Determination of Free and Bound 3-Chloropropane-1,2-diol by Gas Chromatography with Mass Spectrometric Detection using Deuterated 3-Chloropropane-1,2-diol as Internal Standard

Veronika DIVINOVÁ, Blanka SVEJKOVSKÁ, Marek DOLEŽAL and Jan VELÍŠEK

Department of Food Chemistry and Analysis, Faculty of Food and Biotechnological Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

Divinová V., Svejkovská B., Doležal M., Velíšek J. (2004): **Determination of free and bound 3-chloropropane-1,2-diol by gas chromatography with mass spectrometric detection using deuterated 3-chloropropane-1,2-diol as internal standard**. Czech J. Food Sci., **22**: 182–189.

An improved routine, simple and sensitive method is presented for the determination of free and bound 3-chloropropane-1,2-diol (3-MCPD) in different foods using capillary gas chromatography with mass spectrometric detection and deuterated 3-MCPD as internal standard. The optimised method was linear within the working calibration standard concentrations in the range of 0.009–1.3 mg 3-MCPD per 1 kg of sample. The LOD and LOQ were 0.003 μg/kg and 0.009 μg/kg, respectively. Validation of the method was carried out by analysing standards of 3-MCPD, acid-HVP, roasted coffee samples, and the same samples spiked with 3-MCPD. Repeatability (expressed as RSD) of the method was in the range 1.0–4.2%, the average spike recoveries were 99.1–99.5% (RSD = 0.8–1.4%), respectively. 3-MCPD bound in esters with higher fatty acids was isolated as fat, the isolated fat was subjected to methanolysis and 3-MCPD generated was quantified using the same method. The LOD and LOQ were determined to be 1.1 mg/kg of lipids and 3.3 mg/kg of lipids, respectively. Using the optimised method, 20 samples of retail food products were analysed for their free and bound 3-MCPD. All samples contained free 3-MCPD at 9.6–83 μg/kg (RSD = 0.4–7.0%). The level of the bound 3-MCPD varied between the LOD and 2.4 mg/kg with RSD = 0.3–2.4%.

Keywords: 3-chloropropane-1,2-diol (3-MCPD); chloropropanediols; 3-MCPD esters; phenylboronic acid; food analysis

3-Chloropropane-1,2-diol (known as 3-MCPD) was first identified in acid-hydrolysed vegetable protein (acid-HVP) in 1981 (Davídek *et al*. 1982). In view of its toxicity, a regulatory limit of 0.02 mg/kg was adopted for 3-MCPD in soy sauce and acid-HVP (European Commission 2001). Recent studies revealed that elevated levels of 3-MCPD can occur not only in soy sauces and related products (MacArthur *et al*. 2000; Crews *et al*. 2003; Nyman *et al*. 2003) but also in many foods and food ingredients formulated without acid-HVP (Crews *et al*. 2001, 2002; Hamlet *et al*. 2002a,b; Breitling-Utzmann *et al*. 2003). Fatty acid esters of 3-chloropropane-1,2-diol were identified in acid-HVP (Velíšek *et al*.

Partly supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project MSM No. 223300004.

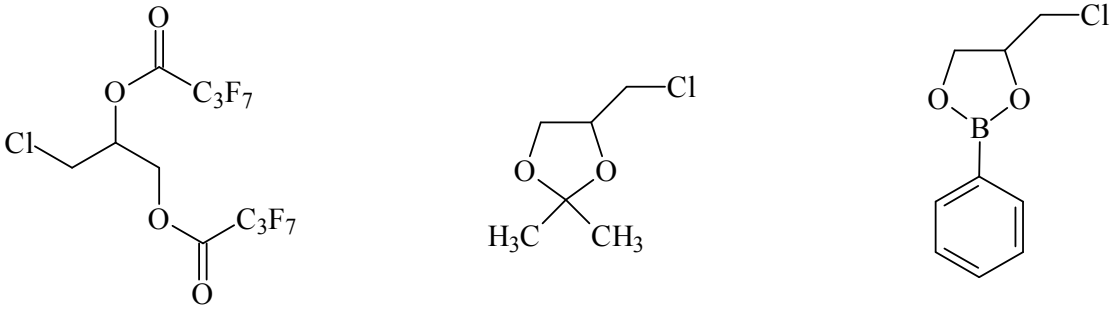
1980) and recently found in a variety of processed foods (Svejkovská *et al*. 2004).

Several analytical methods for the determination of 3-MCPD in acid-HVP, soy sauces, related products, and processed foods have been published; the procedures based on capillary gas chromatography (GC) have been shown to be the methods of choice.

Earlier GC methods used to determine underivatised 3-MCPD in acid-HVP by means of mass spectral (MS) detection (Wittmann 1991) or electrolytic conductivity detection operated in the halogen mode (Spyres 1993) were not sufficiently sensitive or selective for the determination of μg/kg levels of 3-MCPD in diverse foods. Wittmann (1991) reported a method for the determination of 3-MCPD in seasonings and in foodstuffs containing seasonings. After adsorption on a column of kieselguhr (Extrelut), 3-MCPD was partitioned into ethyl acetate and analysed. The limit of detection (LOD) established was 0.1 mg/kg. The modified method reported by Spyres (1993) used 1-chlorotetradecane as the internal standard and the LOD was found to be 0.25 mg/kg.

Recent GC methods and their modifications rely on the formation of volatile and stable 3-MCPD derivatives (Figure 1) that may be readily characterised by selective MS detection. Van Bergen *et al*. (1992) developed a method based on GC of heptafluorobutyrates of chloropropanols in acid-HVP. 3-MCPD was extracted into saline solution and then partitioned into diethyl ether using a solid-phase extraction based on diatomaceous earth (Extrelut). Concentrated extracts were then reacted with heptafluorobutyrylimidazole to give the corresponding diester of 3-MCPD. This ester was analysed using electron capture detection. The LOD was shown to be 0.05–0.1 mg/kg. Hamlet and Sutton (1997) reported a modified GC/MS procedure for the determination of 3-MCPD in acid-HVP and seasonings. Quantification was carried out by the stable isotope internal standard method using deuterium-labeled 3-MCPD (3-MCPD-d_7) added to the sample before extraction. Calibration was linear over the range 5–500 pg/μl with LOD below 0.005 mg/kg. The procedure was extended to cover other food matrices such as flour, bread, meat and starch products (Hamlet 1998) and was shown to have the LOD between 0.003–0.005 mg/kg for all commodities. The method using commercially available deuterium-labeled internal standard (3-MCPD-d_5) was validated by collaborative trial (Brereton *et al*. 2001). The limit of quantification (LOQ) was established as approaching 0.01 mg/kg. The procedure was recently modified (Chung *et al*. 2002) using silica gel as 3-MCPD sorbent and ethyl acetate instead of diethyl ether. The LOQ of this method was found to be about 0.005 mg/kg. Precision of the method was satisfactory at about 5% and the recovery of 3-MCPD from soy sauce spiked at 0.025 mg/kg was 98%. Both methods are also suitable for the determination of 2-chloropropane-1,3-diol (2-MCPD) and other chloropropanols including 1,3-dichloropropan-1-ol (1,3-DCP).

Solid-phase extraction on diatomaceous earth has also been utilised for the GC/MS determination of 3-MCPD as acetonide derivative in soy sauces, seasonings and bouillon (Meierhans *et al*. 1998; Jin *et al*. 2001). The reaction of 3-MCPD with ac-



(a) 3-chloropropane-1,2-diol-di(heptafluoro)butyrate

(b) 4-chloromethyl-2,2-dimethyl-1,3-dioxolane

(c) 4-chloromethyl-2-phenyl 1,3,2-dioxaborolane

Figure 1. Reaction products of 3-MCPD with derivatisation reagents: (a) heptafluorobutyrylimidazole; (b) acetone; (c) phenylboronic acid

etone was catalysed with toluene-4-sulfonic acid and the resulting substituted 1,3-dioxolane was analysed by GC/MS. Quantification was carried out by the external standard method (3-MCPD) with the LOD of 0.001 mg/kg. The method is also suitable for the determination of 2-MCPD but not suitable for the determination of 1,3-DCP since this chloropropanol does not form cyclic acetonide derivative.

3-MCPD was also determined by GC in acid-HVP and in soy sauces using phenylboronic acid (PBA) as a derivatisation reagent, and either flame-ionisation (Plantinga *et al*. 1991) or MS detection (Wu & Zhang 1999). No sample purification was carried out as PBA reacts specifically with diols forming non-polar cyclic derivatives extractable into hexane. Other chloropropanols such as 1,3-DCP cannot be determined by this method. The method of Plantinga *et al*. (1991) showed a standard deviation of 0.019 mg/kg and a repeatability of 0.05 mg/kg at a level of 0.84 mg/kg. The LOD was 0.2 mg/kg. The LOD of a modification of this method used for determination of 3-MCPD in systems simulating processed foods was recently shown to be 0.02 mg/kg with flame-ionisation detection and 0.01 mg/kg with MS detection (Calta *et al*. 2004). Ushijima *et al*. (1995) employed solid-phase extraction and a purification stage described previously. Quantification of 3-MCPD in seasonings was made by the internal standard method using hexane-1,2-diol before derivatisation. GC/MS analysis without purification of a variety of samples comprising soy sauce, bakery products, savoury snacks, Asian convenience products (instant noodle dishes), malt products, and beer was performed by the stable isotope internal standard method using 3-MCPD-d_5 (Breitling-Utzmann 2003). LODs were found to range between 0.003 (soy sauce) and 0.01 (toast) mg/kg, LOQs from 0.01 (soy sauce) to 0.05 (toast) mg/kg.

This paper reports on an improved PBA method using deuterium-labeled internal standard (3-MCPD-d_5) and the in-house validation of the method which eliminates the matrix effects and provides the sensitivity complying with the recently imposed action limit while maintaining the needed selectivity and simplicity. It has been applied to the monitoring of the acid-HVP manufacturing process, the levels of occurrence of 3-MCPD in different foods, and to determine 3-MCPD occuring in foods bound as esters with higher fatty acids.

MATERIAL AND METHODS

Chemicals. 3-Chloropropane-1,2-diol (3-MCPD, > 98%) was purchased from E. Merck (Darmstadt, Germany), 3-MCPD-d_5 (99.4%) from Dr. Ehrenstorfer (Augsburg, Germany), phenylboronic acid (PBA, ≥ 97%) from Fluka Chemie (Buchs, Switzerland). 1,2-Dipalmitoyl-3-chloropropane (96.9%) was synthesised according to Kraft *et al*. (1979) and purified using a silica gel column and light petroleum ether/diethyl ether mixtures. All other reagents and solvents were of analytical purity.

Samples. Acid-HVP, coffee, and soybean oil were purchased from retail outlets in Prague.

Solutions

Internal standard. The stock standard solution containing 0.1 mg of 3-MCPD-d_5 per 1 ml in water was prepared, 5 ml of this solution was transferred to a 50 ml volumetric flask and filled to the mark with water, thus resulting in the final concentration of 10 µg MCPD-d_5/ml. Amount of 50 µl of this final solution were added to each sample.

PBA. 1 g phenylboronic acid was dissolved in 4 ml of an acetone:water mixture (19:1, v/v).

3-MCPD. The stock standard solution containing 1 mg 3-MCPD/ml in 20% (w/v) sodium chloride was prepared. Working standard solutions were prepared for calibration by diluting the stock solution with 20% NaCl to obtain concentrations of 3-MCPD in the range of 0.02–3 µg/ml.

Soybean oil. The stock solution contained 0.01 g soybean oil per 1 ml in tetrahydrofurane (THF).

Sulphuric acid. Sulphuric acid (98%, 1.8 ml) was dissolved in methanol (100 ml).

1,2-Dipalmitoyl-3-chloropropane. The stock standard solution of 1,2-dipalmitoyl-3-chloropropane was prepared in the concentration of 0.5 mg/ml in THF. Mixed working standard solutions were prepared for calibration by diluting the stock standard solution with THF to give levels of this ester ranging from 0.001 to 0.1 mg/ml with soybean oil at 10 mg/ml. 1 ml aliquots of each of the working solutions were then interesterified in a sulphuric acid solution.

Calibration curves. The individual working standard solutions of 3-MCPD (2 ml) were pipetted to a series of 10 ml distillation flasks. The final internal standard solution (50 µl) and PBA solution (0.4 ml) were added and the flasks were heated in a water bath at 90°C for 20 min. After cooling to room temperature, 2 ml hexane was added and the

3-MCPD derivative formed was extracted by vigorous shaking. 1 µl of the hexane layer was analysed by GC/MS. The ratio of the peak area response of 3-MCPD to the peak area response of the internal standard (3-MCPD-d_5) for the quantitation ions was measured for the calibration standards and the calibration graph constructed by plotting the peak area ratios (y-axis) against the amount of 3-MCPD (x-axis).

Aliquots (1 ml) of the working solutions containing 1,2-dipalmitoyl-3-chloropropane and soybean oil were transferred to 10 ml flasks, 1.8 ml of sulphuric acid solution was added and the mixture was heated at 40°C for 16 h. The mixture was then cooled to room temperature, neutralised with 0.5 ml of saturated $NaHCO_3$ solution in water and evaporated to dryness at 55°C using a vacuum rotary evaporator. The residue was dissolved in 2 ml of 20% sodium chloride and the resulting solution was further treated as described above. The ratio of ion areas at m/z 147 (3-MCPD) and at m/z 150 (3-MCPD-d_5) was plotted against the 3-MCPD concentration.

Sample extraction and 3-MCPD derivatisation

Free 3-MCPD. To the sample (about 5 g) placed in a 100 ml beaker, 30 ml of a hexane/acetone mixture (1:1, v/v) and 50 µl of the internal standard final solution were added. The mixture was homogenised for 3 min using the homogeniser Ultra-Thurrax T25 (Janke and Kunkel, IKA-Labortechnik, Switzerland) and filtered through a Büchner funnel. The solid residue in the beaker was washed with two 10 ml portions of the hexane/acetone mixture and the filtrate was transferred into a separatory funnel containing 10 ml water. The lower aqueous layer was separated, the organic layer re-extracted with another 10 ml portion of water and the combined extracts were evaporated in a 100 ml distillation flask under vacuum at 55°C to dryness. The residue was dissolved in 2 ml of the 20% NaCl solution, 0.4 ml of the PBA solution was added and the flask was heated in a water bath maintained at 90°C for 20 min. After cooling to room temperature, 2 ml hexane was added and the derivative of 3-MCPD formed was extracted by vigorous shaking. 1 µl of the hexane layer was analysed by GC/MS.

Bound 3-MCPD. The homogenised sample was extracted with diethyl ether (1:4, w/v) and the extract was filtered through a Büchner funnel. The residue was washed with two portions of diethyl ether (1:1, w/v), the filtrate was extracted in a separatory funnel with water (5:1, v/v) and centrifuged if necessary. The solvent was dried over anhydrous sodium sulphate and evaporated using a vacuum rotary evaporator. The residue obtained (100 mg) was transferred to a 10 ml volumetric flask and dissolved in 10 ml of THF. Aliquots (1 ml) of this solution were interesterified using the procedure described above. The stock standard solution of 3-MCPD-d_5 was prepared using THF as the solvent.

GC/MS analysis. GC/MS analysis was carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Series 5973 quadrupole mass selective detector Agilent 5973 MSD (70 eV) and the data processing system (MSD ChemStation, G1701CA version C.00.00). Gas chromatography was performed on a capillary column SPB™-1 (30 m × 0.25 mm i.d., thickness of 1 µm, Supelco, PA, USA). The injector was held at 250°C (splitless), the column temperature was programmed from 80°C (1 min) to 300°C (37 min) at the rate of 10°C/min. Helium at the flow rate of 0.8 ml/min was used as the carrier gas, 1 µl sample was injected. Quantitative analysis was carried out by monitoring characteristic ions at m/z 147 (3-MCPD) and at m/z 150 (3-MCPD-d_5) (Figure 2). Ions at m/z 91and 196 (3-MCPD) and at m/z 93 and 201 (3-MCPD-d_5) were used as qualifiers.

RESULTS AND DISCUSSION

Free 3-MCPD

PBA readily reacts with 3-MCPD to give a stable derivative 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane (Figure 1) appropriate for GC/MS analysis. PBA was the derivatisation reagent of choice as it is very selective, the product is extractable with non-polar solvents, only a marginal contamination due to the sample constituents occurs and the tedious purification on diatomaceous earth is not obligatory as it is in the methods employing heptafluorobutyrylimidazole as the reagent. In order to eliminate the variations in the LODs and LOQs due to the influences of the different sample matrices mentioned by (Breitling-Utzmann *et al*. 2003), a simple extraction of fat was employed for the sample purification prior to the derivatisation of 3-MCPD.

The method employed was linear within the working calibration standard concentrations ($c_{3\text{-MCPD}}$)

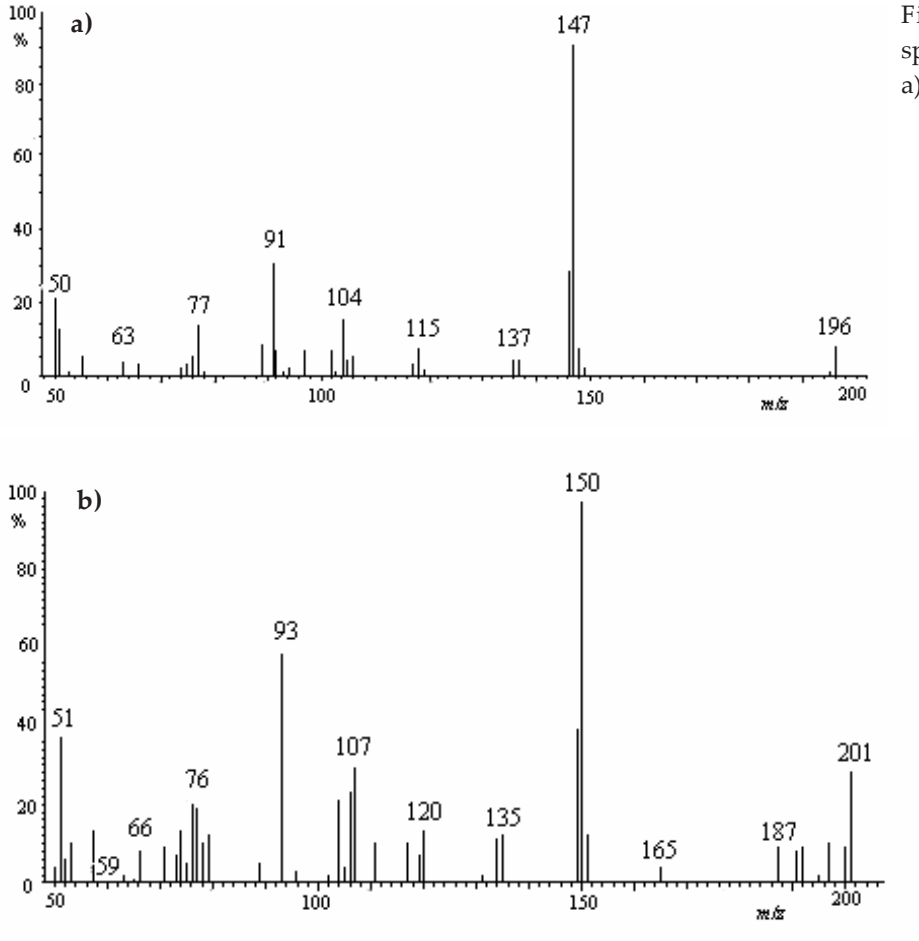


Figure 2. Full scan EI mass spectra for PBA derivatives of a) 3-MCPD and b) 3-MCPD-d_5

in the range of 2E-05 to 3.29E-03 μg 3-MCPD/μl (corresponding to 0.009–1.3 mg 3-MCPD per 1 kg of sample) and had an equation $A_{3\text{-MCPD}}/A_{3\text{-MCPD-}d_5} = 0.0053 \times c_{3\text{-MCPD}}$ (μg 3-MCPD/kg sample). The correlation coefficient was 0.9997. With samples having the level of 3-MCPD higher than 1.3 mg/kg, a lower amount of the sample may be used. The LOD and LOQ were determined using the standard solutions of 3-MCPD. The values were 0.003 μg/kg and 0.009 μg/kg, respectively. The LOD was defined as the concentration of 3-MCPD that would result in a signal for the quantitation ion that was 3 times the peak-to-peak noise of the background signal. The LOQ was defined as the concentration of 3-MCPD that would result in a signal for the same ion that was 10 times the peak-to-peak noise of the background signal.

Validation of the method was carried out by analysing standards, three replicates of blank acid-HVP, and three replicates of roasted coffee samples. The average amount of 3-MCPD in the acid-HVP was 0.0104 mg/kg (the standard error was 5.8E-05) and the relative standard deviation (RSD) was 1.0%. The samples of roasted coffee contained average 0.0161 mg 3-MCPD/kg (the standard error was 39E-05) and the RSD was 4.2%. Repeatability (expressed as RSD) of the method was thus in the range 1.0–4.2% for the target analyte in both samples (acid-HVP and coffee). The samples of acid-HVP were then spiked with either 10 μg/kg or 20 μg/kg and the samples of roasted coffee with 20 μg/kg. All samples were analysed in triplicates. The average spike recoveries of acid-HVP were 99.1% (RSD = 1.0%) and 99.5% (RSD = 0.8%), respectively. The average spike recovery for coffee was 99.3% (RSD = 1.4%).

Bound 3-MCPD

Interesterification of TLC purified natural esters of 3-MCPD isolated from goat milk using 1M HCl in methanol was carried out by Cerbulis *et al*. (1984) to identify the fatty acids and the alcohol moiety. Using the same interesterification process, we found that small amounts of 3-MCPD formed from neutral fats indicating that

Table 1. Interesterification reaction influenced by time

Time (h)	3-MCPD recovered (%)*	RSD (%)
2	32.0	2.0
4	68.1	3.0
8	94.4	1.0
12	97.4	0.4
16	99.4	1.0
20	98.4	1.7
24	97.3	0.4

*3 replications

3-MCPD was generated during the interesterification reaction. Alternatively, interesterification was carried out with methanol and H_2SO_4 as the catalyst at room temperature. Using one of the mixed working standard solutions containing in 1 ml 1,2-dipalmitoyl-3-chloropropane at 0.08 g/kg of soybean oil, the yield of 3-MCPD increased with time during the first 16 hours and then its level decreased again (Table 1). The removal of fatty acid methylesters from the mixture with hexane, the presence of variable levels of natural lipids (soybean oil) and 1,2-dipalmitoyl-3-chloropropane did not significantly influence (t-test, $\alpha = 0.05$) the recovery of 3-MCPD (Table 2). The stability of 3-MCPD formed during interesterification was followed by analysing 2 samples spiked with 3-MCPD and soybean oil (Table 3); no significant decrease of the analyte was observed (t-test, $\alpha = 0.05$).

Using the optimised conditions (interesterification for 16 h, without extraction by hexane, lipids at 10 mg per sample), the method was subjected to validation using standards of 1,2-dipalmitoyl-3-chloropropane and 3-MCPD (Table 2). The calibration curve constructed by using 1,2-dipalmitoyl-3-chloropropane instead of 3-MCPD had an equation $A_{3\text{-MCPD}}/A_{3\text{-MCPD-}d_5} = 0.0052 \times c_{3\text{-MCPD}}$ (μg 3-MCPD/kg sample). The correlation coefficient was 0.9994. Both calibration curves were tested using t-test and no differences at the probability levels of 0.05, 0.01 and 0.001 were found. The LOD and LOQ were determined using the calibration standards of 1,2-dipalmitoyl-3-chloropropane. The LOD, due to a relatively low amount of interesterified fat, was 1.1 mg/kg of lipids when expressed as 3-MCPD, and 5.86 mg/kg of lipids when calculated as 1,2-dipalmitoyl-3-chloropropane. The LOQ was 3.3 mg/kg of lipids when expressed as 3-MCPD and

Table 2. Interesterification reaction influenced by fatty acid methylesters, neutral lipids and amount of 1,2-dipalmitoyl-3-chloropropane

Method No.*	Extraction by hexane	1,2-Dipalmitoyl-3-chloropropane**	Soybean oil (mg/ml)	3-MCPD recovered (%)	RSD (%)
1	yes	1.4	1	98.1	0.9
2	no	1.4	1	100.1	0.5
3	no	0.28	1	100.5	1.5
4	no	0.14	5	99.9	0.6
5	no	0.08	10	99.4	1.0

*3 replications; **expressed in g/kg of soybean oil

Table 3. Stability of 3-MCPD during interesterification

Method No.*	Extraction by hexane	Soybean oil (mg/ml)	3-MCPD ** recovered (%)	RSD (%)
1	no	1	98.8	1.7
2	no	10	99.9	0.5

*3 replications; **0.02 mg in interesterified sample

17.57 mg/kg of lipids when calculated as 1,2-dipalmitoyl-3-chloropropane.

Using the optimised method, 20 samples of retail food products were analysed for their free and bound 3-MCPD. All samples contained free 3-MCPD at 9.6–83 μg/kg (RSD = 0.4–7.0%). The level of the bound 3-MCPD varied between the LOD and 2.4 mg/kg with RSD = 0.3–2.4%.

CONCLUSION

The GC/MS method using PBA as the derivatisation reagent and deuterated 3-MCPD as the internal standard has proved to be a sensitivite and selective method for the determination of trace levels of 3-MCPD in acid-HVP and in different foods. The method has the advantage of minimal cleanup. A routine LOD of about 0.003 mg/kg is sufficient to comply with the recommendations in EC for soy sauce and acid-HVP. The method also has a potential to be used for the determination of 3-MCPD bound in esters with higher fatty acids. A routine LOD was of about 1.1 mg/kg of sample lipids.

References

Breitling-Utzmann C.M., Köbler H., Herbolzheimer D., Maier A. (2003): 3-MCPD – occurrence in bread crust and various food groups as well as formation in toast. Dtsch. Lebensm.-Rdsch., **99**: 280–285.

Brereton P., Kelly J., Crews C., Honour S., Wood R., Davies A. (2001): Determination of 3-monochloropropane-1,2-diol in food and food ingredients using mass spectrometric detection: collaborative study. J. Assoc. Off. Anal. Chem. Int., **84**: 1–11.

Calta P., Velíšek J., Doležal M., Hasnip S., Crews C., Réblová Z. (2004): Formation of 3-chloropropane-1,2-diol in systems simulating processed foods. Eur. Food Res. Technol., **218**: 501–506.

Cerbulis J., Parks O.W., Liu R.H., Piotrowski E.G., Farrell H.M. Jr. (1984): Occurrence of diesters of 3-chloro-1,2-propanediol in the neutral lipid fraction of goat's milk. J. Agric. Food Chem., **32**: 474–476.

Chung W., Hui K., Cheng S. (2002): Sensitive method for the determination of 1,3-dichloropropan-2-ol and 3-chloropropane-1,2-diol in soy sauce by capillary gas chromatography with mass spectrometric detection. J. Chromatogr. A, **952**: 183–192.

Crews C., Brereton P., Davies A. (2001): Effect of domestic cooking on the levels of 3-monochloropropane-1,2-diol in food. Food Addit. Contamin., **18**: 271–280.

Crews C., Hough P., Brereton P., Harvey D., MacArthur R., Matthews W. (2002): Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999–2000. Food Addit. Contamin., **19**: 22–27.

Crews C., Hasnip S., Chapman S., Hough P., Potter N., Todd J., Brereton P., Matthews W. (2003): Survey of chloropropanols in soy sauces and related products purchased in the UK in 2000 and 2002. Food Addit. Contamin., **20**: 916–922.

Davídek J., Velíšek J., Kubelka V., Janíček G. (1982): New chlorine containing organic compounds in protein hydrolysates. In: Baltes W., Czedik-Eysenberg P.B., Pfannhauser W. (eds): Recent Developments in Food Analysis. Proc. Euro Food Chem. I, Vienna, Austria: 17–20 Feb., 1981. Weinheim, Deerfield Beach, Florida: 322–325.

European Commission (2001): Commission Regulation (EC) No 466/2001. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of European Communities. 8th March.

Hamlet C.G. (1998): Analytical methods for the determination of 3-chloro-1,2-propanediol and 2-chloro-1,3-propanediol in hydrolysed vegetable protein, seasonings and food products using gas chromatography/ion trap tandem spectrometry. Food Addit. Contamin., **15**: 451–465.

Hamlet C.G., Sutton P.G. (1997): Determination of the chloropropanols 3-chloro-1,2-propanediol and 2-chloro-1,3-propanediol in hydrolysed vegetable proteins and seasonings by gas chromatography/ion trap tandem spectrometry. Rapid Commun. Mass Spectrom., **11**: 1417–1424.

Hamlet C.G., Jayaratne S.M., Matthews W. (2002a): 3-Monochloropropane-1,2-diol (3-MCPD) in food ingredients from UK food producers and ingredient suppliers. Food Addit. Contamin., **19**: 15–21.

Hamlet C.G., Sadd P.A., Crews C., Velíšek J., Baxter D.E. (2002b): Occurrence of 3-chloropropane-1,2-diol (3-MCPD) and related compounds in foods: a review. Food Addit. Contamin., **19**: 619–631.

Jin Q., Zhang Z., Luo R., Li J. (2001): Survey of 3-monochloropropane-1,2-diol (3-MCPD) in soy sauce and similar products. Wei Shang Yan Jiu, **30**: 60–61.

Kraft R., Brachwitz H., Etzold G., Langen P., Zopfl H.J. (1979): Zur Synthese und Massspektrometrischen Charakterisierung von 2-*O*-acyl-3-halogen-propan-1,2-diolen. J. Prakt. Chem., **321**: 769–774.

MacArthur R., Crews C., Davies A., Brereton P., Hough P., Harvey D. (2000): 3-Monochloropropane-1,2-diol (3-MCPD) in soy sauce and similar products available from retail outlets in the UK. Food Addit. Contamin., **17**: 903–906.

Meierhans D.C., Bruehlmann S., Meili J., Taeschler C. (1998): 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. J. Chromatogr. A, **802**: 325–333.

Nyman P.J., Diachenko G.W., Perfetti G.A. (2003): Survey of chloropropanols in soy sauces and related products. Food Addit. Contamin., **20**: 909–915.

Plantinga W.J., Van Toorn W.G., Van der Stegen G.H.D. (1991): Determination of 3-chloropropane-1,2-diol in liquid hydrolysed vegetable proteins by capillary gas chromatography with flame ionization detector. J. Chromatogr., **555**: 311–314.

Spyres G. (1993): Determination of 3-chloropropane-1,2-diol in hydrolysed vegetable protein by capillary gas chromatography with electrolytic conductivity detection. J. Chromatogr., **638**: 71–74.

Svejkovská B., Novotný O., Divinová V., Réblová Z., Doležal M., Velíšek J. (2004): Esters of 3-chloropropane-1,2-diol in foodstuffs. Czech J. Food Sci., **22**: 190–196.

Ushijima K., Deguchi Y., Kikukawa K., Nomura T., Adachi T. (1995): Analysis for residual 3-chloropropane-1,2-diol in seasonings after derivatisation with phenylboronic acid. Shokuhin Eiseigaku Zasshi, **36**: 360–364.

Van Bergen C.A., Collier P.D., Cromie D.D.O., Lucas R.A., Preston H.D., Sissons D.J. (1992): Determination of chloropropanols in protein hydrolysates. J. Chromatogr., **589**: 109–119.

Velíšek J., Davídek J., Kubelka V., Janíček G., Svobodová Z., Šimicová Z. (1980): New chlorine-containing organic compounds in protein hydrolysates. J. Agric. Food Chem., **28**: 1142–1144.

Wittmann R. (1991): Determination of dichloropropanols and monochloropropandiols in seasonings and in foodstuffs containing seasonings. Z. Lebensm. Unters. Forsch., **193**: 224–229.

Wu H., Zhang G. (1999): Determination of 3-chloropropane-1,2-diol in soy sauce by GC-MS-SIM. Fenxi Ceshi Xuebao (J. Instrum. Anal.), **18**: 64–65.

Received for publication August 16, 2004
Accepted after corrections October 4, 2004

Souhrn

Divinová V., Svejkovská B., Doležal M., Velíšek J. (2004): S**tanovení volného a vázaného 3-chloropropan-1,2-diolu plynovou chromatografií s hmotnostním detektorem, používající deuterovaný 3-chloropropan-1,2-diol jako vnitřní standard.** Czech J. Food Sci., **22**: 182–189.

Pro stanovení volného a vázaného 3-chloropropan-1,2-diolu (3-MCPD) v potravinách byla zavedena rutinní, jednoduchá a citlivá metoda kapilární plynové chromatografie s hmotnostním detektorem, používající deuterovaný 3-MCPD jako vnitřní standard. Optimalizovaná metoda poskytovala lineární odezvu v rozsahu 0,009–1,3 mg 3-MCPD/kg vzorku. Limit detekce byl 0,003 μg/kg vzorku a limit kvantifikace 0,009 μg/kg vzorku. Validace metody byla provedena analýzou standardních roztoků 3-MCPD, analýzou vzorku bílkovinného hydrolyzátu a pražené kávy a stejných vzorků fortifikovaných 3-MCPD. Opakovatelnost vyjádřená jako relativní směrodatná odchylka se pohybovala v rozmezí 1,0–4,2 %, zpětné nálezy fortifikovaných vzorků byly 99,1–99,5 % (relativní směrodatná odchylka RSD = 0,8–1,4 %). 3-MCPD vázaný ve formě esterů s vyššími mastnými kyselinami byl izolován jako tuk, který byl podroben metanolýze, a vzniklý 3-MCPD byl stanoven stejnou metodou. Limit detekce vztažený na tuk vzorku byl 1,1 mg/kg a limit kvantifikace 3,3 mg/kg. Uvedená optimalizovaná metoda byla použita ke zjištění obsahu volného a vázaného 3-MCPD ve 20 vzorcích vybraných potravin. Všechny analyzované vzorky obsahovaly volný 3-MCPD v mezích 9,6–83 μg/kg (RSD = 0,4–7,0 %). Hladiny vázaného 3-MCPD se pohybovaly od hodnoty limitu detekce po 2,4 mg/kg, relativní směrodatná odchylka byla 0,3–2,4 %.

Klíčová slova: 3-chloropropan-1,2-diol (3-MCPD); chloropropandioly; estery 3-MCPD; fenylboronová kyselina; analýza potravin

Corresponding author:

Prof. Ing. Jan Velíšek, DrSc., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 233 335 217, fax: + 420 233 339 990, e-mail: jan.velisek@vscht.cz