Designation: D ~~2330 – 88 (Reapproved 1995)$^{\epsilon 1}$~~ 2330 – 02

Standard Test Method for Methylene Blue Active Substances[1]

This standard is issued under the fixed designation D 2330; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

This standard has been approved for use by agencies of the Department of Defense.

~~ε[1] NOTE—Editorial changes were made throughout in June 1995.~~

1. Scope

1.1 This test method covers the determination of compounds that react with methylene blue under the conditions specified in the test procedure. They are referred to as methylene blue active substances (MBAS), and are calculated and reported in terms of the reference material, linear alkyl benzene sulfonate, LAS.

1.2 This test method is applicable for determining MBAS in water and wastewater. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

1.3 This test method is a simple, rapid, control procedure suitable for monitoring the effectiveness of a biodegradation or other linear alkyl benzene sulfonate (LAS) removal process. For greater specificity and interference removal, the pretreatment procedure in Annex A1 should be used. Data derived without the pretreatment procedure should be interpreted with care. This test method is applicable in the range from 0.03 to 1.5 mg/L for a 100-mL sample.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For a specific hazard statement, see ~~Note 2.~~ 8.3.

2. Referenced Documents

2.1 *ASTM Standards:*

D 459 Terminology Relating to Soap and Other Detergents[2]

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[1] This test method is under the jurisdiction of ASTM Committee D~~-~~19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods ~~of~~ for Analysis for Organic Substances in Water.

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D 1129 Terminology Relating to Water[3]
D 1193 Specification for Reagent Water[3]
D 3370 Practices for Sampling Water from Closed Conduits[3]
D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[3]
D 4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data[3]
~~E~~
D ~~60 Practice~~ 5788 Guide for ~~Photometric and Spectrophotometric Methods for Chemical Analysis of Metals~~ Spiking Organics into Aqueous Samples[4]
D 5789 Practice for Writing Quality Control Specifications for Standard Test Methods for Organic Constituents[4]
D 5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis[4]
E ~~131 Terminology Relating to~~ 60 Practice for Molecular Absorption Spectros~~m~~metric Metho~~p~~ds for Chemical Analy~~-~~sis of Metals, Ores, and Related Materials[5]
E 131 Terminology Relating to Molecular Spectroscopy[6]
E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers~~6~~[6]

[2] *Annual Book of ASTM Standards*, Vol 15.04.
[3] *Annual Book of ASTM Standards*, Vol 11.01.
[4] *Annual Book of ASTM Standards*, Vol ~~03.05.~~ 11.02.
[5] *Annual Book of ASTM Standards*, Vol ~~14.01.~~ 03.05.
[6] *Annual Book of ASTM Standards*, Vol 03.06.

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129 and E 131.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *alkyl benzene sulfonate (ABS)*[7]—the generic name applied to the neutralized product resulting from the sulfonation of a branched-chain alkylated benzene. See also Terminology D 459.

3.2.2 *linear alkyl benzene sulfonate (LAS)*[7]—a form of alkyl benzene sulfonate (ABS) in which the alkyl group is linear rather than a branched chain. See also Terminology D 459.

4. Summary of Test Method [8]

4.1 This test method is based upon the formation of a blue-colored chloroform extractable ion pair by the reaction of cationic methylene blue and an anionic surfactant (including LAS, other sulfonates, and sulfate esters).

4.2 The sample is mixed with an acidified, aqueous solution of methylene blue. Any resulting hydrophobic ion pair which may be formed is extracted successfully with chloroform. The combined chloroform extracts are washed with an acid solution to remove the less hydrophobic ion pairs (having low partition coefficients) that can be formed by potentially interfering substances. The chloroform layer retains the highly hydrophobic methylene blue-LAS ion pairs.

4.3 The intensity of the blue color remaining in the chloroform extract is measured photometrically at the wavelength of maximum absorption near 650 nm. This intensity is related to the concentration of LAS by means of a calibration curve or chart.

5. Significance and Use

5.1 The widespread use and discharge of detergents into surface waters can result in a lowering of its aesthetic quality by foam formation and by causing toxicity to aquatic wildlife. This test method is capable of detecting small concentrations of detergents as MBAS so that they can be controlled to prevent such problems.

5.2 Biodegradable linear alkyl benzene sulfonates (LAS) have replaced the branched-chain alkyl benzene sulfonates (ABS) in detergent formulations, which were more resistant to biodegradation. Differentiation between linear and branched-chain alkyl benzene sulfonates, as well as differentiation of the various positional isomers of either type, is not possible by this test method. While the methylene blue method may be employed to monitor studies designed to measure biodegradability, it cannot be used to predict this quality.

6. Interferences

6.1 Any organic or inorganic compound that will form a chloroform extractible ion pair will interfere by producing high results, unless the ion pair formed is eliminated by the treatment described in 4.1. These positive interferences include organic sulfonates, carboxylates, phosphates, and phenols, as well as inorganic cyanates, chlorides, nitrates, and thiocyanates.

6.2 Any compound effectively competing with methylene blue to form a LAS ion pair will give negative results. This negative interference is demonstrated by some amines and has analytical significance in the case of quaternary ammonium compounds.

6.3 An evaluation of the effect of various potential interferences is summarized in Table 1. The listed compounds, in the concentrations indicated, were added to solutions containing 1 mg/L LAS.

6.4 When interferences are present, the pretreatment procedure described in Annex A1 should be used. Table 2 shows the interferences that can be present even though the pretreatment was used.

6.5 When a concentrated acid chromate cleaning solution is used to clean glassware, including separatory funnels, between samples, care must be taken to completely flush all of the acid chromate cleaning solution from all surfaces and, in particular, from the space between the barrel and plug of the separatory funnel stopcock. Failure to remove the acid can result in an error in results.

6.5.1 Never use a detergent to clean any glassware used in this test method as a detergent is difficult to remove from surfaces. Any residual detergent could cause a high result.

7. Apparatus

7.1 *Filter Photometer or Spectrophotometer*, suitable for measurement at a wavelength in the region near 650 nm and equipped with 50-mm and 10-mm light path absorption cells.

NOTE 1—Photometers and photometric practices prescribed in this test method shall conform to Practice E 60. Spectrophotometers shall conform to Practice E 275.

7.2 *Separatory Funnels*, 250-mL size, Squibb-type, glass-stoppered, preferably with TFE-fluorocarbon stopcocks.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all

[7] For a more complete discussion of terms relating to synthetic detergents and their significance, refer to "Syndets and Waste Disposal" by McKinney, R. E., *Sewage and Industrial Wastes*, Vol 29, Part 6, June 1957, pp. 654–666.

[8] Adopted from "Surfactants (Anionic) Methylene Blue Methods," *Standard Methods for the Examination of Water and Waste Water*, Twelfth Ed., 1965.

TABLE 1 Evaluation of Potential Interferences in the Methylene Blue Method

Added to 1.0 mg/L LAS Solution	Concentration, mg/L	Indicated LAS, mg/L
Acetic acid	100	1.0
Ammonium diethylphosphorodithioate	20	1.1
Benzene sulfonic acid	100	1.3
Cholesterol	100	1.0
2,4-dichlorophenol	100	1.0
Diethanolamine	1000	1.0
Disodium phenylphosphate	10	1.0
Isopropylamine	14	1.0
Leucine	10	1.0
N-1-(naphthylethylenediamine) hydrochloride	100	0.9
Nonyl phenol + 9 EtO	100	1.0
Phenol	100	1.0
Picric acid	5	4.6
Potassium chloride	100	1.0
Potassium cyanate	100	1.0
Potassium nitrate	100	1.0
Potassium thiocyanate	2	1.0
Potassium thiocyanate	100	4.1
Proteins (Knox gelatine)	100	0.9
Sodium dodecyl sulfate	10	14.6
Sodium dodecane sulfonate	5	5.0
Sodium naphthalene sulfonate	5	5.1
Sodium stearate	100	1.0

TABLE 2 Evaluation of Potential Interferences in the Methylene Blue Method with Pretreatment Described in Annex A1

Added to 1.0 mg/L LAS Solution	Concentration, mg/L	Indicated LAS, mg/L
Sodium dodecane sulfonate	5	3.7
Sodium benzene sulfonate	100	1.2
Sodium dodecyl sulfate	10	0.9
Potassium thiocyanate	100	1.0
Picric acid	10	1.0

reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[9] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type II.

8.3 *Chloroform* ($CHCl_3$).

~~NOTE 2—**Warning:**~~ **). (Warning—**Chloroform ($CHCl_3$) is toxic and is suspected of being a possible carcinogen: avoid ingestion, inhalation, or absorption through the skin. Use a well-ventilated fume hood to carry off chloroform vapors during analysis.)

8.4 *Linear Alkyl Benzene Sulfonate Solution, Stock* (1.0 mL = 1.0 mg LAS)—Weigh the amount of reference material[10] necessary to provide the equivalent of 1.000 g of LAS on a 100 % active basis. Dissolve in water and dilute to 1 L, mixing gently to prevent foam formation. Record the molecular weight of the LAS reference material as supplied. The stock solution may be stored at 4°C in the dark for 12 months in a well-stoppered flask without deterioration.[11]

8.5 *Linear Alkyl Benzene Sulfonate Standard Solution* ,(1.0 mL = 0.01 mg LAS)—Dilute 10.0 mL of the foam-free stock solution (8.4) to 1 L with water that has been previously adjusted to pH 2 with sulfuric acid and mix. The standard solution may be stored at 4°C in the dark for at least 12 months in a well-stoppered flask without deterioration.[11]

8.6 *Methylene Blue Solution* (30 mg/L)—Dissolve 0.1 g of methylene blue chloride in 100 mL of water. Transfer 30 mL of this solution to a 1-L volumetric flask and add 500 mL of water. Add carefully 50 mL of 14 % sulfuric acid stock solution (8.10) and 50 g of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$). Shake until solution is complete and then dilute to 1 L with water and mix.

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[9] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[10] Linear alkyl benzene sulfonate reference material may be obtained from the United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

[11] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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8.7 *Phenolphthalein Indicator Solution* (5.0 g/L)—Dissolve 0.5 g of phenolphthalein in 50 mL of 95 % ethyl alcohol and dilute to 100 mL with water and mix.

NOTE ~~3~~2—Specially denatured ethyl alcohol conforming to Formula No. 3A or 30 of the U. S. Bureau of Alcohol, Tobacco, and Firearms may be substituted for 95 % ethyl alcohol.

8.8 *Phosphate Wash Solution*—Dissolve 50 g of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) in 500 mL of water in a 1-L volumetric flask. Add carefully 50 mL of 14 % sulfuric acid stock solution (8.10) and dilute to volume with water and mix. The solution has a pH of approximately 1.8.

8.9 *Sodium Hydroxide Solution* (10 g/L)—Dissolve 10 g of sodium hydroxide (NaOH) in water, dilute to 1 L and mix.

8.10 *Sulfuric Acid Stock Solution* (14 % volume per volume)—Add carefully 140 mL of concentrated sulfuric acid (H_2SO_4, sp gr 1.84) to 700 mL of cold (0 to 5°C) water with good stirring, dilute to 1 L with water and mix.

8.11 *Sulfuric Acid Solution, Dilute* (0.7 % volume per volume)—Dilute carefully 50 mL of 14 % sulfuric acid stock solution (8.10) to 1 L with water and mix.

9. Sampling

9.1 Collect the sample in accordance with Practices D 3370.

9.2 Samples may be preserved against biological oxidation by adding concentrated sulfuric acid (H_2SO_4) to adjust the sample to pH 2 or less and storing at 4°C. Analyze the preserved sample as soon as possible, or within ~~1~~ one week after collection. Data on decomposition are not available.

9.3 Rinse the sample container and cap well to free them of detergent if they have been used previously and cleaned prior to recycling.

10. Preparation of Apparatus

10.1 *Glassware Conditioning*:

10.1.1 All glassware used for the determination of LAS should be free of scratches and etch marks because of the tendency of surface-active materials to adsorb on this type of surface. All volumetric flasks and photometer cells, projected for use in LAS determinations, should, as instructed herein, be preconditioned as follows: Obtain the chloroform extract from 12.0 mL of the standard LAS solution as described in 11.4. Transfer sequentially to each of the volumetric flasks and photometer cells and permit a minimum contact time, in each case, of 5 min. Rinse thoroughly with chloroform and drain (**Warning**—see ~~Note 2).~~ 8.3).

11. Calibration

11.1 Prepare a series of standards by adding the standard solution (8.5) from a 25-mL buret to a series of 250-mL separatory funnels (see 6.5) and dilute the standards to 100 mL volume with water, yielding solutions as follows:

Standard, mL (1.0 mL = 0.01 mg LAS)	LAS, mg (per 100 mL of extract)
0.00	0.00
1.00	0.01
3.00	0.03
5.00	0.05
7.00	0.07
9.00	0.09
12.00	0.12

NOTE ~~4~~3—If desired, additional standards in the range from 0.00 to 0.12 mg of LAS may be prepared for the calibration series.

11.2 Add 3 drops of phenolphthalein solution (8.7) and just enough sodium hydroxide solution (8.9) to produce a pink color. Add dilute sulfuric acid solution (8.11), in small increments until the pink color is barely discharged.

11.3 Add 25 mL of methylene blue solution (8.6) and mix. Add 25 mL of chloroform (**Warning**—see ~~Note 2)~~ 8.3) and mix thoroughly for 30 s with shaking. Vent carefully, permit the phases to separate and then drain the chloroform layer into a second 250-mL separatory funnel (see 6.5). Leave any emulsion layer in the first separatory funnel. Repeat the extraction, serially, with two additional 25-mL portions of chloroform.

NOTE ~~5~~4—Vent the separatory funnel through the stopcock with the funnel tip directed away from the face to avoid contact with any sample spray (**Warning**—see ~~Note 2).~~ 8.3).

11.4 Add 50 mL of phosphate wash solution (8.8) to the combined chloroform extracts in the second separatory funnel and shake vigorously for 30 s (see Note ~~5).~~ 4). Hold the separatory funnel in a vertical position and swirl the contents. Permit settling for 1 min. Filter the chloroform layer through a glass wool plug into a conditioned (see 10.1) 100-mL volumetric flask. Add 20 mL of chloroform to the second separatory funnel and repeat the shaking, swirling, and settling steps (see Note ~~5).~~ 4). Combine the chloroform layer through the glass wool into the volumetric flask. Add additional chloroform as needed to bring the flask to 100-mL volume and mix thoroughly.

11.5 Using a 50-mm (Note ~~6)~~ 5) light-path cell, at 650-nm wavelength, set the photometer to zero absorbance with the extract of the calibration blank.

NOTE ~~6~~5—If a shorter 10-mm light path cell is employed, the volumes of standard LAS solution selected for the calibration should be proportionately increased.

11.6 Measure the absorbance of each of the extracts. Because of a tendency to fade slowly, the absorbance of the extracted methylene blue complex should be measured within 30 min after formation. Prepare a calibration curve by plotting photometer readings in absorbance against concentration of LAS in milligrams per 100 mL of extract on rectilinear graph paper, and record the molecular weight of the LAS reference material, as supplied,[9] on the graph.

NOTE ~~7~~6—If the scale of the photometer reads in percent transmittance, plot the results on semilog paper, using the vertical log axis for transmittance and the horizontal linear axis for concentration in milligrams of LAS per 100 mL of extract.

NOTE ~~8~~7—A separate calibration curve must be made for each photometer and each cell used. Each calibration curve must be checked periodically to ensure repeatability. If a subsequent calibration curve does not reproduce the previous curve, then recheck the curve again. Make sure that the LAS reference material as supplied has the same molecular weight as that which was used to produce the previous curve.

12. Procedure

12.1 Select a volume of sample consistent with the anticipated LAS content. If the LAS concentration is not expected to exceed 1 mg/L, use a 100-mL sample. For LAS in the 10 mg/L range, use a 10-mL sample diluted to 100 mL with water. The sensitivity of the method may be improved in the cases of relatively unpolluted waters by concentrating larger sample volumes to 100 mL by evaporation.

12.2 Process the sample or a set of samples, a quality control standard (see Annex A2), selected from near the midscale of the series used to prepare the calibration chart in 11.1, and a parallel procedure blank, using 100 mL of water, in 250-mL separatory funnels as outlined in 11.2 to 11.6.

NOTE ~~9~~8—If an excessive amount of emulsion forms with a sample in 11.3 and it is evident that a substantial loss of MBAS will occur, then the analyst is advised to use well-known techniques in an attempt to break the emulsion. Several known techniques are (*1*) the brief local application of heat by a hot-water stream applied to the outside of the separatory funnel in the area of the emulsion layer and (*2*) filtering the emulsion through a wad of glass wool to remove particulate matter, etc. If the emulsion cannot be broken, then make a note recording the fact, or else, make another attempt at analysis by using a smaller sample size.

13. Calculations

13.1 Calculate and express as MBAS, the apparent concentration of linear alkyl benzene sulfonate as follows:

$$\text{MBAS, mg/L} = W \times 1000/S$$

where:

W = LAS in the sample from the calibration chart for the appropriate absorption cell, milligrams, and
S = sample volume selected in accordance with 12.1, millilitres.

13.2 Follow the procedures outlined in Annex A2 and Annex A3: to evaluate the quality control standard, to determine if the method is under control, and to accept or reject the results from that set of analyses.

14. Report

14.1 Include in the report the molecular weight (mw) of the LAS used to prepare the calibration curve in Section 11. Report results as:

MBAS (calculated as LAS, mw ___) = ___ mg/L

15. Precision and Bias [10]

15.1 Seven operators from seven laboratories determined three concentration levels of LAS on ~~3~~ three days in reagent water and in selected water matrices.

15.2 The overall and single-operator precision of this test method within its designated range for reagent water and selected water matrices varies with the quantity tested in accordance with the values cited in Table 3 .

TABLE 3 Precision

Amount added, mg/L	Overall, S_t	Single-Operator, S_o
	Reagent Water	
0.23	±0.042	±0.021
0.78	±0.044	±0.028
1.28	±0.203	±0.091
	Matrix Water	
0.23	±0.045	±0.039
0.78	±0.131	±0.102
1.28	±0.063	±0.054

15.3 Recoveries of known amounts of LAS, calculated as MBAS, from reagent water (Types I, II, and III) and selected water matrices (drinking, natural, and treated waste) were as shown in Table 4 .

TABLE 4 Recovery

Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant, 95 % Confidence Level
		Reagent Water	
0.23	0.225	−2.2	no
0.78	0.756	−3.1	yes
1.28	1.223	−4.5	no
		Matrix Water	
0.23	0.224	−2.6	no
0.78	0.742	−4.9	no
1.28	1.037	−19.0	yes

15.4 These collaborative test data were obtained on reagent grade water, drinking, natural, and treated waste waters. It is the user's responsibility to ensure the validity of this test method for water of untested matrices.

~~15.5 The QA/QC portion of this test method has not been completely established at this time. Until such time as these procedures are established, it is recommended that the user use Practices D 4210 and D 3856 as guides for establishing QA/QC.~~

~~15.6 Before this test method is applied to the analysis of samples, the analyst shall establish his own precision and bias data. Analysts performing this test method will be requiredto measure their performance against the performance level achieved by the laboratories that participated in the ASTM round robin study done on this test method.~~

~~15.7 A known standard shall be run each day that an analysis is done. The known standard shall conform with Annex A1 of this test method before an analysis is acceptable.~~

~~15.8 Other formal QA/QC procedures will be incorporated at such time as they have been officially accepted by the Society.~~

16. Quality Assurance / Quality Control

16.1 It is recommended that the user use D 3856, D 4210, D 5788, and D 5847 as guides for the establishment of QA/QC.

16.2 Before this test method is applied to the analysis of samples, the analyst shall establish his own precision and bias data. Analysts performing this test method will be required to measure their performance against the performance level achieved by the laboratories that participated in the ASTM interlaboratory study done on this test method.

16.3 A known standard shall be run each day that an analysis is performed. The known standard shall conform with Annex A2 of this test method before the analysis is acceptable.

16.4 *Demonstration of Analyst Proficiency*:

16.4.1 Demonstrate the competence of the analyst before this method is used to generate reportable data (Practice D 5789, Section 9).

16.4.2 Verify the procedure to be used by analyzing standard solutions in the expected range.

16.4.3 Analyze in duplicate six samples of known or nearly the same concentration by the method.

16.4.4 Calculate the standard deviation of the data (D 3856, D 4210, D 5789, and D 5847). If the value obtained is within that given in the procedure for single operator precision, the analyst can be considered "competent."

NOTE 9—If this is the first data generated in the laboratory, construct a preliminary control chart (D 3856, D 4210).

16.5 *Demonstration of Laboratory Proficiency*

16.5.1 Initially analyze five or six samples in duplicate to obtain a crude estimate of population standard deviation. If the method is used routinely, continue to accumulate additional data until at least 40 data points are obtained (D 4210, Section 5).

16.5.2 Construct a control chart with upper and lower limits from the data obtained (D 3856, Section 11 and D 4210, Section 9).

16.5.3 To monitor precision and bias, analyze the following in duplicate: a standard solution, a sample of known value, a spiked sample (D 5788), a field blank, and a method blank each day (or every 20 routine samples).

16.5.4 Calculate the relative range value (R) for each set of duplicate analyses. If the R's are greater than the upper control limit, the precision is judged out–of–control, and analyses should be discontinued until the problem is resolved.

16.5.5 Calculate the percent recovery (P) for the standard and the spiked sample. If the recoveries are not within 100 ± 10 %, the analyses should be discontinued until the reason is found.

17. Keywords

167.1 chloroform; extraction; hydrophobic ions; linear alkyl benzene sulfonate; methylene blue; spectrophotometer

ANNEXES

(Mandatory Information)

A1. PRETREATMENT PROCEDURE FOR SAMPLES CONTAINING INTERFERENCES

A1.1 Scope

A1.1.1 This procedure is the pretreatment of MBAS in all waters and wastewaters that contain interfering substances, including those noted in 6.1, 6.2, and Table 1. As noted in Table 2, this procedure may not eliminate all interferences. It is the user's responsibility to evaluate the effectiveness of this procedure.

A1.2 Summary of Procedure

A1.2.1 The selected sample is hydrolyzed by boiling under partial reflux with hydrochloric acid. The residual products are neutralized to a controlled pH value, and reacted with 1-methylheptylamine. The resulting ion pairs are extracted into a chloroform phase and evaporated to dryness on a steam bath. The amine component of the ion pair is removed by boiling in an aqueous alkaline media and the isolated MBAS are then determined as described in 11.2 to 11.6.

A1.3 Interferences

A1.3.1 Any compound that survives the purification steps described in A1.2.1 and which, subsequently, will satisfy the limitations imposed in 4.2 will interfere. Current knowledge indicates that such compounds are limited to alkyl sulfonates, and to a much lesser degree, to aryl sulfonates. In addition, other untested hydrophobic sulfonates, with any degree of hydrophilic substitution, may interfere.

A1.3.2 The increased specificity attainable by the use of this procedure is illustrated in Table 2. The listed compounds, in the concentrations indicated, were added to solutions containing 1.0 mg/L LAS.

A1.4 Apparatus

A1.4.1 *pH Meter*.

A1.4.2 *Hot Plate*, adjustable.

A1.4.3 *Steam Bath*.

A1.5 Reagents

A1.5.1 *Hydrochloric Acid* (sp gr 1.19)—Concentrated hydrochloric acid (HCl).

A1.5.2 *1-Methylheptylamine*. [12]

A1.5.3 *Amine-Chloroform Mixture* (0.12 % volume per volume)—Pipet 1.2 mL of 1-methylheptylamine to a glass-stoppered bottle that contains 1000 mL of chloroform and mix. Prepare fresh daily. Store in a well-ventilated fume hood. Handle with care (**Warning**, see ~~Note 2).~~ 8.3).

A1.5.4 *Phosphate Buffer Solution* (pH 7.5 ± 0.1)—Dissolve 10 g of potassium dihydrogen phosphate (KH_2PO_4) in 700 mL of water and adjust the pH value to 7.5 ± 0.1 by adding approximately 250 mL of sodium hydroxide solution (9.7). Dilute to 1 L with water and mix.

A1.6 Calibration

A1.6.1 Select the volumes of standard LAS solution suggested in 11.1 and dilute each to 100 mL with water in a series of 400-mL beakers.

A1.6.2 Process each in accordance with A1.7.1 to A1.7.5.

A1.7 Procedure

A1.7.1 Select a volume of sample consistent with the anticipated LAS concentration (see 12.1), transfer to a 400-mL beaker and dilute to 100-mL volume with water. Process in parallel in additional beakers, a selected quality control standard (see 12.2) as well as a procedure blank, each made to 100-mL volume with water (see 11.1). Add 30 mL of HCl (sp gr 1.19), and cover with watch glasses. Boil, using an adjustable hot plate, at a slow rate, in a fume hood, so that at least 1 h is required to reduce the solutions to no less than 4 to 5 mL-volume (Note A1.1). Additional water may be added, as required, to satisfy the above time limitation.

NOTE A1.1—If the sample goes dry in this step, loss of MBAS will occur and low results will be reported.

A1.7.2 Cool and rinse the watch glasses and beaker walls with water from a wash bottle until the residue in the beakers is diluted to approximately 25 mL. Add 3 drops of phenolphthalein solution (8.7) and make the solutions definitely alkaline by adding

[12] Fisher Scientific No. 2439 has been found satisfactory for this purpose; other sources may be equally suitable.

sodium hydroxide solution (8.9). Boil for 2 min, with the watch glasses in place, and then transfer quantitatively, by water washing to 250-mL separatory funnels. Reserve the beakers for subsequent use in A1.7.4.

A1.7.3 Add additional water, if needed, so that the total volume in each separatory funnel is about 100 mL. Carefully add dilute H_2SO_4 solution (8.11) until the pink phenolphthalein color is barely discharged. Add 10 mL of phosphate buffer solution (A1.5.4).

A1.7.4 Extract each of the solutions, in the separatory funnels (see Note ~~4),~~ 3), serially with four 25-mL portions of the amine-chloroform mixture (A1.5.3) (**Warning**, see ~~Note 2).~~ 8.3). Shake thoroughly, but not vigorously, for 1 min after each addition. On each occasion allow the chloroform layer to separate and then drain to the respective 400-mL reserved beaker from A1.7.2. Leave any emulsion layer in the separatory funnel with the aqueous phase (Note ~~9).~~ 8). Add 5 mL of the sodium hydroxide solution (8.9) to the combined chloroform extracts and evaporate the chloroform on a steam bath in a fume hood. Dilute the alkaline residues to about 50 mL with water, cover with watch glasses, and boil for 15 min on a hot plate in a fume hood. Replace evaporation losses by washing down walls of beakers with water after boiling for 10 min.

A1.7.5 Transfer quantitatively, after cooling, to 250-mL separatory funnels (Note A1.2). Dilute with water to about 100 mL and add 3 drops of phenolphthalein (8.7) solution. Add dilute H_2SO_4 solution (8.11) in small increments, until the pink color is barely discharged. Complete the analysis in accordance with 11.3 to 11.6. Use the processed water blank to set the photometer to zero absorbance.

NOTE A1.2—If the separatory funnels intended for use are the same ones used for the amine extractions, rinse thoroughly with a solution consisting of 1 part HCl (sp gr 1.19) in 10 parts of methanol. Follow with copious water rinses. The same cleaning procedure is effective in removing adsorbed LAS residues and methylene blue stains from glassware.

A1.8 Precision and Bias

A1.8.1 The information supplied is only meant to show what may happen when the pretreatment procedure is employed. It is for informational use only.

A1.8.2 Two operators from two laboratories determined three concentration levels of LAS on ~~3~~ three days in reagent water and in selected water matrices.

A1.8.3 Four laboratories with single operators, agreed to cooperate in the interlaboratory testing of this procedure.

A1.8.4 The overall and single-operator precision of this procedure within its designated range for reagent water and selected water matrices varies with the quantity tested in accordance with the values cited in Table A1.1.

A1.8.5 Recoveries of known amounts of LAS, calculated as MBAS, from reagent water (Type I, II) and selected water matrices (drinking, treated waste) were as shown in Table A1.2.

A1.8.6 These collaborative test data were obtained on reagent grade water, drinking and treated wastewaters. For other matrices, these data may not apply.

TABLE A1.1 Precision with Pretreatment Procedure

Amount Added, mg/L	Overall, S_t	Single Operator, S_o
	Reagent Water	
0.23	±0.026	±0.013
0.78	±0.066	±0.037
1.28	±0.231	±0.039
	Matrix Water	
0.23	±0.021	±0.018
0.78	±0.069	±0.022
1.28	±0.033	±0.037

TABLE A1.2 Recovery with Pretreatment Procedure

Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant, 95 % Confidence Level
	Reagent Water		
0.23	0.208	−9.6	no
0.78	0.745	−4.5	no
1.28	1.235	−3.5	no
	Matrix Water		
0.23	0.175	−23.9	yes
0.78	0.623	−20.1	yes
1.28	1.022	−20.2	yes

A2. QUALITY CONTROL

A2.1 A quality control standard, near the midscale of the calibration chart, is analyzed with each set of samples to ensure that the method is under control (see 12.2).

A2.1.1 If the calibration factor (F_i) of the quality control standard (see A3.1) falls within the 95 % confidence interval (see A3.4) of the calibration factor that was determined when the calibration chart was established (see 11.6), then the results obtained in that set of samples are accepted as valid.

A2.1.2 If the calibration factor (F_i) of the quality control standard falls outside of the 95 % confidence interval of the calibration chart's factor, then the results obtained in that set of samples are suspect and steps must be taken to determine if they are to be rejected and the samples reanalyzed.

A2.1.2.1 Take the necessary action to determine why the calibration factor of the quality control standard did not fall within the previously determined 95 % confidence interval.

A2.1.2.2 Take the necessary corrective action to ensure that the calibration factors of future quality control standards fall within the previously determined 95 % confidence interval.

A3. DETERMINATION OF THE CALIBRATION FACTOR'S 95 % CONFIDENCE INTERVAL

A3.1 Determine the calibration factor of each individual standard solution used to prepare the calibration chart (see 11.6) by means of the following equation:

$$F_i = \frac{W}{A}$$

where:
F_i = calibration factor of a given individual standard solution,
W = LAS in the given standard solution, mg, and
A = absorbance of a given standard solution as determined in 11.6.

A3.2 Determine the estimated standard deviation of the calibration factor from all of the standard solutions measured in the same absorption cell, that was used to prepare the calibration chart in 11.6 by means of the following equation:

$$s = \sqrt{(F_i - \bar{F})^2/n - 1}$$

where:
s = estimated standard deviation of the results from the calibration series of standard solutions,
F_i = each individual calibration factor as determined A3.1,
$\bar{F}$ = average of all the calibration factors in the series, and
n = number of calibration factors in the series.

A3.3 Determine the 95 % confidence limits for the estimated standard deviation by means of the following equation:

$$95\ \%\ CL = \pm\ s(t)$$

where:
95 % CL = 95 % confidence limits for a single determination of a standard solution,
s = definition given in A3.2, and
t = Student's t which for 5 df (n − 1) and 95 % confidence limits = 2.57 (Note A3.1).

NOTE A3.1—The values for Student's t will vary depending upon the number (n) of standard calibration solutions in the series that are used to determine the estimated standard deviation in A3.2. This method, in 16.1 recommends that at least six standard solutions be used in that series and Note

~~4~~ 3 indicates that additional standard solutions may be prepared and analyzed, if desired. If *n* exceeds six, then consult any standard volume on statistics to determine other values of Student's *t* when *n* does differ from six.

A3.4 Determine the lower and upper limits of the 95 % confidence interval of the calibration chart's average absorptivity factor respectively by subtracting and adding the 95 % limit to the average absorptivity factor ($\bar{F}$) which was determined in A3.2.