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**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. (See end of Document for details) View outstanding changes*

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Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents (Text with EEA relevance)

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## ANNEX VIII

### TEST METHODS AND ANALYTICAL METHODS

#### 2. Determination of anionic surfactants in biodegradability tests

##### 2.1. Principle

The method is based on the fact that the cationic dye methylene blue forms blue salts with anionic surfactants (MBAS), which can be extracted with chloroform. To eliminate interference, the extraction is first effected from alkaline solution and the extract is then shaken with acidic methylene blue solution. The absorbency of the separated organic phase is measured photometrically at the wavelength of maximum absorption of 650 nm.

##### 2.2. Reagents and equipment

###### 2.2.1. Buffer solution pH 10

Dissolve 24 g sodium bicarbonate,  $\text{NaHCO}_3$  AR, and 27 g anhydrous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) AR in deionised water and dilute to 1 000 ml.

###### 2.2.2. Neutral methylene blue solution

Dissolve 0,35 g methylene blue AR in deionised water and dilute to 1 000 ml. Prepare the solution at least twenty-four hours before use. The absorbency of the blank chloroform phase, measured against chloroform must not exceed 0,015 per 1 cm of layer thickness at 650 nm.

###### 2.2.3. Acidic methylene blue solution

Dissolve 0,35 g methylene blue AR in 500 ml deionised water and mix with 6,5 ml  $\text{H}_2\text{SO}_4$  ( $d = 1,84$  g/ml). Dilute to 1 000 ml with deionised water. Prepare the solution at least twenty-four hours before use. The absorbency of the blank chloroform phase, measured against chloroform must not exceed 0,015 per 1 cm of layer thickness at 650 nm.

###### 2.2.4. Chloroform (trichloromethane) AR freshly distilled

###### 2.2.5. Dodecyl benzene sulphonic acid methyl ester

###### 2.2.6. Ethanolic potassium hydroxide solution, KOH 0,1 M

###### 2.2.7. Ethanol pure, $\text{C}_2\text{H}_5\text{OH}$

###### 2.2.8. sulphuric acid, $\text{H}_2\text{SO}_4$ 0,5 M

###### 2.2.9. Phenolphthalein solution

Dissolve 1 g phenolphthalein in 50 ml ethanol and add 50 ml deionised water while stirring continuously. Filter off any precipitate obtained.

###### 2.2.10. Methanolic hydrochloric acid: 250 ml hydrochloric acid AR and 750 ml methanol

###### 2.2.11. Separating funnel, 250 ml

###### 2.2.12. Graduated flask, 50 ml

###### 2.2.13. Graduated flask, 500 ml

###### 2.2.14. Graduated flask, 1 000 ml

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- 2.2.15. Round-bottomed flask with ground glass stopper and reflux condenser, 250 ml; boiling granules
- 2.2.16. pH meter
- 2.2.17. Photometer for measurements at 650 nm, with 1 to 5 cm cells
- 2.2.18. Qualitative grade filter paper
- 2.3. Procedure

The samples for analysis must not be taken through a layer of foam.

After thorough cleaning with water, the equipment used for the analysis must be thoroughly rinsed with methanolic hydrochloric acid (2.2.10) and then with deionised water before using.

Filter the activated sludge plant influent and effluent to be examined immediately on sampling. Discard the first 100 ml of the filtrates.

Place a measured volume of the sample, neutralised if necessary, into a 250 ml separating funnel (2.2.11). The volume of sample should contain between 20 and 150 g of MBAS. At the lower MBAS content, up to 100 ml of sample may be used. When using less than 100 ml, dilute to 100 ml with deionised water. Add to the sample 10 ml of buffer solution (2.2.1), 5 ml of neutral methylene blue solution (2.2.2) and 15 ml of chloroform (2.2.4). Shake the mixture uniformly and not too vigorously for one minute. After phase separation, run the chloroform layer into a second separating funnel, containing 110 ml of deionised water and 5 ml of acidic methylene blue solution (2.2.3). Shake the mixture for one minute. Pass the chloroform layer through a cotton-wool filter previously cleaned and wetted with chloroform into a graduated flask (2.2.12).

Extract the alkaline and acid solutions three times, using 10 ml of chloroform for the second and third extractions. Filter the combined chloroform extracts through the same cotton wool filter and dilute to the mark in the 50 ml flask (2.2.12) with chloroform used for rewashing the cotton wool. Measure the absorbency of the chloroform solution with a photometer at 650 nm in 1 to 5 cm cells against chloroform. Run a blank determination through the whole procedure.

#### 2.4. Calibration curve

Prepare a calibration solution from the standard substance dodecylbenzene sulphonic acid methyl ester (tetrapropylene type mol. wt. 340) after saponification into the potassium salt. The MBAS is calculated as sodium dodecyl benzene sulphonate (mol. wt. 348).

From a weighing pipette, weigh 400 to 450 mg of dodecyl-benzene-sulphonic-acid-methyl-ester (2.2.5) to the nearest 0,1 mg in a round-bottomed flask and add 50 ml of ethanolic potassium hydroxide solution (2.2.6) and some boiling granules. After mounting the reflux condenser, boil for one hour. After cooling, wash the condenser and ground glass joint with about 30 ml of ethanol, and add these washings to the contents of the flask. Titrate the solution with sulphuric acid against phenolphthalein until it becomes colourless. Transfer this solution to a 1 000 ml graduated flask (2.2.14), dilute to the mark with deionised water and mix.

Part of this surfactant stock solution is then further diluted. Withdraw 25 ml, transfer to a 500 ml graduated flask (2.2.13), dilute to the mark with deionised water and mix.

This standard solution contains:

$$\frac{E \times 1,023 \text{ mg MBAS per ml}}{20000}$$

where E is the sample weight in mg.

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To establish the calibration curve, withdraw 1, 2, 4, 6, 8 ml portions of the standard solution and dilute each to 100 ml with deionised water. Then proceed as stated under item 2.3 including a blank determination.

#### 2.5. Calculation of results

The amount of anionic surfactant (MBAS) in the sample is read from the calibration curve (2.4). The MBAS content of the sample is given by:

$$\frac{\text{mg MBAS} \times 1000}{V} = \text{MBAS mg / l}$$

where: V = ml volume of the sample used.

Express the results as sodium dodecylbenzene sulphonate (MW 348).

#### 2.6. Expression of results

Express the results as MBAS mg/l to the nearest 0,1.

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### 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\(9\)\(b\)](#)