ANNEX VIII

TEST METHODS AND ANALYTICAL METHODS

4. Preliminary treatment of anionic surfactants to be tested

4.1. Preliminary notes

4.1.1. Treatment of samples

The treatment of anionic surface-active agents and formulated detergents prior to the determination of primary biodegradability in the confirmatory test is:

Products	**Treatment**
Anionic surfactants	None
Formulated detergents	Alcoholic extraction followed by separation of the anionic surfactants by ion exchange

The purpose of the alcoholic extraction is to eliminate the insoluble and inorganic ingredients of the commercial product, which in some circumstances might upset the biodegradability test.

4.1.2. Ion-exchange procedure

Isolation and separation of anionic surface active agents from soap, non-ionic and cationic surfactants are required for correct biodegradability tests.

This is achieved by an ion-exchange technique using a macro-porous exchange resin and suitable eluants for fractional elution. Thus soap, anionic and non-ionic surfactants may be isolated in one procedure.

4.1.3. Analytical control

After homogenising, the concentration of anionic surfactants in the synthetic detergent is determined according to the MBAS analytical procedure. The soap content is determined by a suitable analytical method.

This analysis of the products is necessary to calculate the quantities required for preparing fractions for the biodegradability test.

Quantitative extraction is not necessary; however, at least 80 % of the anionic surfactants should be extracted. Usually, 90 % or more is obtained.

4.2. Principle

From a homogeneous sample (powders, dried pastes and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the synthetic detergent sample.

The ethanol extract is evaporated to dryness, dissolved in an isopropanol/water mixture and the solution obtained is passed through a strongly acidic cation exchange/macro-porous anion exchange combination heated to 50 ° C. This temperature is necessary to prevent the precipitation of any fatty acids which may be present in acidic media.

Any non-ionic surfactants remain in the effluent.

Soap fatty acids are separated by extraction with ethanol containing CO_2. The anionic surfactants are then obtained as ammonium salts, by elution with an aqueous isopropanolic solution of ammonium bicarbonate. These ammonium salts are used for the degradation test.

Cationic surfactants that might upset the biodegradability test and the analytical procedure are eliminated by the cation exchanger placed above the anion exchanger.

4.3. Chemicals and equipment

4.3.1. Deionised water

4.3.2. Ethanol, 95 % (v/v) C_2H_5OH (permissible denaturant: methyl ethyl ketone or methanol)

4.3.3. Isopropanol/water mixture (50/50 v/v):

— 50 parts by volume isopropanol, $CH_3CHOH.CH_3$, and

— 50 parts by volume water (4.3.1)

4.3.4. Solution of carbon dioxide in ethanol (approximately 0,1 % CO_2): using a delivery tube with a built-in sinter, pass carbon dioxide, CO_2, through the ethanol (4.3.2) for ten minutes. Use fresh solutions only

4.3.5. Ammonium bicarbonate solution (60/40 v/v): 0,3 mol NH_4HCO_3 in 1 000 ml of an isopropanol/water mixture consisting of 60 parts by volume isopropanol and 40 parts by volume water (4.3.1)

4.3.6. Cation exchanger (KAT), strongly acidic, resistant to alcohol (50-100 mesh)

4.3.7. Anion exchanger (AAT), macro-porous, Merck Lewatit MP 7080 (70-150 mesh) or equivalent

4.3.8. Hydrochloric acid, 10 % HCl (w/w)

4.3.9. 2 000 ml round-bottomed flask with ground glass stopper and reflux condenser

4.3.10. 90 mm diameter suction filter (heatable) for filter papers

4.3.11. 2 000 ml filter flask

4.3.12. Exchange columns with heating jacket and tap: inner tube 60 mm in diameter and 450 mm in height (see Figure 4)

4.3.13. Water-bath

4.3.14. Vacuum drying oven

4.3.15. Thermostat

4.3.16. Rotary evaporator

4.4. Preparation of extract and separation of anionic active agents

4.4.1. Preparation of extract

The quantity of surfactants necessary for the biodegradation test is about 50 g MBAS.

Normally, the quantity of product to be extracted will not exceed 1 000 g, but it may be necessary to extract further quantities of sample. For practical reasons, the quantity of product used should in most cases be limited to 5 000 g in preparing extracts for the biodegradation test.

Experience has shown that there are advantages in using a number of small extractions rather than one large extraction. The exchanger quantities specified are designed for a working capacity of 600-700 mmoles of surfactants and soap.

4.4.2. Isolation of alcohol-soluble constituents

Add 250 g of the synthetic detergent to be analysed to 1 250 ml ethanol, heat the mixture to boiling point and reflux for one hour with stirring. Pass the hot alcoholic solution through a coarse-pored suction filter heated to 50 ° C and filter rapidly. Wash the flask and suction filter with approximately 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products to be analysed, make sure that not more than 55 g anionic surfactants and 35 g soap are contained in the sample. Evaporate this weighed sample to dryness. Dissolve the residue in 2 000 ml ethanol and proceed as described above. In the case of powders of low apparent density (< 300 g/l) it is recommended to increase the ethanol ratio in the relation 20:1. Evaporate the ethanolic filtrate to dryness, preferably by means of a rotary evaporator. Repeat the operation if a greater quantity of extract is required. Dissolve the residue in 5 000 ml isopropanol/water mixture.

Preparation of ion-exchange columns

4.4.3. CATION-EXCHANGE COLUMN

Place 600 ml cation-exchange resin (4.3.6) in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid (4.3.8). Allow to stand for at least two hours, with occasional stirring.

Decant the acid and transfer the resin into the column (4.3.12) by means of deionised water. The column should contain a glass-wool plug.

Wash the column with deionised water at a rate of 10-30 ml/min until the eluate is free of chloride.

Displace the water with 2 000 ml isopropanol/water mixture (4.3.3) at a rate of 10-30 ml/min. The exchange column is now ready for operation.
ANION-EXCHANGE COLUMN

Place 600 ml anion-exchange resin (4.3.7) in a 3 000 ml beaker and cover by adding 2 000 ml deionised water.

Allow the resin to swell for at least two hours.

Transfer the resin into the column by means of deionised water. The column should contain a glass-wool plug.

Wash the column with 0,3 M ammonium bicarbonate solution (4.3.5) until free of chloride. This requires about 5 000 ml solution. Wash again with 2 000 ml deionised water. Displace the water with 2 000 ml isopropanol/water mixture (4.3.3) at a rate of 10-30 ml/min. The exchange column is now in the OH-form and ready for operation.

Ion-exchange procedure

4.4.4. Connect the exchange columns so that the cation-exchange column is placed on top of the anion-exchange column.

Heat the exchange columns to 50 ° C using thermostatic control.

Heat 5 000 ml of the solution obtained in item 4.4.2 to 60 ° C and pass the solution through the exchanger combination at a rate of 20 ml/min. Wash the columns with 1 000 ml hot isopropanol/ water mixture (4.3.3).

To obtain the anionic surface active agents (MBAS), disconnect the KAT column. Using 5 000 ml ethanol/CO_2 solution at 50 ° C (4.3.4), elute the soap fatty acids out of the KAT column. Reject the eluate.

Then elute the MBAS out of the AAT column with 5 000 ml ammonium bicarbonate solution (4.3.5). Evaporate the eluate to dryness using a steam bath or in a rotary evaporator.

The residue contains the MBAS (as ammonium salt) and possible non-surfactant anionics that have no detrimental effect on the biodegradation test. Add deionised water to the residue until a definite volume is obtained and determine the MBAS content in an aliquot. The solution is used as a standard solution of the anionic synthetic detergents for the biodegradation test. The solution should be kept at a temperature below 5 ° C.

Regeneration of ion exchange resins

4.4.5. The cation exchanger is rejected after use.

Passing an additional quantity of ammonium bicarbonate solution (4.3.5) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionic surfactants (methylene blue test) regenerates the anion-exchange resin.

Then pass 2 000 ml isopropanol/water mixture (4.3.3) down the anion exchanger to wash. The anion exchanger is again ready for operation.

Preliminary treatment of non-ionic surfactants to be tested

Changes to legislation:
There are outstanding changes not yet made to Regulation (EC) No 648/2004 of the European Parliament and of the Council. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by S.I. 2019/672 reg. 22
- Annex 1 para. 1 words substituted by S.I. 2019/672 reg. 23(2)(a)
- Annex 1 para. 1 words substituted by S.I. 2019/672 reg. 23(2)(b)
- Annex 1 para. 1 words substituted by S.I. 2019/672 reg. 23(2)(c)
- Annex 1 para. 2 words substituted by S.I. 2019/672 reg. 23(3)(a)
- Annex 1 para. 2 words substituted by S.I. 2019/672 reg. 23(3)(b)
- Annex 2 s. A words substituted by S.I. 2019/672 reg. 24(2)
- Annex 2 s. B words substituted by S.I. 2019/672 reg. 24(2)
- Annex 2 s. D words substituted by S.I. 2019/672 reg. 24(2)
- Annex 2 s. C words substituted by S.I. 2019/672 reg. 24(3)
- Art. 2(9) word omitted by S.I. 2019/672 reg. 6(2)(a)
- Art. 2(9) words substituted by S.I. 2019/672 reg. 6(2)(b)
- Art. 2(9) words substituted by S.I. 2019/672, reg. 6(2) (as substituted) by S.I. 2020/1617 reg. 2(4)(a)
- Art. 2(9a) words substituted by S.I. 2019/672 reg. 6(3)
- Art. 2(9A) words substituted in earlier amending provision S.I. 2019/672, reg. 6(3) by S.I. 2020/1617 reg. 2(4)(b)
- Art. 2(10) words inserted by S.I. 2019/672 reg. 6(4)
- Art. 2(10) words inserted by S.I. 2019/672, reg. 6(4) (as substituted) by S.I. 2020/1617 reg. 2(4)(c)
- Art. 2(13)-(15) inserted by S.I. 2019/672 reg. 6(5)
- Art. 2(16) inserted in earlier amending provision S.I. 2019/672, reg. 6(5) by S.I. 2020/1617 reg. 2(4)(d)
- Annex 3 Pt. B words omitted by S.I. 2019/672 reg. 25(3)(c)
- Annex 3 Pt. A para. 2 words substituted by S.I. 2019/672 reg. 25(2)(a)
- Annex 3 Pt. A para. 3 words substituted by S.I. 2019/672 reg. 25(2)(b)
- Annex 3 Pt. A para. 4 words substituted by S.I. 2019/672 reg. 25(2)(c)
- Annex 3 Pt. A para. 5 words substituted by S.I. 2019/672 reg. 25(2)(d)
- Annex 3 Pt. B para. 1 words substituted by S.I. 2019/672 reg. 25(3)(a)
- Annex 3 Pt. B para. 2 words substituted by S.I. 2019/672 reg. 25(3)(b)
- Art. 3(1)(a) substituted by S.I. 2019/672 reg. 7(2)(b)
- Art. 3(1)(a) words substituted in earlier amending provision S.I. 2019/672, reg. 7(2)(b) by S.I. 2020/1617 reg. 2(5)
- Art. 3(1)(b) words substituted by S.I. 2019/672 reg. 7(2)(c)
- Art. 3(1)(c) words substituted by S.I. 2019/672 reg. 7(2)(d)
- Art. 3A inserted by S.I. 2019/672, reg. 7A (as inserted) by S.I. 2020/1617 reg. 2(6)
- Annex 4 point 3 words inserted by S.I. 2019/672 reg. 26(8)(b)
- Annex 4 words omitted by S.I. 2019/672 reg. 26(6)
- Annex 4 point 1 heading words omitted by S.I. 2019/672 reg. 26(7)
- Annex 4 point 3 words omitted by S.I. 2019/672 reg. 26(8)(a)
- Annex 4 words omitted by S.I. 2019/672 reg. 26(13)
- Annex 4 words substituted by S.I. 2019/672 reg. 26(2)
- Annex 4 words substituted by S.I. 2019/672 reg. 26(3)
- Annex 4 words substituted by S.I. 2019/672 reg. 26(4)
- Annex 4 words substituted by S.I. 2019/672 reg. 26(5)
- Annex 4 point 4.1.2 words substituted by S.I. 2019/672 reg. 26(9)(a)
- Annex 4 point 4.1.2 words substituted by S.I. 2019/672 reg. 26(9)(b)
- Annex 4 point 4.1.3 words substituted by S.I. 2019/672 reg. 26(10)
- Annex 4 point 4.2.2 words substituted by S.I. 2019/672 reg. 26(11)(a)

- Annex 4 point 4.2.2 words substituted by S.I. 2019/672 reg. 26(11)(b)
- Annex 4 point 4.2.2 words substituted by S.I. 2019/672 reg. 26(11)(c)
- Annex 4 point 4.2.2 words substituted by S.I. 2019/672 reg. 26(11)(d)
- Annex 4 point 4.2.3 words substituted by S.I. 2019/672 reg. 26(12)(a)
- Annex 4 point 4.2.3 words substituted by S.I. 2019/672 reg. 26(12)(b)
- Annex 5 words omitted by S.I. 2019/672 reg. 27
- Annex 7 Pt. B words inserted by S.I. 2019/672 reg. 28(3)(b)
- Annex 7 Pt. A words substituted by S.I. 2019/672 reg. 28(2)(a)(i)
- Annex 7 Pt. A words substituted by S.I. 2019/672 reg. 28(2)(a)(ii)
- Annex 7 Pt. A words substituted by S.I. 2019/672 reg. 28(2)(b)(i)
- Annex 7 Pt. A words substituted by S.I. 2019/672 reg. 28(2)(b)(ii)
- Annex 7 Pt. B words substituted by S.I. 2019/672 reg. 28(3)(a)
- Annex 7 Pt. C words substituted by S.I. 2019/672 reg. 28(4)
- Annex 7 Pt. D words substituted by S.I. 2019/672 reg. 28(5)
- Annex 8 words substituted by S.I. 2019/672 reg. 29
- Art. 10(3)(4) inserted by S.I. 2019/672 reg. 13(4)
- Art. 15(3)-(8) inserted by S.I. 2019/671 reg. 3(4)
- Art. 15(3) words omitted in earlier amending provision S.I. 2019/671, reg. 3(4) by S.I. 2020/1617 reg. 3(2)(b)(i)
- Art. 15(6)(7) omitted in earlier amending provision S.I. 2019/671, reg. 3(4) by S.I. 2020/1617 reg. 3(2)(b)(ii)
- Art. 18A inserted by S.I. 2019/672 reg. 21
- Art. 18A(1) words omitted in earlier amending provision S.I. 2019/672, reg. 21 by S.I. 2020/1617 reg. 2(9)(a)
- Art. 18A(3) substituted in earlier amending provision S.I. 2019/672, reg. 21 by S.I. 2020/1617 reg. 2(9)(b)