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SLOVENSKI STANDARD
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Krma: metode vzorčenja in analize - Določevanje mineralnih olj nasičenih ogljikovodikov (MOSH) in mineralnih olj aromatskih ogljikovodikov (MOAH) z analizo on-line HPLC-GC-FID

Animal feeding stuffs: Methods of sampling and analysis - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis

Futtermittel: Probenahme- und Untersuchungsverfahren - Bestimmung von mineralölgesättigten Kohlenwasserstoffen (MOSH) und mineralölaromatischen Kohlenwasserstoffen (MOAH) mit Online-Analyse durch HPLC-GC-FID

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Aliments pour animaux - Méthodes d'échantillonnage et d'analyse - Détermination des hydrocarbures saturés d'huile minérale (MOSH) et des hydrocarbures aromatiques d'huile minérale (MOAH) par analyse CLHP CG FID en ligne

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 327.

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European foreword

This document (prEN 17517:2020) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, the secretariat of which is held by NEN.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a standardization request given to CEN by the European Commission and the European Free Trade Association.

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Introduction

WARNING — The method described in this document implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this document to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

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1 Scope

This document specifies a method for the determination of saturated and aromatic hydrocarbons (from C10 to C50) in feed. The method has been interlaboratory validated with online-HPLC-GC-FID – see [1], [2] and [3]. This method is not intended to be applied to other matrices.

The method can be used for the analysis of mineral oil saturated hydrocarbons (MOSH) and/or mineral oil aromatic hydrocarbons (MOAH).

The method is applicable for feed materials, in particular vegetable oils and other fat rich feed materials, compound feeds and pre-mixtures. It is not applicable to additives or deodistillates.

The method has been tested in an interlaboratory study via the analysis of both naturally contaminated and spiked samples (pre-mixture, soybean meal, sunflower seeds, chicken feed, pig feed, vegetable oil) ranging from 3 mg/kg to 286 mg/kg for MOSH and from 1 mg/kg to 16 mg/kg for MOAH.

According to the results of the interlaboratory study, the method has been proven suitable for MOSH and MOAH mass concentrations, each above 10 mg/kg. However, the method was not fully validated during the collaborative study for the premixture sample due to too low concentrations of MOSH and MOAH. The method was also not fully validated during the collaborative study for the sunflower seeds sample due to a too low concentration of MOAH.

NOTE The conclusions regarding MOAH are based on 4 analyte / matrix combinations while according to the IUPAC protocol [4] expects this to be a minimum of 5.

In case of suspected interferences from natural sources, the fossil origin of the MOSH and MOAH fraction can be verified by examination of the pattern by GC-MS.

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For the determination of MOSH and MOAH in edible fats and oils, another CEN standard is also available: EN 16995. For more information see [5].

Annex C proposes a manual alternative method to online HPLC-GC-FID analysis that can be used as a screening method.

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2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
mineral oil saturated hydrocarbons
MOSH
paraffinic (open-chain, usually branched) and naphthenic (cyclic, alkylated) hydrocarbons

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3.2
mineral oil aromatic hydrocarbons
MOAH
aromatic mainly alkylated hydrocarbons

3.3
unresolved complex mixture
UCM
complex mixture of saturated or aromatic hydrocarbons not resolved by gas chromatography such as branched paraffins, alkylated naphthenes and alkylated aromatics

4 Principle

The fatty material is extracted from the commodity using organic solvent. After concentration of part of the solvent, the extract is submitted to an epoxidation step. The fractions of MOSH and MOAH are isolated and separated by an HPLC-GC-FID system. MOSH and MOAH fractions are separated on a silica gel column using a *n*-hexane/dichloromethane gradient and each transferred as 450 µl fractions to GC using the Y-interface [6], while triglycerides are kept on the HPLC column. Solvent vapours are discharged via a solvent vapour exit located between the uncoated pre-column and the GC separation column. Volatile components are retained by solvent trapping applying partially concurrent eluent evaporation. High boiling components are spread over the entire length of the flooded zone and refocused by the retention gap technique [2].

The area attributed to mineral oil is calculated by subtraction of peaks due to *n*-alkanes (naturally occurring hydrocarbons), terpenes, squalene and its isomerization products, sterenes and olefins with the structure of carotenoids. MOSH and MOAH are quantitated by internal standard added before analysis. Verification standards are added for monitoring proper HPLC fractionation and GC transfer conditions.

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Epoxidation is a purification step that is necessary for the quantification of MOAH. This purification step allows the elimination of olefins like squalene, which elute within the MOAH fraction and interfere with quantification (e.g. olive oil, palm oil). Epoxidation also removes certain olefins co-eluting with the MOSH fraction, therefore epoxidation also may be used as a purification step for the MOSH fraction. The epoxidation step is the best compromise to remove olefins, even though it is not fully quantitative and the efficiency may be sample dependent. Depending on the sample, this reaction may induce the epoxidation of a part of the MOAH or incomplete removal of the interfering olefins.

5 Reagents

WARNING — The method described in this document implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this document to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

Unless otherwise specified, use only reagents of recognized analytical grade.

5.1 Demineralized water, stored in a glass bottle

5.2 *n*-Hexane, trace organic analysis grade, for pesticide residue analysis

n-Hexane purity can be checked by concentrating 30 ml of *n*-hexane mixed with 25 µl of internal standard solution (5.16) and 2 drops of keeper (5.27) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.10). Take care that in the evaporation step the residue is not evaporated to dryness to avoid loss of volatile hydrocarbons. The signal abundance of

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the residue after evaporation should not exceed a tenth of the signal abundance obtained at the quantification limit.

5.3 Toluene

5.4 1,1,2-Trichloroethane

5.5 Perylene (Per), purity ≥ 99 %

5.6 *α*-Cholestane (Cho), purity ≥ 97 %

5.7 *n*-Undecane (*n*-C11), purity ≥ 98 %

5.8 *n*-Tridecane (*n*-C13), purity ≥ 97 %

5.9 Tri-tert-butylbenzene (TBB)

5.10 Bicyclohexyl (CyCy), purity ≥ 99 %

5.11 1-Methylnaphthalene (1-MN), purity ≥ 95 %

5.12 2-Methylnaphthalene (2-MN), purity ≥ 97 %

5.13 Pentylbenzene (5-PB), purity ≥ 96 %

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5.14 Stock solutions, mass concentration ρ = 10 mg/ml

Prepare individual stock solutions by weighing, to the nearest 1 mg, 100 mg of *n*-C11 (5.7), *n*-C13 (5.8), TBB (5.9), CyCy (5.10), 1-MN (5.11), 2-MN (5.12) and 5-PB (5.13) into a 10 ml volumetric flask and dilute to the mark with 1,1,2-trichloroethane (5.4) or toluene (5.3). Store the solutions at room temperature. If crystals precipitate during storage, warm the solution until everything has dissolved.

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5.15 Internal standard solution 1 (ISTD1)

Weigh, to the nearest 0,5 mg, 12 mg of Per (5.5) and Cho (5.6) in a volumetric flask of 20 ml (6.21), to which 600 µl of each stock solution (5.14) is added with the exception of *n*-C13, of which 300 µl is added. Fill the volumetric flask up to 20 ml with 1,1,2-trichloroethane (5.4) or toluene (5.3). Resulting mass concentrations are for *n*-C13: ρ = 150 µg/ml, for *n*-C11, TBB, CyCy, 1-MN, 2-MN and 5-PB: ρ = 300 µg/ml and for Per, Cho: ρ = 600 µg/ml.

NOTE 1 This document mixture is available.[1]

5.16 Internal standard solution 2 (ISTD2)

Dilute the ISTD1 solutions by a factor of 10, e.g. 1 ml filled up to 10 ml with *n*-hexane (5.2). Resulting mass concentrations are for *n*-C13: ρ = 15 µg/ml, for *n*-C11, TBB, CyCy, 1-MN, 2-MN and 5-PB: ρ = 30 µg/ml and for Per, Cho: ρ = 60 µg/ml.

5.17 Chloroperbenzoic acid (CPBA), purity 70 % to 75 %

5.18 CPBA solution, ρ = 0,2 g/ml in absolute ethanol

[1] Restek Corp., Cat.# 31070 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

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For example 5 g of CPBA (5.17) in 25 ml of absolute ethanol (5.22). The solution can be used for up to one week.

5.19 Carrier gas for gas chromatography, preferably hydrogen, purity ≥ 99,995 %

5.20 Auxiliary gases for flame ionization detector, hydrogen, air, and nitrogen suitable for gas chromatography

5.21 Alkane standard mixture C10 to C40, solution of equal concentration in an apolar solvent, ρ = 1 µg/ml

5.22 Ethanol, absolute

NOTE 2 The ethanol purity can be checked by concentrating 50 ml of ethanol mixed with 25 µl of internal standard solution (5.16) and 2 drops of keeper (5.27) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.10).

5.23 *n*-Pentacontane (C50), purity ≥ 98 %

5.24 *n*-Pentacontane (C50) solution in toluene, ρ approximately 10 µg/ml

Weigh 2 mg of C50 (5.23) in a volumetric flask of 20 ml (6.21) and dilute to the mark with toluene (5.3). Proceed to a second dilution of 1 ml in a 10 ml volumetric flask (6.21). Store the solutions at room temperature.

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NOTE 3 Solubility of pentacontane in toluene is limited at room temperature. However, the concentration of the solution of pentacontane does not need to be accurate as it is used only to determine the limit of integration for mineral oil peak.

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NOTE 4 It is also possible to use a commercial mixture of *n*-alkanes from C12 to C60 that contains *n*-pentacontane.[2]

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5.25 Sodium carbonate solution, ρ = 0,1 g/ml in water (5.1)

5.26 Dichloromethane (DCM), trace organic analysis grade, purity ≥ 99 %

DCM purity can be checked by concentrating 50 ml of DCM mixed with 25 µl of internal standard solution (5.16) and 2 drops of keeper (5.27) using a rotary evaporator, dissolving the residue in 0,2 ml of *n*-hexane and the analysis of 50 µl by online-HPLC-GC-FID (6.10). Take care that in the evaporation step the residue is not evaporated to dryness to avoid loss of volatile hydrocarbons. The signal abundance of the residue after evaporation should not exceed a fifth of the signal abundance obtained at the quantification limit.

5.27 Keeper solvent

The keeper is a solvent that will not evaporate or evaporate to a lesser degree during the evaporation step, e.g. bis(2-ethylhexyl) maleate. A keeper is used to enhance the recovery of volatile compounds.

[2] ASTM D5442 C12-C60 Qualitative Retention Time Mix available by e.g. Supelco Cat.# 500623 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

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6 Apparatus

IMPORTANT — The glassware used for the determination shall be thoroughly cleaned and rinsed with *n*-hexane (5.2) before use so that it is free from impurities.

Usual laboratory apparatus and, in particular, the following. The glassware shall be thoroughly cleaned and rinsed with *n*-hexane (5.2) or baked in an oven before use so that it is free from impurities.

6.1 Mill with stainless-steel rotor, for grinding to at least 1 mm

6.2 Magnetic stirrer

6.3 Magnetic stir bars

6.4 Analytical balance, reading accuracy 0,000 1 g

6.5 Round-bottomed flasks, 250 ml capacity

6.6 Glass vials with screw caps, 15 ml and 40 ml capacity

6.7 Centrifuge and centrifuge tubes

6.8 Automatic evaporator (optional)[3]

6.9 Glass sample vials, volume of 2 ml

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6.10 High performance liquid chromatograph, coupled with gas chromatograph and flame ionization detector (HPLC-GC-FID)

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6.11 Data acquisition system, with the possibility of manual integration

6.12 LC column, 5 µm (250 mm x 2 mm inner diameter (i.d.)) or equivalent

The silica gel column shall have a capacity to retain 20 mg fat. [4]

6.13 Uncoated precolumn, 10 m x 0,53 mm or equivalent[5]

6.14 Capillary column 1, capable for temperatures up to 350 °C

The column should have the following characteristics: 100 % dimethylpolysiloxane or 95 % dimethyl / 5 % phenyl methylpolysiloxane stationary phase, a length of 15 m, an internal diameter of 0,32 mm or 0,25 mm and a film thickness 0,10 µm to 0,15 µm or equivalent.

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[3] MicroDancer, IR-Dancer (e.g. Zinser) or Syncore Analyst (Büchi) are examples of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

[4] LiChrospher Si 60 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

[5] Hydroguard® MXT® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

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6.15 Capillary column 2, from transfer valve to first Y-piece, fused silica (FS) methyl silicone deactivated (length 1 m, outside diameter (o.d.) 0,27 mm, inner diameter (i.d.) 0,1 mm)

6.16 Capillary column 3, for hydrogen carrier gas, FS methyl silicone (length 1 m, o.d. 360 µm, i.d. Twenty-five µm)

6.17 Capillary column 4, for solvent vapour exit, FS methyl silicone (length 1 m, o.d. 0,68 mm, i.d. 0,53 mm)

The columns given in 6.15, 6.16 and 6.17 have proven to be suitable for the analysis. However these columns can be adjusted in accordance with the characteristics of the HPLC-GC apparatus and the analytical conditions.

6.18 Restriction capillary column, transfer valve and solvent vapor exit, FS uncoated (length 1 m, o.d. 360 µm, i.d. 50 µm)

6.19 Microsyringe, 5 µl to 100 µl capacity, suitable for injection in liquid chromatography

6.20 Pasteur pipette, glass

The use of plastic pasteur pipettes and polyethylene film shall be avoided.

6.21 Volumetric flasks, various sizes

7 Sampling

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The sample should be truly representative and not damaged or changed during transport or storage.

Samples should be packed in glass bottles or aluminium foil in order to prevent additional contamination. Plastic and paper packaging are unsuitable.

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Sampling is not part of the method specified in this document. A recommended sampling method is given in EN ISO 6497 [7].

8 Preparation of the test sample

Prepare the test sample in accordance with EN ISO 6498.

Grind the laboratory sample (typically 50 g) to a particle size of at least 1 mm in the mill (6.1) in order to ensure representative data. Mix the sample thoroughly.

9 Preparation of the analytical sample

9.1 Fat extraction from feed sample

9.1.1 Fatty material extraction for samples with fat content lower than 30 %

Weigh 5 g of milled sample in a 250 ml round-bottomed flask (6.5), add a magnetic stir bar (6.3). Add 500 µl ISTD2 (5.16) and 100 ml of *n*-hexane (5.2) to the sample and mix for 1 h with a magnetic stirrer (6.2).

Transfer 20 ml of the solvent phase into a 40 ml glass vial (6.6) and wash with 5 ml of demineralized water (5.1). Centrifuge for 2 min at a speed of 2500 rpm and transfer 10 ml of sample extract to a 15 ml glass vial (6.6) and concentrate the solvent down to 1 ml (triglycerides from the sample act as a keeper) under a stream of nitrogen, using either a water bath at 35 °C or an automatic evaporator (6.8).