SLOVENSKI STANDARD
SIST EN 14573:2005

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Živila - Določevanje 3-monokloropropan-1,2-diola z GC/MS

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

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

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

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

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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.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2005, and conflicting national standards shall be withdrawn at the latest by April 2005.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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.