric flask and making up to the required volume with water. Spiking solutions with concentrations of 40 and 4 mg l^{-1} were prepared by serial dilution of the stock solution with water as appropriate. These solutions were used to spike samples in the range 10 μg kg^{-1}–10 mg kg^{-1}.

The stock solutions, calibrating solutions and the spiking solutions were kept refrigerated (4°C) and were prepared at least every two months.

2.5. Sample preparation

According to expected MCPD levels or the desired limit of detection, two very similar procedures were applied: for routine analysis, Extrelut 3 columns were used, whereas for ultimate sensitivity, Extrelut 20 columns were used.

Liquid samples: an 8-g amount of liquid hydrolysate and 16 g of saturated sodium chloride solution were weighed into a test tube and mixed for approx. 20 s on a vortex-mixer.

Viscous samples: a 12-g amount of viscous hydrolysate, such as pastes, and 12 g of saturated sodium chloride solution were weighed into a beaker and sonicated in an ultrasonic bath for approx. 15 min at 40°C. If necessary, the mixture was homogenised with a Polytron blender, followed by centrifugation until a clear supernatant was obtained.

Solid samples: a 7-g amount of solid hydrolysate and 35 g of saturated sodium chloride solution were weighed into a beaker and sonicated in an ultrasonic bath for approx. 30 min at 40°C. If necessary, the mixture was homogenised with a Polytron blender, followed by centrifugation until a clear supernatant was obtained.

A 3-g amount (20 g for Extrelut 20 columns) of the resulting liquor was weighed into an Extrelut 3 column and allowed to equilibrate for 15 min. The column was eluted with 25 ml of diethyl ether (130 ml of diethyl ether for Extrelut 20 columns) and the eluent was collected in a 25-ml test tube. For flow restriction, extra small bore needles (20×0.4 mm) were used, because they resulted in a higher extraction efficiency than the standard needles supplied with Extrelut columns. The resulting extract was then evaporated to dryness at 30°C under nitrogen. Special care was taken to avoid excessive evaporation times.

2.6. Derivatisation

To the residual extract, 1 ml of a solution containing 1 mg ml^{-1} of toluene-4-sulfonic acid monohydrate in acetone was added, the test tube was closed with a stopper and mixed for approx. 15 s on a vortex-mixer. The derivatisation was then carried out at 40°C for 90 min, with occasional shaking.

After cooling to ambient temperature, 3 ml of saturated sodium chloride solution and 1 ml of *n*-hexane were added and the test tube was shaken for another 15 s. The aqueous phase was removed and the organic phase was dried by adding approx. 300 mg of anhydrous sodium sulphate and shaking the mixture on a vortex-mixer. The organic phase was finally transferred to a 1.5-ml crimp vial and used directly for GC analysis. Extracts were kept refrigerated (4°C) and were found to be stable for up to five days.

2.7. Analysis by GC–MS

A 1-μl volume of derivatised extracts was injected in splitless mode (splitless time, 0.5 min; split ratio, 1:20) into a DB-1701 capillary column (30 m×0.32 mm, 0.25 μm, J&W Scientific, Part No. 123-0732) that was fitted in a Hewlett-Packard 5890 Series I gas chromatograph. Helium (99.999%) was used as the carrier gas at a head pressure of 40 kPa, resulting in a flow-rate of 2.2 ml min^{-1}. The injector temperature was set to 250°C and the oven was programmed from 50°C (after 1 min) to 100°C at 5°C min^{-1} and afterwards to 250°C (for 5 min) at 20°C min^{-1}. The gas chromatograph was coupled via a direct interface (transfer line temperature, 280°C) to a Hewlett-Packard 5970 B mass selective detector, which was operated in the electron impact (EI) mode. The ions monitored in low resolution single ion monitoring were m/z 135 and 137, each measured with a dwell time of 400 ms. Derivatised 3-MCPD and 2-MCPD had retention times of 5.63 and 5.91 min, respectively, under the oven programming conditions described above.

2.8. Confirmation by GC–MS and GC–MS with high resolution

The identities of derivatised 3-MCPD and 2-MCPD were confirmed by GC–MS using a Finnigan MAT 95 double focusing mass spectrometer linked to a Hewlett-Packard 5890 series II gas chromatograph and operated in the EI mode at an electron energy of 70 eV, an emission energy of 1 mA and a source temperature of 250°C.

A 1-μl volume of derivatised extracts was separated on a DB-5 capillary column (30 m×0.25 mm, 0.25 μm, J&W Scientific, Part No. 122-5032) under the following conditions: Injector temperature, 250°C (splitless mode for 0.5 min; split ratio, 1:50); carrier gas, helium (99.999%) at 80 kPa head pressure, resulting in a flow-rate of 1.4 ml min^{-1}; temperature programme, 50°C, held for 1 min, then increased at 5°C min^{-1} to 100°C, then increased by 20°C min^{-1} to 250°C and finally held for 5 min. Retention times of derivatives were as follows: 3-MCPD, 6:10; 2-MCPD, 6:37.

Confirmation analysis was performed by scanning the mass spectrometer from m/z 35 to 200 with a scan speed of 1 s per decade.

Quantitative analysis in the very low μg kg^{-1} range was performed in the multiple ion detection (MID) mode with lock mass technique, using ions m/z 118.9920 and 149.9904 of perfluorotributylamine, which was used as the calibration compound. The instrument was operated at a resolution of 7500, monitoring the ions with accurate masses of 135.0213 and 137.0183 at a cycle time of 0.5 s, resulting in a dwell time of 198 ms per ion.

3. Results and discussion

3.1. Chromatography of MCPD derivatives

The separation of derivatised 3-MCPD in a sample of liquid HVP under optimum conditions is shown in Fig. 1. The chromatography allows the interference-free detection of derivatised 3-MCPD when the ions at m/z 135 and 137 were monitored. The separation of derivatised 3-MCPD and 2-MCPD in a sample of soy sauce is demonstrated in Fig. 2. Because of the considerable structural differences between the two derivatives shown in Fig. 3, the separation is easily achieved on capillary columns of low and medium polarity. All chromatograms were virtually free of any interferences, due to the excellent selectivity that was achieved by operating the mass spectrometer in high resolution multiple ion detection mode.

Quantification of 3-MCPD and 2-MCPD in samples was based on external standards using the calibration solutions described above. Due to the lack of 2-MCPD of appropriate purity, quantification of 2-MCPD was also performed with calibration data obtained from derivatised 3-MCPD. Calibration data showed excellent linearity of the detector response over the concentration range of 0.01–5 mg l^{-1}. For quantification, two calibration plots, covering the concentration ranges of 0–0.25 mg l^{-1} and 0.25–5 mg l^{-1}, respectively, with regression coefficients of 0.9997 and better, were used.

3.2. Validation

The analytical method described was validated in detail using our in-house validation procedure con-

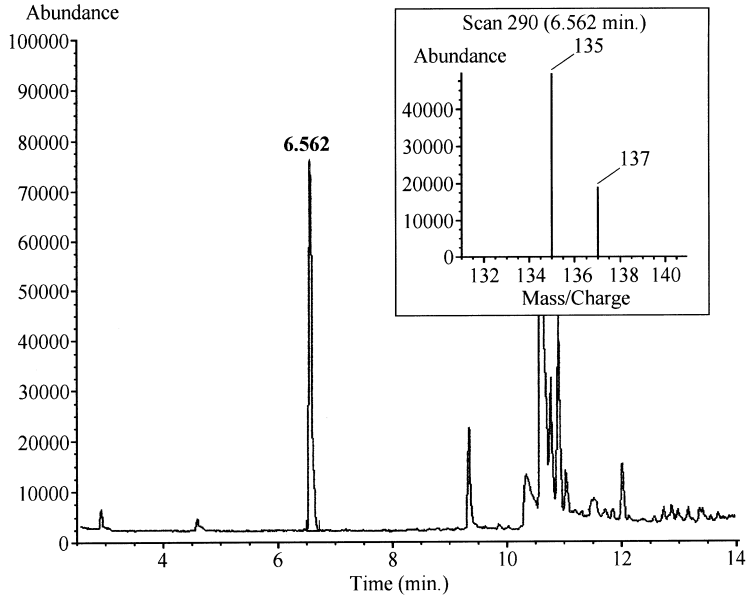

Fig. 1. Sample chromatogram of derivatised 3-MCPD in a liquid HVP sample containing 50 μg kg^{-1} 3-MCPD (signal at 6.562 min). The column used was a DB-1701 (30 m×0.32 mm, 0.25 μm), the carrier pressure was 30 kPa helium, the oven was programmed from 40°C (after 1 min) to 100°C at 5°C min^{-1}, and then to 250°C (for 5 min) at 20°C min^{-1}.

forming to the quality system SN EN 45001/ISO 25 [17], under which our laboratories have operated since 1995.

Limits of detection were established by the analysis of liquid HVP samples, virtually free of any MCPD contamination, which had been spiked with low concentrations of 3-MCPD and the results were compared with replicate analyses ($n=6$) of blank samples of saturated sodium chloride solution. The limit of detection was defined as the minimal analyte concentration that corresponded to the mean detector response of the replicate blank samples plus three standard deviations. A limit of detection of 10 μg kg^{-1} could be achieved for the procedure with Extrelut 3 columns, using low resolution mass spectrometry for detection. By applying the Extrelut 20 column procedure, this value could be reduced to a limit of detection of 1 μg kg^{-1}. For ultimate sensitivity, a limit of detection of 0.1 μg kg^{-1} was easily achieved when high resolution mass spectrometry was used, where amounts of 250 fg of injected 3-MCPD derivative could be detected with a signal-to-noise ratio of better than 5:1.

Limits of quantification and the working range were established by replicate analysis ($n=6$) of liquid HVP samples spiked with desired concentrations of 3-MCPD. They were accepted if the repeatability, r_{95}, according to ISO 5725-2 [18], which is defined as the absolute difference at the 95% level of probability between two results obtained under repeatability conditions, and accuracy of results were within acceptable limits. For the Extrelut 3 column procedure, a limit of quantification of 50 μg kg^{-1} was reached with a relative repeatability, r_{95}, of 18% and a mean recovery of 131%. The limit of quantification for the Extrelut 20 column procedure was 5 μg kg^{-1}. Within the established working range of 50 μg kg^{-1}–10 mg kg^{-1} for the Extrelut 3 column procedure, recoveries were found to be between 85–131%, with a maximum relative repeatability, r_{95}, of 21%.

Ruggedness of the analytical method was checked according to the procedure proposed by Youden and Steiner [19]. Seven different variables of the sample preparation, which are supposed to have an influence on the quality of results, were identified and limits

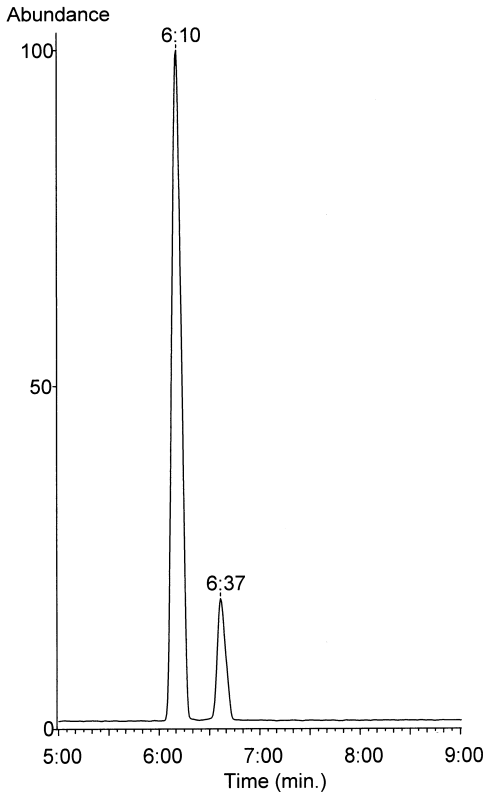

Fig. 2. Sample chromatogram of derivatised 3-MCPD and 2-MCPD in a soy sauce sample containing 15.8 μg kg^{-1} 3-MCPD (signal at 6:10 min) and 2.1 μg kg^{-1} 2-MCPD (signal at 6:37 min). Detection was on a Finnigan MAT 95 with multiple ion detection under the conditions outlined in the text.

within which they should be maintained during routine analysis were agreed. By replicate analysis ($n=8$) of a liquid HVP sample, while varying the different variables, it could be shown that the analytical method is robust, with comparable quality

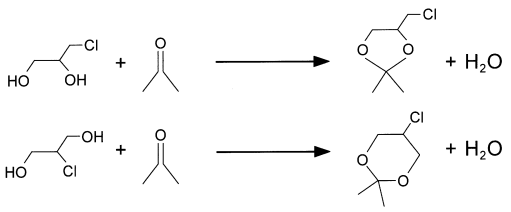

Fig. 3. Reaction of 3-MCPD and 2-MCPD with acetone.

to the repeatability studies, when maintaining the variables within the limits presented in Table 1.

3.3. GC–MS confirmation procedures

Identities of derivatised 3-MCPD and 2-MCPD found by GC–MS with single ion monitoring were confirmed by full scan GC–MS of calibration solutions and extracts of contaminated samples. The EI mass spectra of derivatised 3-MCPD shown in Fig. 4 was unambiguously matched with the corresponding NIST library spectrum. No molecular ion was observed at m/z 150. The spectrum exhibits two abundant signals at m/z 135 and 137, corresponding to the ion species $C_5H_8O_2^{35}Cl$ and $C_5H_8O_2^{37}Cl$, respectively, which were attributed to fragments of the molecular ion after the loss of a methyl group.

3.4. Results of ring test studies

The analytical method was tested by participating in two ring test studies for method evaluation. In the first study, ten unknown samples of liquid protein hydrolysate, obtained by fermentation and spiked with known quantities of 3-MCPD in the range 0.1–1 mg l^{-1}, were analysed with the Extrelut 3 column procedure, whereas in the second study, the Extrelut 20 column procedure was applied for another eight unknown samples of liquid HVP, spiked with known quantities of 3-MCPD in the range 5–50 μg kg^{-1}. In these studies, there were five and four pairs of identical samples, respectively, whose identities were not known to the participants in advance.

Measured results, listed in Tables 2 and 3, respectively, were provided with a measurement uncertainty of 20%, which was deduced from the repeatability study of the validation procedure, and the results were in excellent agreement with the true values. The maximum relative deviation of results found with the Extrelut 3 column procedure was 13% compared to the true values and individual results of identical samples were in good agreement.

Results obtained with the Extrelut 20 column procedure were also in good agreement to the true values, when corrected for the blank value.

Table 1
Ruggedness testing of the analytical method

Variable	Lower limit	Upper limit
Equilibration time of Extrelut column	13 min	18 min
Elution volume	23 ml	27 ml
Volume after evaporation	0 μl[a]	20 μl
Evaporation temperature	25°C	35°C
Time of derivatisation	70 min	100 min
Temperature of derivatisation	35°C	45°C
Drying with anhydrous sodium sulphate	150 mg	650 mg

[a] Sample was evaporated for an additional 5 min after reaching the point of dryness.

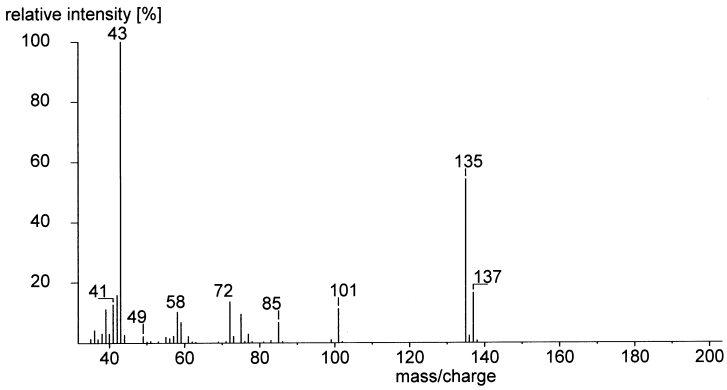

Fig. 4. Mass spectrum of derivatised 3-MCPD.

Table 2
Ring test study using the Extrelut 3 column procedure

Sample	Spiking level (mg l^{-1})	Found (±measurement uncertainty)	
1/10	0.308	0.27±0.06	0.31±0.07
2/6	1.029	0.96±0.20	1.14±0.24
3/8	0.206	0.18±0.04	0.18±0.04
4/9	0.617	0.55±0.12	0.57±0.12
5/7	0.103	0.09±0.02	0.10±0.02

3.5. Results of finished goods

In a limited survey carried out in 1994, the analytical method was used to analyse twenty samples of different finished goods, such as liquid seasonings, soy sauces and bouillon cubes, which were purchased from the local retail market. These products are known to contain protein hydrolysates

Table 3
Ring test study using the Extrelut 20 column procedure

Sample	Spiking level (μg kg^{-1})	Found (±measurement uncertainty)	
A/D	10.04	15.9±3.2	15.5±3.1
B/E	5.02	9.2±1.8	10.1±2.0
C/G	25.10	33.2±6.6	31.2±6.2
F/H	50.20	58.0±11.6	57.7±11.5
Blank	–	3.9±0.8 (n=4)	

Table 4
Survey of finished goods

Sample	Number of samples	3-MCPD ($\mu g\ kg^{-1}$)	2-MCPD ($\mu g\ kg^{-1}$)
Soy sauce	13	4720	256
		2130	125
		1010	51
		15.8	2.1
		2.1	0.3
		1.4	0.5
		1.1	0.3
		0.9	0.3
		0.9	n.d.[a]
		0.8	0.5
		0.7	n.d.
		0.7	n.d.
		0.4	n.d.
Seasoning	4	428	125
		1.9	n.d.
		1.6	n.d.
		0.6	0.2
Bouillon	3	0.4	0.3
		n.d.	n.d.
		n.d.	n.d.

[a] Not detectable; limit of detection was 0.1 $\mu g/kg$.

or have the potential from the way they are produced to contain traces of chloropropanediols. From earlier investigations, it was known that expected levels of 3-MCPD and 2-MCPD are generally very low in these types of sample. Therefore, the survey was performed with the procedure using high resolution MS, as described above, to achieve a low limit of detection. The results are summarised in Table 4.

3-MCPD was found in each sample of soy sauce and seasoning and in one sample of bouillon, whereas 2-MCPD was significantly less abundant and could not be detected in eight samples. Soy sauces with 3-MCPD concentrations above 1 mg kg^{-1} are supposed to be products of a hydrochloric acid hydrolysis. 3-MCPD concentrations in the low $\mu g\ kg^{-1}$ range indicate a manufacturing process where soy sauces are obtained by fermentation. The same considerations seem to apply for liquid seasonings, where the MCPD concentration in one sample was found to be considerably higher than in the other samples.

4. Conclusions

Analysis of different products for 3-MCPD and 2-MCPD can be carried out with good recoveries and good accuracy in the very low $\mu g\ kg^{-1}$ range by the procedure described. The method is fast and was shown to be very reliable. Monitoring of finished goods revealed the presence of 3-MCPD and 2-MCPD (at very low levels) in almost all samples under investigation. MCPDs seem to be ubiquitous contaminants that are not only present in hydrolysed proteins manufactured under acidic conditions, but seem to be inevitable in mixtures containing residual lipids.

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