BRITISH STANDARD

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14573:2004

Foodstuffs — Determination of 3-monochloropropane-1,2-diol by GC/MS

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ICS 67.050

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National foreword

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EN 14573

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Foodstuffs - Determination of 3-monochloropropane-1,2-diol by GC/MS

Produits alimentaires - Dosage du 3-monochloropropane-1,2-diol par CG/SM

Lebensmittel - Bestimmung von 3-Monochlorpropan-1,2-diol mit GC/MS

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Contents

Foreword

This document (EN 14573:2004) has been prepared by Technical Committee CEN/TC 275 “Food Analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This document specifies a gas chromatographic method using mass spectrometric detection for the determination of 3-monochloropropane-1,2-diol (3-MCPD) in hydrolysed vegetable proteins and other foodstuffs [1]. The method has been validated in interlaboratory studies for malt extract, soup powder, bread crumbs, salami sausage, cheese alternative and hydrolysed vegetable protein.

2 Principle

The sample is mixed with a deuterated internal standard, with sodium chloride solution and with a solid support material. The mixture is transferred to a chromatographic column and is first extracted with a mixture of n-hexane and diethyl ether for removing non-polar components. Then, 3-MCPD is eluted with diethyl ether, the eluate is concentrated and an aliquot portion is derivatized using heptafluorobutyrylimidazole. The solution is analyzed by gas chromatography using mass spectrometric detection [2].

3 Reagents

3.1 General

Unless otherwise specified, use reagents of recognized analytical quality and distilled or demineralized water.

Take every precaution to avoid possible contamination of water, solvents, inorganic salts etc. by plastics and rubber materials. Use only glass containers for storage and handling of all water and reagents.

WARNING — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

3.2 n-Hexane, glass distilled

3.3 Diethyl ether, glass distilled

3.4 2,2,4-Trimethylpentane

3.5 Ethyl acetate

3.6 Solvent mixture, n-hexane (3.2) and diethyl ether (3.3) 9+1 (*V/V*)

3.7 Sodium chloride solution, 290 g of sodium chloride dissolved in 1 l of water

3.8 Sodium sulfate, anhydrous

3.9 Heptafluorobutyrylimidazole

3.10 Solid support material, Extrelut® [1], refill packs, approx. 20 g

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[1] Extrelut® is the trade name of a suitable product available commercially from E. Merck, Darmstadt (Germany). This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

3.11 3-Monochloropropane-1,2-diol (3-MCPD)

3.12 d5-3-Monochloropropane-1,2-diol (d5-3-MCPD), minimum 98 % isotopic purity

The stability of d5-3-Monochloropropane-1,2-diol (d5-3-MCPD) is limited and should be checked.

3.13 3-MCPD stock solution, ρ(3-MCPD) = 1 mg/ml

Weigh 25 mg of 3-MCPD (3.11) and dilute to the mark with ethyl acetate (3.5) in a 25 ml volumetric flask.

3.14 3-MCPD standard solutions

Dilute 10 ml of the 3-MCPD stock solution (3.13) to the mark with ethyl acetate (3.5) in a 100 ml volumetric flask. From this dilution, transfer volumes of each 0 µl, 12,5 µl, 25 µl, 125 µl, 250 µl and 500 µl into 25 ml volumetric flasks and dilute to the mark with 2,2,4-trimethylpentane (3.4) to give 0 µg/ml, 0,05 µg/ml, 0,10 µg/ml, 0,50 µg/ml, 1,0 µg/ml, and 2,0 µg/ml 3-MCPD.

3.15 d5-3-MCPD stock solution, ρ(d5-3-MCPD) = 1 mg/ml

Weigh 25 mg of d5-3-MCPD (3.12) and dilute to the mark with ethyl acetate (3.5) in a 25 ml volumetric flask.

3.16 d5-3-MCPD internal standard solution, ρ(d5-3-MCPD) = 10 µg/ml

Dilute 1,0 ml of the d5-3-MCPD stock solution (3.15) to the mark with ethyl acetate (3.5) in a 100 ml volumetric flask.

3.17 Nitrogen

4 Apparatus

4.1 General

Use usual laboratory equipment and, in particular, the following.

4.2 Ultrasonic bath

4.3 Vortex shaker

4.4 High-speed laboratory blender

4.5 Centrifuge, capable to be spun at a rotational frequency of at least 3500 min^{-1}, with centrifuge tubes of capacity 100 ml.

4.6 Filter paper, fast flow rate

4.7 Chromatographic column, 2 cm internal diameter, 40 cm long, with a sintered glass disk and tap.

4.8 Rotary evaporator, with a water bath and with evaporation flasks of capacity 250 ml.

4.9 Syringe, 1 ml, gas-tight

4.10 Aluminium block heater

4.11 Glass vials, 2 ml and 4 ml, with screw caps

4.12 Gas chromatograph, equipped with a split/splitless injector and connected with a mass spectrometer, capable of selected ion monitoring or full scanning at high sensitivity.

5 Procedure

5.1 Preparation of samples

Grind dry samples such as stock cubes and cereals to a fine consistency. Mince or grate bread, cheese, salami and fish samples to a homogenous mixture. Mix all samples thoroughly before analysis.

If analysis cannot proceed immediately, store all samples in air-tight containers, frozen if necessary.

5.2 Extraction

5.2.1 Hydrolyzed vegetable protein, soy sauce, soup powder and malt extract

Weigh 5 g of soup powder, 8 g of hydrolyzed vegetable protein or soy sauce or 10 g of malt extract each to the nearest 0,01 g and add 100 µl of the d5-3-MCPD internal standard solution (3.16). Add sodium chloride solution (3.7) to give a total weight (sample plus sodium chloride solution) of 20 g. Blend all components to obtain a homogenous mixture, using a spatula for crushing all small lumps. Allow the mixture to stand for 10 min in an ultrasonic bath (4.2).

5.2.2 Flour, starch, cereals and bread

Weigh 10 g of the sample to the nearest 0,01 g and add 100 µl of the d5-3-MCPD internal standard solution (3.16). Add sodium chloride solution (3.7) to give a total weight (sample plus sodium chloride solution) of 40 g. Blend all components to obtain a homogenous mixture, using a spatula for crushing all small lumps. Allow the mixture to stand for 10 min in an ultrasonic bath (4.2). Cover the mixture with a watch glass and leave it to soak overnight.

5.2.3 Salami and cheese

Weigh 20 g of the sample to the nearest 0,01 g and add 100 µl of the d5-3-MCPD internal standard solution (3.16). Add sodium chloride solution (3.7) to give a total weight (sample plus sodium chloride solution) of 70 g. Blend all components to obtain a homogenous mixture, using a further 10 g of sodium chloride solution if necessary. Transfer the mixture to a centrifuge tube (4.5) and centrifuge at 3500 min^{-1} for 20 min. Decant the supernatant layer into a beaker, avoiding the transfer of solid material and visible fat. Weigh a 20 g portion of the supernatant into a 250 ml beaker.

5.2.4 Cream, butter, margarine and other yellow fats

Weigh 20 g of the sample to the nearest 0,01 g and add 100 µl of the d5-3-MCPD internal standard solution (3.16). Add sodium chloride solution (3.7) to give a total weight (sample plus sodium chloride solution) of 50 g. Heat the mixture at 45 °C until the fat has melted. Blend it for 2 min in a high-speed blender (4.4) and allow it to stand in a refrigerator for 1 h or until the fat layer has solidified. Decant the supernatant sodium chloride solution and weigh a 20 g portion of the supernatant into a 250 ml beaker.

5.3 Column chromatography

Take 20 g of the mixture derived from 5.2.1 or 5.2.2 or the solution derived from 5.2.3 or 5.2.4, add the contents of an Extrelut refill pack (3.10) and mix all components thoroughly with a spatula. Add the mixture to the chromatographic column (4.7), agitate the column filling by hand briefly to compact the contents, top it with

a 1 cm layer of sodium sulfate (3.8) and leave it for 15 min to 20 min.

Extract non-polar components with 80 ml of solvent mixture (3.6) with unrestricted flow, except for powdered soup samples where the flow is restricted to approximately 8 ml/min to 10 ml/min. Close the tap of the column when the solvent mixture reaches the sodium sulfate layer and discard the collected washings. Next, elute the column with 250 ml diethyl ether (3.3) at a flow rate of approximately 8 ml/min and collect the eluate in a 250 ml volumetric flask. Dilute the eluate to the mark with diethyl ether, add 15 g sodium sulfate (3.8) to the flask and allow the mixture to stand for 10 min to 15 min.

Filter the dried solution through a filter paper (4.6) into a 250 ml round-bottomed flask. Concentrate the filtrate to approximately 5 ml in a rotary evaporator (4.8) with approximately 35 °C water bath temperature. Do not allow the solution to run dry. Transfer the concentrate to a 10 ml volumetric flask, rinsing the round-bottomed flask with diethyl ether, and dilute the solution up to the mark with diethyl ether. Add a small quantity (spatula tip) of sodium sulfate, shake well and allow to stand for 5 min to 10 min.

5.4 Derivatization

5.4.1 Derivatization of the sample solution

Using a gas-tight syringe (4.9), transfer 1 ml of the solution derived from 5.3 to a 4 ml vial (4.11) and evaporate it just to dryness using a gentle stream of nitrogen (3.17).

NOTE It has been shown by some users of the method that the use of 2,2,4-trimethylpentane (3.4) as a keeper can avoid losses.

To the residue, immediately add 1 ml of 2,2,4-trimethylpentane (3.4) and 0,05 ml of heptafluorobutyrylimidazole (3.9) and seal the vial. Shake the vial for a few seconds using a vortex shaker (4.3) and heat it for 20 min in an aluminium block heater (4.10) at 70 °C. Allow the mixture to cool to below 40 °C. Add 1 ml of distilled water, shake the vial on a vortex shaker for 30 s, allow the phases to separate and repeat shaking. Remove the upper 2,2,4-trimethylpentane phase to a 2 ml vial (4.11), add a small quantity (spatula tip) of sodium sulfate, shake, and allow to stand for 2 min to 5 min. Transfer the solution to a new vial (2 ml) for gas-chromatographic analysis.

5.4.2 Derivatization of standard solutions

To a set of six 4-ml vials (4.11), transfer 100 µl each of the 3-MCPD standard solutions (3.14), 10 µl of the d5-3-MCPD internal standard solution (3.16) and 0,9 ml of 2,2,4-trimethylpentane (3.4). Add 0,05 ml of heptafluorobutyrylimidazole and seal the vial. Shake the vial for a few seconds using a vortex shaker (4.3) and heat it for 20 min in an aluminium block heater (4.10) at 70 °C. Allow the mixture to cool to below 40 °C. Add 1 ml of distilled water, shake the vial on a vortex shaker for 30 s, allow the phases to separate and repeat shaking. Remove the upper 2,2,4-trimethylpentane phase to a 2 ml vial (4.11), add a small quantity (spatula tip) of sodium sulfate, shake, and allow to stand for 2 min to 5 min. Transfer the solution to a new vial (2 ml) for gas-chromatographic analysis.

Run method blanks comprising 20 g sodium chloride solution (3.7) parallel to each batch of samples.

5.5 Gas chromatography/Mass spectrometry

Inject equal volumes of the sample test solutions derived from 5.4.1 and of the standard solutions derived from 5.4.2 into the gas chromatograph (4.12). Appropriate gas chromatographic operating conditions are given in Annex A.

For quantification, run a chromatogram in the SIM mode and use m/z 253 for 3-MCPD and m/z 257 for d5-3-MCPD.

For confirmation, use m/z 453, 291, 289, 275 and 253 for 3-MCPD.

NOTE 1 The molar masses M are 502 g/mol for 3-MCPD-diheptafluorobutyryl-ester and 507 g/mol for the d5-3-MCPD derivative. For the fragments, m/z 453 is from [M – CH_2Cl], m/z 289 and m/z 291 are from [M – C_3F_7COO, Cl-35/Cl-37], m/z 275 is from [M – $C_3F_7COOCH_2$], and m/z 253 is from [M – C_3F_7COO – HCl]. With the d5-3-MCPD derivative, m/z 257 is from [M – C_3F_7COO – DCl].

NOTE 2 For d5-3-MCPD, m/z 456 may also be useful.

For confirming peak identity only, run a chromatogram of a standard solution of adequate concentration in the full scanning mode over the range of approximately m/z 100 to m/z 500.

NOTE 3 GC-MS/MS is also suitable for quantification and confirmation of the peak identity.

6 Evaluation of results

6.1 Calculation

In the chromatograms obtained for the standard solutions in the SIM mode, measure the areas of the peaks of 3-MCPD (m/z 253) and of d5-3-MCPD (m/z 257) and calculate the ratio of both areas m/z 253 to m/z 257. Construct a calibration graph by plotting this peak area ratio against the mass in micrograms of 3-MCPD in the vial. Calculate the slope of the calibration graph.

Calculate the mass fraction $w_{(3\text{-}MCPD)}$ of 3-MCPD, in milligrams per kilogram of sample, using equation (1):

$$w_{(3-MCPD)} = \frac{A}{A'} \times \frac{10}{b \times m} \qquad (1)$$

where:

A is the peak area of 3-MCPD at m/z 253 obtained from the sample test solution;
A' is the peak area of d5-3-MCPD at m/z 257 obtained from the sample test solution;
b is the slope of the calibration graph;
m is the sample mass, in grams;
10 is the dilution factor.

The recovery of the internal standard and 3-MCPD should be checked regularly.

6.2 Confirmation of peak identity

From the chromatograms obtained for standard and sample test solutions, calculate the peak area ratios of the responses at m/z 291, 289, 275, 253 and 453 relative to the response of the base peak. At least two of the four ion abundance ratios for the sample should be in the range of ± 20 % of the mean of the ion abundance ratios for the standards.

In the case of a full scanned and background subtracted spectrum ensure that a satisfactory library fit against a standard is achieved.

7 Precision

7.1 General

Details of the interlaboratory test of the precision of the method according to the harmonized protocol [3] are summarized in Annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than given in Annex B.

7.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit *r* in not more than 5 % of the cases.

The values are:

Hydrolyzed vegetable protein	$\bar{x}$ = 0,029 mg/kg	*r* = 0,006 mg/kg
Malt extract	$\bar{x}$ = 0,055 mg/kg	*r* = 0,008 mg/kg
Soup powder	$\bar{x}$ = 0,045 mg/kg	*r* = 0,011 mg/kg
Dried bread crumbs	$\bar{x}$ = 0,030 mg/kg	*r* = 0,007 mg/kg
Salami	$\bar{x}$ = 0,016 mg/kg	*r* = 0,005 mg/kg
Cheese alternative	$\bar{x}$ = 0,043 mg/kg	*r* = 0,013 mg/kg

7.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit *R* in not more than 5 % of the cases.

The values are:

Hydrolyzed vegetable protein	$\bar{x}$ = 0,029 mg/kg	*R* = 0,010 mg/kg
Malt extract	$\bar{x}$ = 0,055 mg/kg	*R* = 0,021 mg/kg
Soup powder	$\bar{x}$ = 0,045 mg/kg	*R* = 0,022 mg/kg
Dried bread crumbs	$\bar{x}$ = 0,030 mg/kg	*R* = 0,018 mg/kg
Salami	$\bar{x}$ = 0,016 mg/kg	*R* = 0,017 mg/kg
Cheese alternative	$\bar{x}$ = 0,043 mg/kg	*R* = 0,027 mg/kg

8 Test report

The test report shall contain at least the following information:

— all information necessary for the identification of the sample;

— a reference to this document;

— the results and the units in which the results have been expressed;

— date and type of sampling procedure (if known);

— date of receipt of sample in the laboratory;

— date of test;

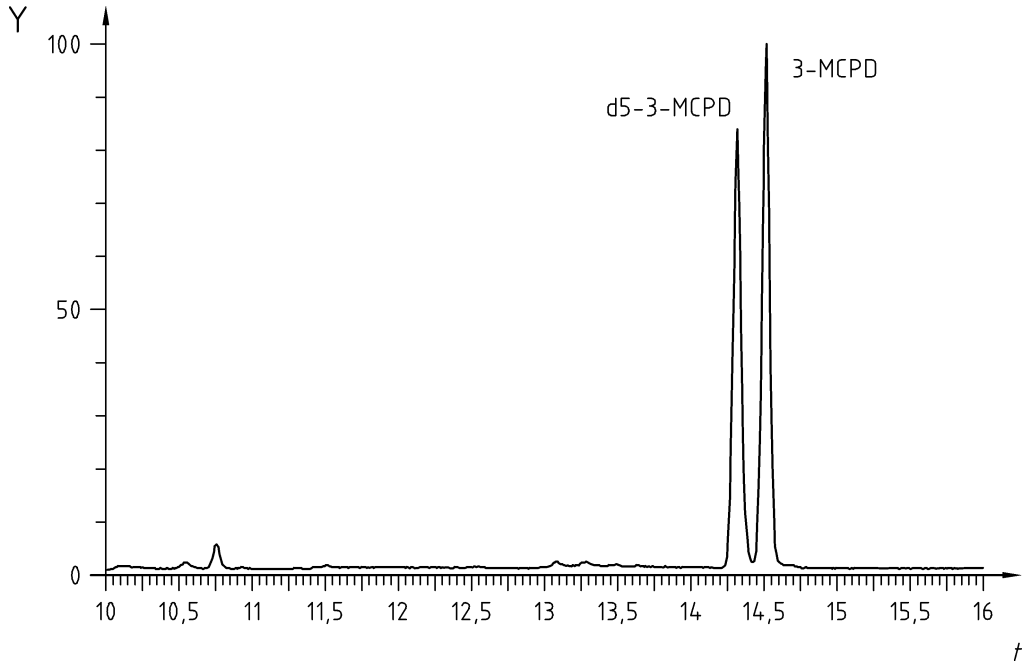
— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional which might have influenced the results.

Annex A
(informative)

Example of appropriate GC/MS operating conditions

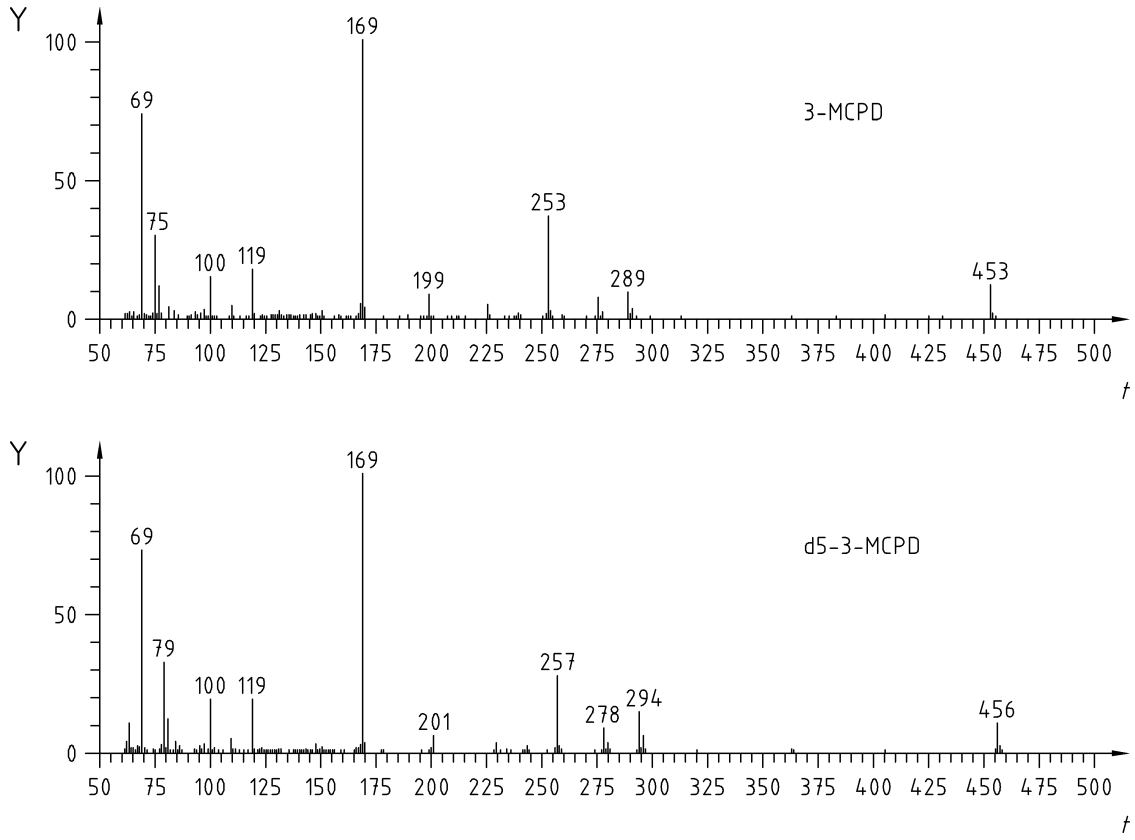
Column size	30 m long, 0,25 mm inner diameter
Column stationary phase	DB 5-MS or equivalent (methyl silicone with 5 % of phenyl groups), 0,25 µm film thickness
Column temperature	50 °C for 1 min, programmed to rise from 50 °C to 90 °C with 2 °C/min and from 90 °C to 270 °C at maximum heating rate, isothermal at 270 °C for 10 min
Injector temperature	270 °C
Injection volume	1 µl in splitless mode with a 40 s splitless period
Carrier gas	Helium, 1 ml/min
Transfer line temperature	270 °C
Ionisation	Positive electron impact
Scanning	SIM mode (selected ion monitoring) m/z 453, 291, 289, 275 and 253 (3-MCPD) and m/z 257 (d5-3-MCPD)



Key

Y peak height
t time (min)

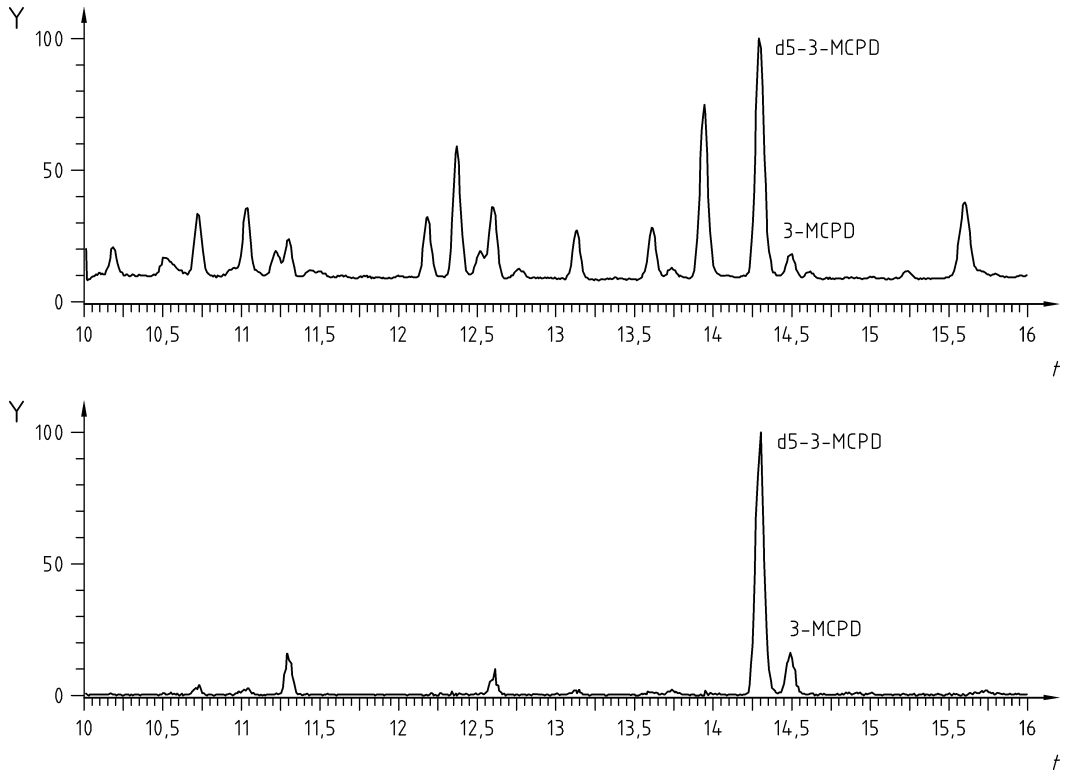
Figure A.1 —Total ion current chromatogram of d5-3-MCPD and 3-MCPD



Key

Y peak height
t fragments (m/z)

Figure A.2 —Mass spectra of 3-MCPD and d5-3-MCPD



Key

Y peak height
t time (min)

Figure A.3 —Chromatograms of soy sauce containing 0,024 mg/kg 3-MCPD; total ion current chromatogram (top) and m/z 257 + 257

Annex B
(informative)

Precision data

The following parameters have been defined in an interlaboratory test in accordance with the AOAC harmonized protocol [3]. The test was conducted by the Central Science Laboratory (CSL) of the then Ministry of Agriculture, Fisheries and Food (MAFF) in Norwich, UK. 12 laboratories (6 UK, 1 Swiss, 1 Japanese, 2 USA, 1 Netherlands and 1 from the European Commission) were asked to analyse 12 samples of 6 test materials (as known duplicates or split level samples).

Sample	Hydrolysed vegetable protein	Malt extract	Soup powder	Dried bread crumbs	Salami	Cheese alternative
Year of interlaboratory test	1997					
Number of samples	2	2	2	2	2	2
Number of laboratories	12	12	12	12	12	12
Number of laboratories retained after elimination of outliers	10	11	11	12	12	11
Number of eliminated laboratories	2	1	1	0	0	1
Number of accepted results	10	11	11	12	12	11
Mean value $\bar{x}$ mg/kg	0,029	0,055	0,045; 0,041	0,030	0,016	0,043
Repeatability standard deviation s_r mg/kg	0,002	0,003	0,004	0,003	0,002	0,005
Repeatability relative standard deviation RSD_r %	7,5	4,9	8,9	8,4	11,6	10,6
Repeatability limit *r* mg/kg	0,006	0,008	0,011	0,007	0,005	0,013
Reproducibility standard deviation s_R mg/kg	0,004	0,007	0,008	0,006	0,006	0,010
Reproducibility relative standard deviation RSD_R %	12,8	13,3	18,6	20,8	38,6	22,3
Reproducibility limit *R* mg/kg	0,010	0,021	0,022	0,018	0,017	0,027
RSD_R expected from Horwitz equation %	27,3	24,8	25,7	27,1	29,8	25,7
Horrat value (RSD_R observed/RSD_R expected)	0,47	0,54	0,72	0,77	1,30	0,87

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[2] W. Horwitz (ed.): Official Methods of Analysis of AOAC International, 17th edition, revision 1, 2002. Method 2000.01, Chapter 48.1.06.

[3] Guidelines for collaborative study procedures to validate characteristics of a method of analysis. In: W. Horwitz (ed.): Official Methods of Analysis of AOAC International, 17th edition, revision 1, 2002, Vol. I, Annex D, p. 1 – 10.

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