BS EN 13273:2001

Surface active agents — Determination of the content of non-ionic substances in anionic surface active agents by high performance liquid chromatography (HPLC)

The European Standard EN 13273:2001 has the status of a
British Standard

ICS 71.100.40

National foreword

This British Standard is the official English language version of EN 13273:2001.

The UK participation in its preparation was entrusted to Technical Committee CII/34, Methods of test for surface active agents, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

Additional information

Some textual errors were discovered when the English language version of EN 13273:2001 was adopted as the national standard. Some of the chemical names are not consistently presented and do not conform to IUPAC recommendations. These textual errors have been reported to CEN in a proposal to amend the text of the European Standard.

Cross-references

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This British Standard, having been prepared under the direction of the Sector Committee for Materials and Chemicals, was published under the authority of the Standards Committee and comes into effect on 15 June 2001

Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 14, an inside back cover and a back cover.

The BSI copyright date displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments

ISBN 0 580 37402 5

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 13273

April 2001

ICS 71.100.40

English version

Surface active agents — Determination of the content of non-ionic substances in anionic surface active agents by high performance liquid chromatography (HPLC)

Agents de surface — Détermination de la teneur en substances non-ioniques dans les agents de surfaces anioniques par chromatographie liquide à haute performance (CLHP)

Grenzflächenaktive Stoffe — Bestimmung des Gehaltes von nichtionischen Substanzen in anionischen grenzflächenaktiven Stoffen mittels Hochleistungs-Flüssigchromatographie (HPLC)

This European Standard was approved by CEN on 20 January 2001.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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

Ref. No. EN 13273:2001 E

Contents

Foreword

This European Standard 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 October 2001, and conflicting national standards shall be withdrawn at the latest by October 2001.

Annexes A, B and C are informative.

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

1 Scope

This European Standard specifies a method for the determination of the contents of non-ionic substances in anionic surface active agents (sulfates, sulfonates and ethoxysulfates) by high performance liquid chromatography (HPLC).

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

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

A specified mass of sample is dissolved and then made up to standard volume with methanol. For sulfonic acids only, the sample is neutralized with aqueous sodium hydroxide solution prior to the dissolution. The solution is then analysed by reverse phase high performance liquid chromatography (RP-HPLC). An octadecylsilane bonded stationary phase, methanol/water mobile phase, differential refractometer detector and external means of quantification are employed. A back flush technique is used to “focus” the non-ionic components after all the active matter components and water have eluted from the column.

4 Reagents

During the analysis, only use reagents of recognized analytical grade and water complying with grade 3 as defined in EN ISO 3696.

4.1 **Methanol**, CH_4O HPLC grade.

4.2 **Sodium hydroxide** NaOH $c(\mathrm{NaOH}) = 1$ mol/l solution.

4.3 **Phenolphthalein indicator, ethanolic solution,** $C_{20}H_{14}O_4$, $w(C_{20}H_{14}O_4) = 0{,}1$ g/100 g.

4.4 **Calibration substances**, based on the feedstock from which the surface active agent/sulfonic acid has been produced, e.g.:

- parent linear alkyl benzene (LAB) for alkyl benzene sulfonates;
- parent alcohols for alcohol sulfates;
- parent alcohol ethoxylates for alcohol ethoxy sulfates.

5 Apparatus

Ordinary laboratory apparatus and the following.

5.1 **High performance liquid chromatograph**, suitable for analysis according to the conditions given in 6.3.

5.2 **Data logger/plotter** giving the chromatographic peak areas.

5.3 **Burette**, capacity 10 ml with polytetrafluorethylene (PTFE) key, never lubricated with stopcock grease.

5.4 **Syringe**, capacity 1 ml with “Luer” fitting.

5.5 **Needle** with “Luer” fitting to suit sample injection valve, or use an automated injector.

5.6 **Syringe filter or membrane** suitable for use with methanol, 0,45 µm, with “Luer” fittings.

6 Calibration

6.1 General

Always wash glassware thoroughly with water and then acetone (propan-2-one). Dry thoroughly.

6.2 Determination of the optimum backflush time

Prepare and analyse the appropriate calibration substance (4.4) according to clause 7, but keep the backflush valve in position 1 (see Figure A.1) throughout the complete analysis. The chromatogram of the calibration substance alkyl ether sulfate, type 2, used in the ring test is shown in Figure B.1.

Note the elution time just before the first peak of the calibration substance appears. This is the backflush valve switching time.

The above valve switching time needs only be determined if the method is being set up or if it becomes apparent that the retention times have changed during routine use.

6.3 Determination of response factors

Determine the response factors for each type of the surface active agents as follows.

Prepare a calibration solution by weighing (0,15 ± 0,02) g of the calibration substance (4.4) to the nearest 0,1 mg into a 100 ml volumetric flask. Dissolve with methanol (4.1) and make up to volume.

Load the syringe (5.4) with 1 ml of the calibration solution and mount a new syringe filter fitted with the needle (5.5).

Use the following conditions:

Column:

Type	HPLC column with appropriate fittings. A radial compression type is suitable if used with appropriate conditions.
Length × internal diameter	Dimensions which enable results similar to the chromatograms in annex B. For a radial compression column, 100 mm × 8 mm is suitable. For conventional columns, 250 mm × 4,6 mm is suitable.
Stationary phase	Octadecylsilyl bonded micro-particulate silica of mean particle size not more than 5 µm. This should be of the completely-end-capped or deactivated type.

Mobile Phase:	
Composition	A mixture of methanol and water capable of use with the chosen HPLC column so as to give results similar to the examples in annex B. A mixture of methanol : water of 85:15 parts by volume can be suitable
Flow rate	1 ml/min to 2 ml/min to the nearest 0,01 ml/min.
Detector:	
Type	Differential refractometer
Sensitivity	A setting which gives a display of peak sizes such that deviations from normal appearance can be readily seen.
Temperatures:	
Column	30,0 °C NOTE Thermostatting of the column gives improved stability, but the method can be used with the column at ambient temperatures.
Detector	40,0 °C
Injection system:	High pressure syringe-loading injection valve or a suitable automated injector, fitted with an injection loop of appropriate volume, for example 50 µl.
Backflush valve:	A 6 port high pressure switching valve for HPLC, fitted with a very low volume by-pass loop.
Valve positioning:	Position 1 at the beginning of the analysis. Switch to position 2 at time A (see Figures A.1 and B.1) Switch to position 1 on completion of the analysis (typical analysis time: 20 min).

Run the chromatograph in accordance with the manufacturer's instructions. When injecting the blend ensure the sample loop is completely flushed by dispensing the total syringe volume taking care not to introduce any air bubbles. An example chromatogram is given in Figure B.1.

NOTE The calibration peak elutes at a run time twice that of the backflush valve switch time. This time can vary depending on the sample type and is derived in 6.2.

The response factor, F, is calculated using the equation (1):

$$F = \frac{m_{\mathrm{c}}}{A_{\mathrm{c}}} \qquad (1)$$

where:

m_{c} is the mass of the calibration substance used, in grams;

A_{c} is the area of the calibration peak, in arbitrary units.

7 Sampling and preparation of the test sample

Sample the material to be tested in accordance with ISO 607.

8 Procedure

8.1 Determine the optimum backflush time from the calibration carried out in 6.2.

8.2 Weigh (1 ± 0,1) g of the sample to the nearest 0,1 mg into a 25 ml volumetric flask.

8.3 For sulfonic acids only, add one drop of phenolphthalein indicator (4.3) and then add 2,00 ml of sodium hydroxide solution (4.2). Swirl to dissolve. When completely dissolved, titrate dropwise with sodium hydroxide solution (4.2) to the end point characterized by faint pink colour (total titrant addition typically about 3,2 ml). For the other surface active agents, continue to 8.4.

Use a burette (5.3).

8.4 Dissolve and make up to volume with methanol (4.1). Allow to stand for a short time such that any precipitate formed settles to the bottom of the flask.

8.5 Load the syringe (5.4) with 1 ml of supernatant from the sample solution. Mount a new syringe filter or membrane (5.6). Use this to deliver a filtered sample portion for use in the analysis. Where possible, this should be done by direct delivery into the injection valve, via the needle (5.5).

8.6 Analyse the sample according to the conditions given in 6.3. When injecting the sample ensure the sample loop is completely flushed by dispensing the total syringe volume taking care not to introduce any air bubbles.

NOTE 1 An example chromatogram is given in Figure B.2 and Figure B.4. If the valve was not used, a chromatogram as shown in Figure B.3 should result.

NOTE 2 The operation of the backflush valve should be automated.

9 Calculation and expression of results

The content of non-ionic substances w_{NIS} of the sample expressed in grams per 100 g is calculated using the equation (2):

$$w_{\mathrm{NIS}} = \frac{A_s \times F \times 100}{m_s \times 4} \quad (2)$$

where:

A_s is the area of the peak of non-ionic substances, as used for A_c (see 6.3) in arbitrary units;

F is the response factor of the calibration solution (6.3);

m_s is the mass of the sample (8.2) in grams.

Report the result to the nearest 0,001 g/100 g.

10 Precision

10.1 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.

From the data given in annex C, the relative repeatability limits are:

- 12 % of the mean value at a level of 2 % to 3 % on active matter;
- 19 % of the mean value at a level below 1 % on active matter.

10.2 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 cases.

From the data given in annex C the relative reproducibility limits are:

- 20 % of the mean value at a level of 2 % to 3 % on active matter;
- 40 % of the mean value at a level below 1 % on active matter.

11 Test report

The test report shall include the following information:

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

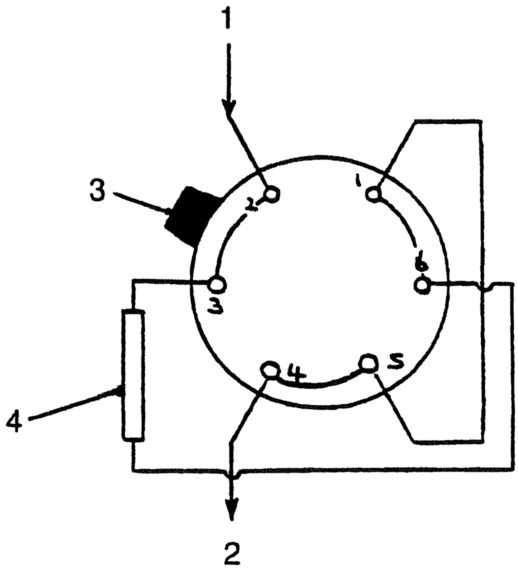
b) a reference to this European Standard;

c) the tests results;

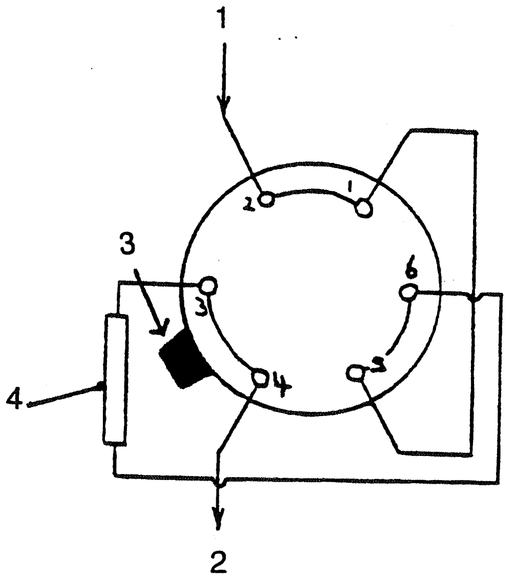
d) details of any operations not specified in this European Standard, and any operations regarded as optional, as well as any incidents likely to have affected the results.

Annex A
(informative)

Flow diagram for the backflush valve in position 1 and position 2



a) flow with valve in position 1



b) flow with valve in position 2

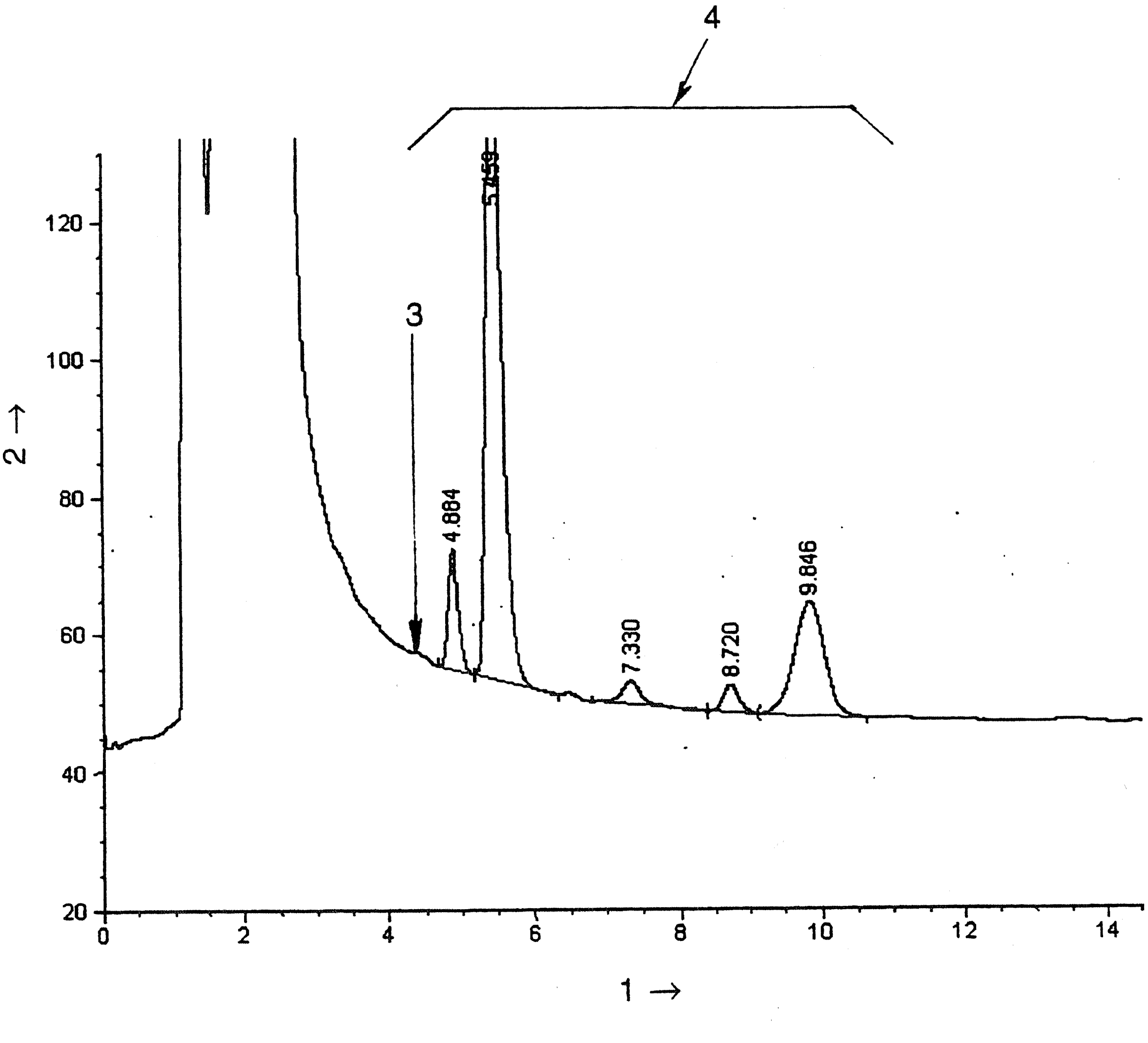
Key for a) and b)

1 From injection
2 To detection
3 Valve handle
4 Column

Figure A.1 — Flow diagram for the backflush valve (viewed from the back of the valve)

Annex B
(informative)

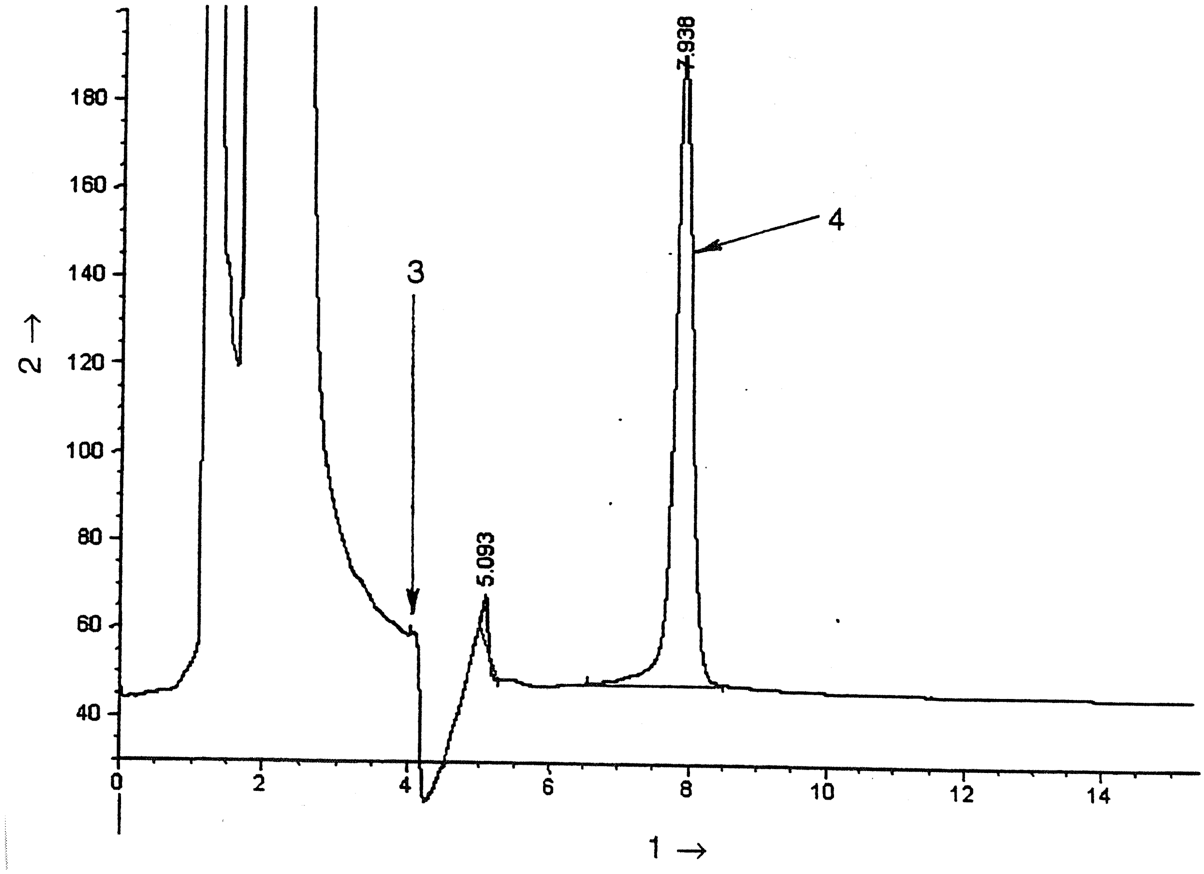
Example chromatograms



Key

1 Running times, in minutes
2 Response, in millivolts
3 Switch time
4 Calibration peaks

Figure B.1 — C12/14 alcohol ethoxylate, backflush valve "OFF"



Key

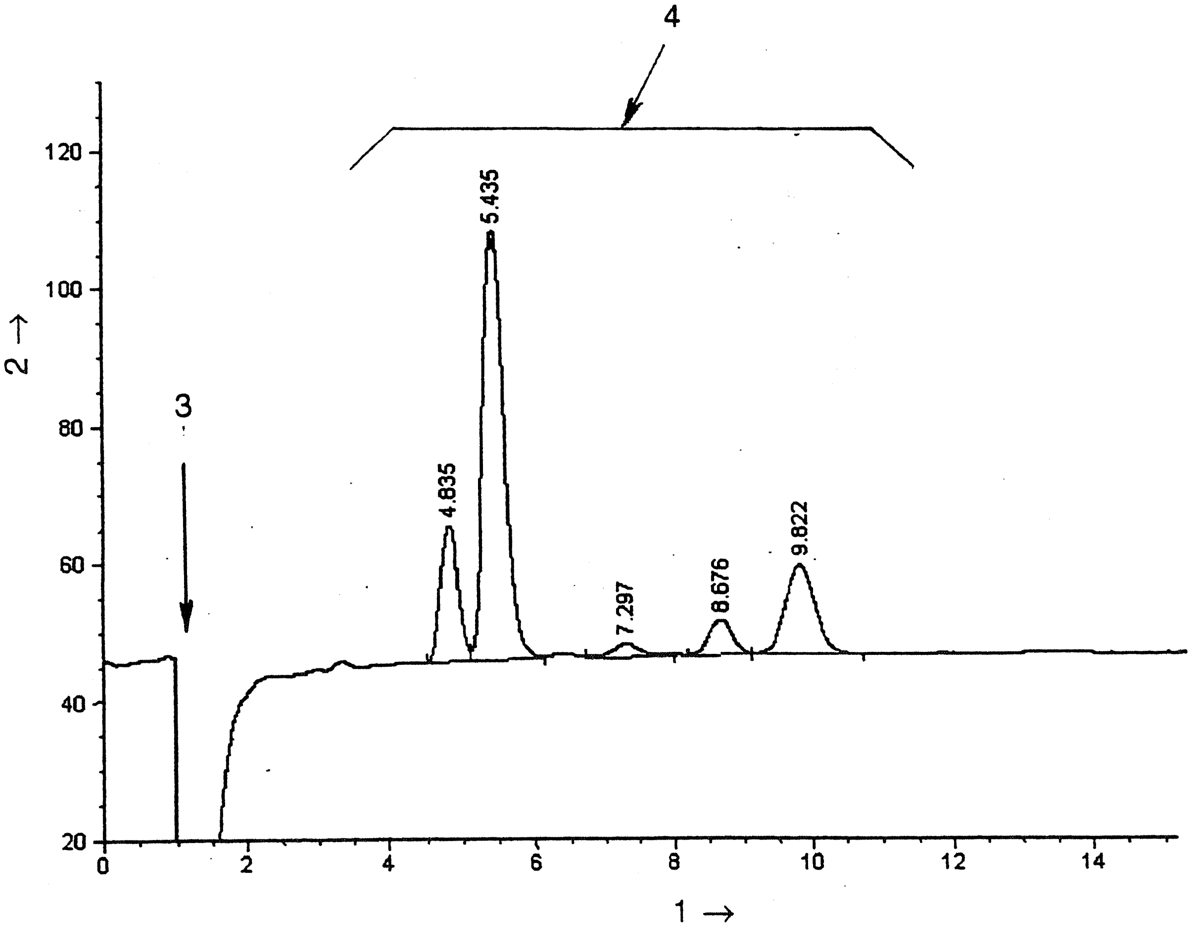
1 Running time, in minutes

2 Response, in millivolts

3 Backflush valve “ON”

4 Single peak of calibration substances

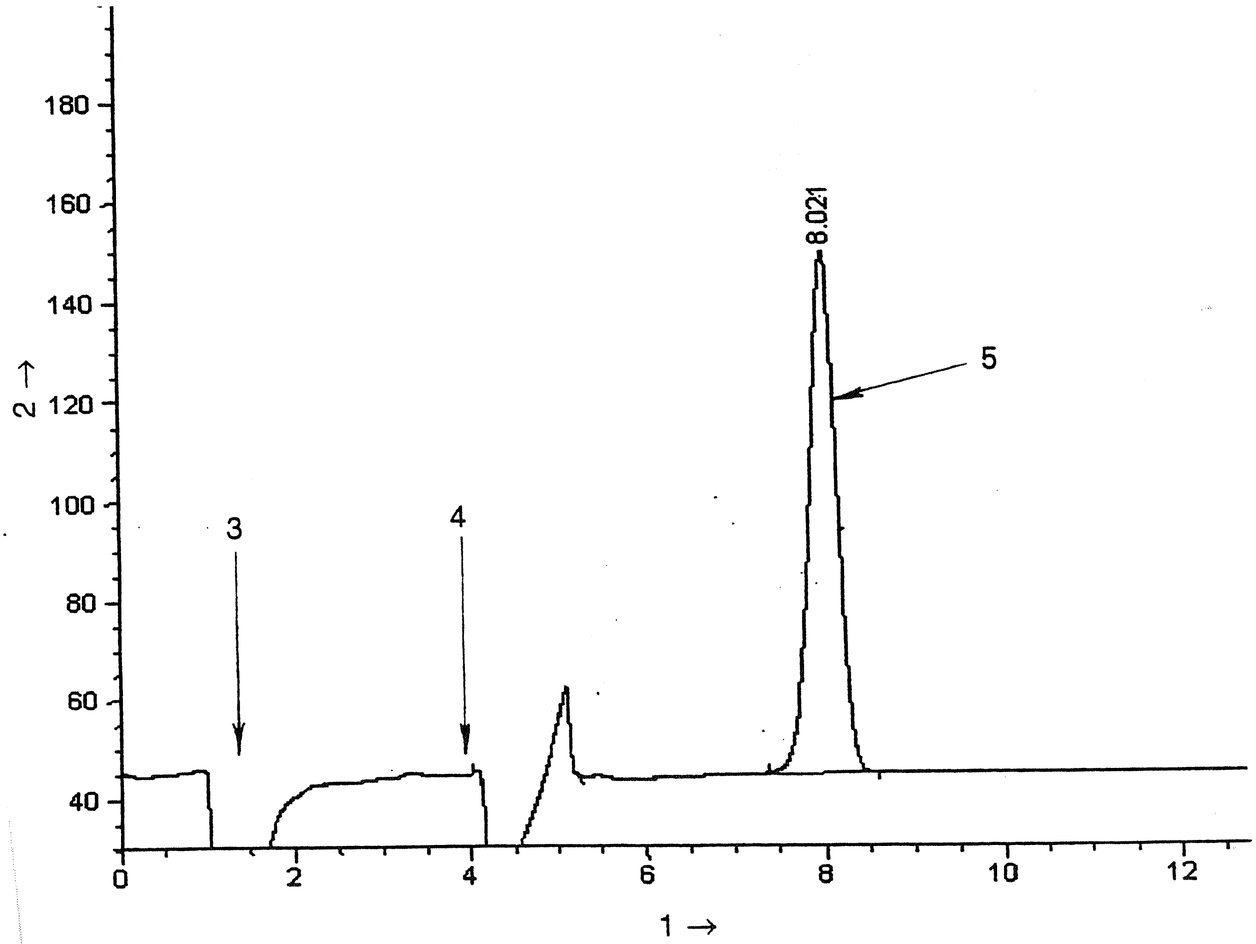
Figure B.2 — C12/14 alcohol ethoxylate, backflush valve “ON” at 4 min.



Key

1 Running time, in minutes
2 Response, in millivolts
3 Active matter
4 Peaks of non-ionic substances

Figure B.3 — C12/14 alcohol sulfate, backflush valve "OFF"



Key

1 Running time, in minutes
2 Response, in millivolts
3 Active matter
4 Backflush valve "ON"
5 Single peak of non-ionic substances

Figure B.4 — C12/14 alcohol sulfate, backflush valve "ON" at 4 min.

Annex C
(informative)

Ring test results

The ring test were carried out by CESIO/AIS in 1996 by using back flush.

Table C.1 — Ring test results

	Alkyl ether sulfate type 1	**Alkyl ether sulfate type 2**	**Primary alcohol sulfate**	**Linear alkyl benzene sulfonic acid**
Number of laboratories participating	8	8	7	8
Number of individual values not eliminated	31	31	25	25
Number of individual values of all laboratories	31	31	26	31
Mean value (g/100 g)	2,9	2,7	0,7	2,1
Repeatability standard deviation: s_r (g/100 g)	0,09	0,11	0,05	0,12
Repeatability limit r: r = 2,8 s_r (g/100 g)	0,26	0,31	0,14	0,33
Repeatability coefficient of variation (%)	3,2	4,0	6,7	5,7
Reproducibility standard deviation: s_R (g/100 g)	0,2	0,15	0,1	0,28
Reproducibility limit R: R = 2,8 s_R(g/100 g)	0,58	0,41	0,29	0,78
Reproducibility coefficient of variation (%)	7,0	5,4	14,4	13,5

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