SLOVENSKI STANDARD
SIST EN 15608:2008

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Površinsko aktivne snovi - Kvantitativno določevanje prostih maščobnih kislin v alkilamidopropilbetainih - Metoda plinske kromatografije

Surface active agents - Quantitative determination of free fatty acid in alkylamidopropylbetaines - Gas-chromatographic method

Grenzflächenaktive Stoffe - Quantitative Bestimmung freier Fettsäure in Alkylamidopropylbetainen - Gaschromatographisches Verfahren

Agents de surface - Dosage quantitatif des acides gras libres dans les alkylamidopropylbétaines - Méthode par chromatographie en phase gazeuse

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ICS:

71.100.40	Površinsko aktivna sredstva	Surface active agents

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English Version

Surface active agents - Quantitative determination of free fatty acid in alkylamidopropylbetaines - Gas-chromatographic method

Agents de surface - Dosage quantitatif des acides gras libres dans les alkylamidopropylbétaïnes - Méthode par chromatographie en phase gazeuse

Grenzflächenaktive Stoffe - Quantitative Bestimmung freier Fettsäure in Alkylamidopropylbetainen - Gaschromatographisches Verfahren

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

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Contents

Foreword

This document (EN 15608:2008) has been prepared by Technical Committee CEN/TC 276 “Surface active agents”, the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by November 2008, and conflicting national standards shall be withdrawn at the latest by November 2008.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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1 Scope

This European Standard specifies a procedure for the determination of the content of free fatty acid, FFA, in alkylamidopropylbetaines, which is defined as being the amount of fatty acid expressed in grams per 100 g of product.

This method has been validated for the determination of fatty acids from C_6 to C_{20} in a total concentration range from 0,02 g to more than 3,0 g fatty acid per 100 g of product.

2 Normative references

The following referenced documents are indispensable for the application 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 3696, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

ISO 607, *Surface active agents and detergents – Methods of sample division*

3 Principle

The free fatty acids are extracted with petroleum ether at acidic pH. Then the extracted fatty acids are derivatised and subsequently analysed by GLC-FID. The chromatogram resolves the different acids according to their alkyl chain length. For quantification the sum of the peak areas of all fatty acid homologues is related to the peak area of the internal standard tridecanoic acid.

4 Reagents

4.1 General

WARNING — Your attention is drawn to the regulations covering the handling of hazardous substances. Technical, organisational and personal protection measures should be observed.

During the analysis, unless otherwise specified, use only reagents of recognized analytical grade and that have been checked in advance as to not interfere with the analytical results and water complying with grade 1 as defined in EN ISO 3696.

4.2 Tridecanoic acid, purity ≥ 99 % (m/m) (CAS number: 638-53-9).

4.3 Petroleum ether (40 ºC to 60 ºC) (CAS number: 101316-46-5).

4.4 Ethanol (CAS number: 64-17-5).

4.5 HCl, *c* = 37% (m/m) (CAS number: 7647-01-0).

4.6 Internal Standard Solution

Weigh to the nearest 0,1 mg, 0,3 g of pure tridecanoic acid (4.2) in a 25 ml volumetric flask and make up to the mark with petroleum ether. This is the Internal Standard Solution.

4.7 TMPAH, Trimethylphenylammonium hydroxide solution, *c*(TMPAH) about 0,5 M in methanol (CAS number: 1899-02-1).

4.8 Methanol water free (CAS number: 67-56-1).

4.9 Methyl tert-butyl ether water free (CAS number: 1634-04-4).

4.10 Phenolphthalein (CAS number: 77-09-8), 1 % in MeOH.

5 Apparatus

Ordinary laboratory apparatus and the following.

5.1 15 ml glass vial with threaten cap.

5.2 Gas-chromatograph, equipped with capillary split injector and a flame ionisation detector.

5.3 Capillary fused silica column, capable of the separation characteristics shown in the chromatogram of Annex A.

6 Sampling and preparation of the test sample

The test sample shall be prepared and stored in accordance with ISO 607.

7 Procedure

7.1 Fatty acid extraction

Weigh to the nearest 0,1 mg, 1,0 g of sample in a 15 ml glass vial with threaten cap. For samples having an expected content lower than 1 g of free fatty acid per 100 g of sample, weigh about 2 g of sample.

Add 5 ml of a water-ethanol 1:1 (v/v) mixture, and homogenize.

Add with a precision pipette, 500 µl aliquot of Internal Standard Solution (4.6).

Add 0,5 ml of HCl (4.5) to acidify the media, and homogenize.

The pH-value of the solution shall be below 2.

Add 5 ml of Petroleum Ether.

Shake the tube vigorously (about 1 min) and let stand until phase separation (2 min are usually enough).

7.2 Preparation of the Methyl Ester (Methylation by TMPAH)

NOTE Prior to analysis, the fatty acids extracted from the sample are methylated to ensure good GC peak allowing a good quantitative analysis. Methylation can easily be done by TMPAH with the reaction being performed in the GLC injector. However, as this procedure requires high temperatures the subsequent use of an apolar GLC column (100% dimethyl polysiloxane) is recommended. Alternatively the methylation can be performed as liquid reaction with methanol with boron trifluoride as catalyst.

Transfer 500 µl of the extract (7.1) to an autosampler vial (2 ml).

Add 500 µl of a mixture of methanol/methyl tert-butyl ether (4.8/4.9) 1:1 and mix carefully.

Subsequently add two drops of the phenolphthalein solution (4.10).

Finally TMPAH (4.7) is added drop wise until the solution is no longer discoloured and remains magenta.

Analyse the solution directly by injecting 2 µl into the GC at an injector temperature of at least 310 °C.

7.3 Gas Chromatography GC

The analysis of the derivatives is performed by capillary gas chromatography.

There are various separation columns of different polarity capable to separate fatty acid methyl esters. However their application may be restricted by the methylation procedure (see 7.2 and Annex B.3).

In either case the retention times of the individual fatty acids (C6 to C20 including all of the relevant unsaturated species) have to be determined by a reference analysis of a standard FAME (fatty acid methyl esters) mixture.

An appropriate GC analysis on a non polar separation column including all parameters is shown in Annex A.

8 Calculation and expression of results

The total free fatty acid, w, is obtained from the GLC chromatogram and calculated as follows in grams per 100 g sample:

$$w = \frac{\sum_i A_i}{A_{istd}} \cdot \frac{m_{istd} \cdot R_{istd}}{m_s} \qquad (1)$$

where

A_i is the area of each one of the peaks corresponding to fatty acid homologues except that of the internal standard;

A_{istd} is the area of the internal standard peak;

m_s is the sample weight (7.1), in grams;

m_{istd} is the weight of Internal standard in the aliquot (7.1), in grams;

R_{istd} is the Internal standard purity in grams per 100 grams.

The relative response factors of all the fatty acid homologues respectively the tridecanoic acid internal standard are assumed to be equal for C_6 to C_{20} fatty acids (see Annex B).

9 Precision

9.1 Repeatability limit

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not exceed the repeatability limit, *r*, in more than 5 % of cases.

9.2 Reproducibility limit

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will not exceed the reproducibility limit, *R*, in more than 5 % of the cases.

10 Test report

The test report shall include the following information:

a) all information necessary for the identification of the sample tested;

b) reference to this European Standard (EN 15608:2008);

c) test results;

d) details of any operation not specified in this European Standard or in the European Standards to which reference is made, and any operations regarded as optional, as well as any incidents like to have affected the results.