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

ICS 71.100.40

BSI British Standards

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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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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.

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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:

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

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;

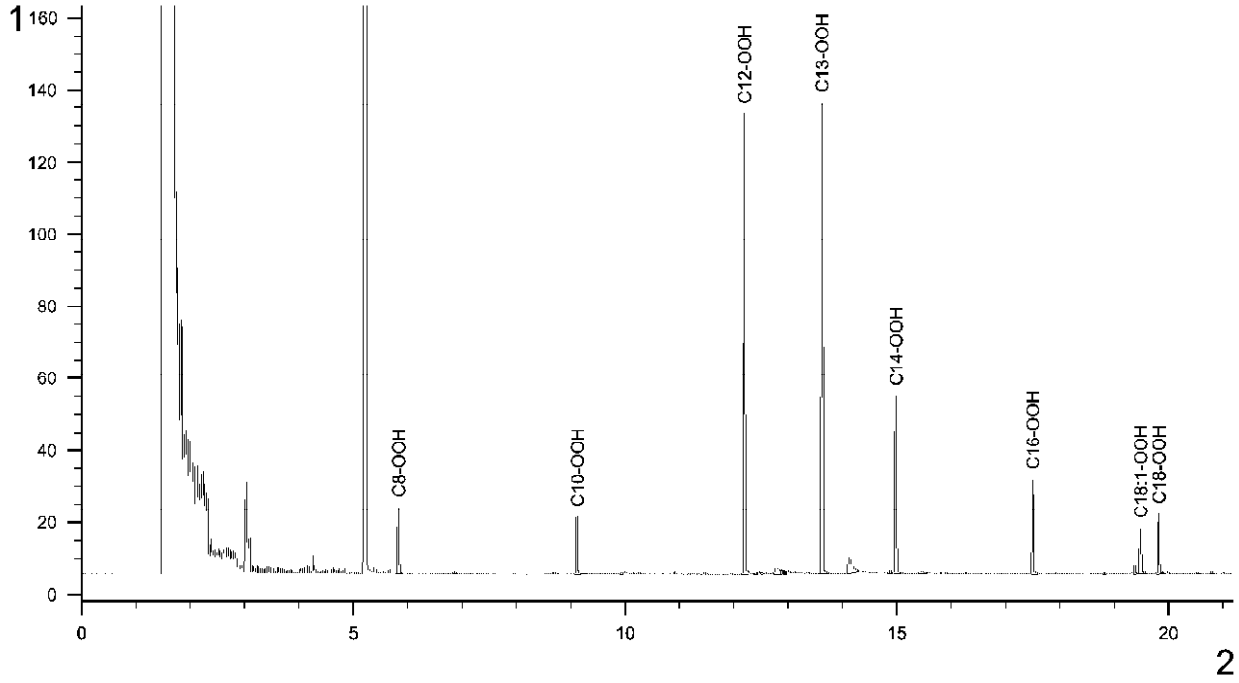
b) reference to this European Standard (EN 15608:2008);

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.

Annex A
(informative)

Reference GLC Chromatogram



Key

1 response
2 retention time

Figure A.1 - Chromatogram of a betaine sample containing 2 % FFA

A.1 GC Conditions

Inject 2 µl of sample extract into the gas chromatograph, the following conditions have been found suitable:

Column	HP-1[1)] (30 m, 0,32 mm, 0,25 µm)
Head P	15 psi
T_{inlet}/T_{FID}	310 ºC
T_1 (t_1)	80 ºC (2 min)
Rate	8 ºC/min
T_2 (t_2)	300 ºC (20 min).
Post run	305 ºC (10min)
Split	20 ml/min Helium

[1)] HP1(100% dimethyl polysiloxane) is the trade name of product supplied by Agilent Technologies This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named.

Annex B
(informative)

Method validation

B.1 Calibration

The individual calibration factors of C8- C20 acid versus C13 acid may differ from 0,85 l to 1,1 depending on the chain length and the cleanness of the GC equipment .

For coconut fatty acids an overall average correction factor of 0,97 to 1,03 depending on the individual chain length distribution is achieved.

For practical reasons a relative response factor of coconut fatty acids to tridecanoic acid is assumed as 1.

This overcomes the necessity of calibration measurements for all individual fatty acids.

This may be acceptable unless betaines with a fatty acid distribution differing from coconut fatty acid have to be analysed.

For a maximum precision or if the chain length distribution differs from coconut fatty acid, a suitable calibration is advised.

B.2 Statistical and other data derived from the results of interlaboratory tests

The interlaboratory test was carried out in 2006 by CESIO (Comité Européen des agents de Surface et de leurs Intermédiaires Organiques)/AISE (International Association for Soaps, Detergents and Maintenance Products) WG "Surfactant Analysis". The test samples were commercial alkylamidopropylbetaines. The results of interlaboratory test were evaluated in accordance with ISO 5725-2 (see Table B.1).

Table B.1 — Results of interlaboratory test

	Tegobetaine F50	**Tegobetain CK**
Number of participating laboratories	5	5
Number of accepted test results	30	30
Mean value (% w/w)	2,02	0,64
Repeatability standard deviation (s_r)	0,053	0,038
Repeatability coefficient of variation	2,6	5,9
Repeatability limit (r) (2,8 x s_r)	0,149	0,105
Reproducibility standard deviation (s_R)	0,111	0,072
Reproducibility coefficient of variation	5,5	11,3
Reproducibility limit (R) (2,8 x s_R)	0,311	0,201

B.3 Remarks

The procedure described has been validated for its recovery. For one step extraction as is described in 7.1, the average recovery has been of 90 %.

The extraction of free fatty acids in one step has been introduced for practical reasons although short chain fatty acids (C_8-C_{12}) are not fully extracted.

A higher recovery (> 97 %) is achievable if the extraction is performed in 3 steps, each with 2 ml of petroleum ether solvent.

Bibliography

[1] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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