Designation: D5315 – 04 (Reapproved 2024)

Standard Test Method for Determination of N-Methyl-Carbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post-Column Derivatization[1]

This standard is issued under the fixed designation D5315; 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.

1. Scope

1.1 This is a high-performance liquid chromatographic (HPLC) test method applicable to the determination of certain n-methylcarbamoyloximes and n-methylcarbamates in ground water and finished drinking water (**1**).[2] This test method is applicable to any carbamate analyte that can be hydrolyzed to a primary amine. The following compounds have been validated using this test method:

Analyte	Chemical Abstract Services Registry Number [A]
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Baygon	114-26-1
Carbaryl	63-25-2
Carbofuran	1563-66-2
3-Hydroxycarbofuran	16655-82-6
Methiocarb	2032-65-7
Methomyl	16752-77-5
Oxamyl	23135-22-0

[A] Numbering system of Chemical Abstracts, Inc.

1.2 This test method has been validated in a collaborative round-robin study (**2**) and estimated detection limits (EDLs) have been determined for the analytes listed in 1.1 (Table 1). Observed detection limits may vary between ground waters, depending on the nature of interferences in the sample matrix and the specific instrumentation used.

1.3 This test method is restricted to use by, or under the supervision of, analysts experienced in both the use of liquid chromatography and the interpretation of liquid chromatograms. Each analyst should demonstrate an ability to generate acceptable results with this test method using the procedure described in 12.3.

1.4 When this test method is used to analyze unfamiliar samples for any or all of the analytes listed in 1.1, analyte identifications should be confirmed by at least one additional qualitative technique.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Additional guidance on laboratory safety is available and suitable references for the information are provided (**3-5**).

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D1192 Guide for Equipment for Sampling Water and Steam in Closed Conduits (Withdrawn 2003)[4]

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3370 Practices for Sampling Water from Flowing Process Streams

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

E682 Practice for Liquid Chromatography Terms and Relationships

[1] 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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[2] The boldface numbers in parentheses refer to the references at the end of this test method.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] The last approved version of this historical standard is referenced on www.astm.org.

TABLE 1 Relative Retention Times for the Primary and Confirmation Columns and EDLs for the 10 Carbamate Pesticides

Analyte	Retention Time (minutes)		
	Primary [A]	Confirmation [B]	EDL [C]
Aldicarb	27.0	21.4	1.0
Aldicarb sulfone	15.2	12.2	2.0
Aldicarb sulfoxide	15.0	17.5	2.0
Baygon (Propoxur)	29.6	23.4	1.0
Carbaryl	30.8	25.4	2.0
Carbofuran	29.3	24.4	1.5
3-Hydroxycarbofuran	23.3	19.0	2.0
Methiocarb	34.9	28.6	4.0
Methomyl	18.4	14.8	0.50
Oxamyl	17.4	14.6	2.0

[A] Primary column—250 mm by 4.6 mm inside diameter Altex Ultrasphere ODS, 5 µm.
[B] Confirmation column—250 mm by 4.6 mm inside diameter Supelco LC-1, 5 µm.
[C] Estimated method detection limit in micrograms per litre.

2.2 *U.S. Environmental Protection Agency Standard:*[5]
EPA Method 531.1 Revision 3.0, USEPA, EMSL-Cincinnati, 1989
EPA Method 531.2 Revision 1.0, USEPA, EMSL-Cincinnati, 2001

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of water terms used in this standard, refer to Terminology D1129.

3.1.2 For definitions of other terms used in this standard, refer to Practice E682.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *calibration standard* (CAL), *n*—a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes; CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2.2 *field duplicates* (FD1 and FD2), *n*—two separate samples collected at the same time, placed under identical circumstances, and treated exactly the same throughout field and laboratory procedures; analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.2.3 *field reagent blank* (FRB), *n*—reagent water placed in a sample container in the laboratory and treated in all respects as a sample, including being exposed to sampling site conditions, storage, preservation, and all analytical procedures; the purpose of the FRB is to determine whether method analytes or other interferences are present in the field environment.

3.2.4 *internal standard, n*—a pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other analytes and surrogates that are components of the same solution; the internal standard must be an analyte that is not a sample component.

3.2.5 *laboratory duplicates* (LD1 and LD2), *n*—two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures; analyses of LD1 and LD2 provide a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2.6 *laboratory-fortified blank* (LFB), *n*—an aliquot of reagent water to which known quantities of the test method analytes are added in the laboratory; the LFB is analyzed exactly as a sample is; its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise methods at the required test method detection limit.

3.2.7 *laboratory-fortified sample matrix* (LFM), *n*—an aliquot of an environmental sample to which known quantities of the test method analytes are added in the laboratory; the LFM is analyzed exactly as a sample is; its purpose is to determine whether the sample matrix contributes bias to the analytical results; the background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.2.8 *laboratory performance check solution* (LPC), *n*—a solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.2.9 *laboratory reagent blank* (LRB), *n*—an aliquot of reagent water treated exactly the same as a sample, including being exposed to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples; the LRB is used to determine whether method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2.10 *primary dilution standard solution, n*—a solution of several analytes prepared in the laboratory from stock standard solutions and diluted as necessary to prepare calibration solutions and other necessary analyte solutions.

3.2.11 *quality control sample* (QCS), *n*—a sample matrix containing test method analytes or a solution of test method analytes in a water miscible solvent that is used to fortify water or environmental samples; the QCS is obtained from a source external to the laboratory and is used to check the laboratory performance with externally prepared test materials.

3.2.12 *stock standard solution, n*—a concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound; stock standard solutions are used to prepare primary dilution standards.

3.2.13 *surrogate analyte, n*—a pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction; it is measured with the same procedures used to measure other sample components; the purpose of a surrogate analyte is to monitor the method performance with each sample.

[5] Available from United States Environmental Protection Agency (EPA), William Jefferson Clinton Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

4. Summary of Test Method

4.1 The water sample is filtered, and a 200 µL to 400 µL aliquot is injected onto a reverse phase HPLC column. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed with sodium hydroxide (2.0 g/L NaOH) at 95 °C. The methylamine formed during hydrolysis is reacted with o-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative that is detected by a fluorescence detector (**5**).

4.2 This test method is applicable to any carbamate analyte that can be hydrolyzed to a primary amine, not necessarily methylamine.

5. Significance and Use

5.1 N-methylcarbamates and n-methylcarbomoyloximes are used in agriculture as insecticides and herbicides. They are sometimes found in both surface and ground waters and can be toxic to animals and plants at moderate to high concentrations. The manufacturing precursors and degradation products may be equally as hazardous to the environment.

6. Interferences

6.1 Test method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatuses that lead to discrete artifacts or elevated baselines in liquid chromatograms. Specific sources of contamination have not been identified. All reagents and apparatus must be routinely demonstrated to be free of interferences under the analysis conditions by running laboratory reagent blanks in accordance with 12.2.

6.1.1 Glassware must be cleaned scrupulously. Clean all glassware as soon as possible after use by rinsing thoroughly with the last solvent used in it.

6.1.2 After drying, store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

6.1.3 The use of high-purity reagents and solvents helps to minimize interference problems.

6.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately after a sample containing relatively high concentrations of analytes. A preventive technique is between-sample rinsing of the sample syringe and filter holder with two portions of water. Analyze one or more laboratory method blanks after analysis of a sample containing high concentrations of analytes.

6.3 Matrix interference may be caused by contaminants present in the sample. The extent of matrix interference will vary considerably from source to source, depending upon the water sampled. Positive analyte identifications must be confirmed using the alternative conformational columns, or LC/MS.

6.4 The quality of the reagent water used to prepare standards and samples must conform to Specification D1193, especially in TOC content. High reagent water TOC causes a deterioration of column selectivity, baseline stability, and analyte sensitivity.

6.5 Eliminate all sources of airborne primary amines, especially ammonia, which are absorbed into the mobile phases and effect sensitivity.

7. Apparatus

7.1 *Sampling Equipment:*

7.1.1 *Sample Bottle,* 60 mL screw cap glass vials[6] and caps[7] equipped with a PTFE-faced silicone septa. Prior to use, wash the vials and septa as described in 6.1.1.

7.2 *Filtration Apparatus:*

7.2.1 *Macrofiltration Device,* to filter derivatization solutions and mobile phases used in HPLC. It is recommended that 47 mm, 0.45 µm pore size filters be used.[8]

7.2.2 *Microfiltration Device,* to filter samples prior to HPLC analysis. Use a 13 mm filter holder[9] and 13 mm diameter, 0.2 µm polyester filters.[10]

7.3 *Syringes and Valves:*

7.3.1 *Hypodermic Syringe,* 10 mL, glass, with Luer-Lok[11] tip.

7.3.2 *Syringe Valve,* three-way.[12]

7.3.3 *Syringe Needle,* 7 cm to 10 cm long, 17 gauge, blunt tip.

7.3.4 *Micro Syringes,* various sizes.

7.4 *Miscellaneous:*

7.4.1 *Solution Storage Bottles,* amber glass, 10 mL to 15 mL capacity with TFE-fluorocarbon-lined screw cap.

7.5 *High-Performance Liquid Chromatograph (HPLC):*

7.5.1 *HPLC System,*[13] capable of injecting 200 µL to 1000 µL aliquots and performing ternary linear gradients at a constant flow rate. A data system is recommended for measuring peak areas. Table 2 lists the retention times observed for test method analytes using the columns and analytical conditions described below.

7.5.2 *Column 1 (Primary Column),* 250 mm long by 4.6 mm inside diameter, stainless steel, packed with 5 µm C-18 material.[14] Mobile phase is established at 1.0 mL/min as a

[6] Sample bottle vial, Pierce No. 13075, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent, has been found suitable for use.

[7] Sample bottle cap, Pierce No. 12722, available from Pierce Chemical Co., 3747 N. Meridian Rd., Rockford, IL 61101, or equivalent, has been found suitable for use.

[8] Millipore Type HA, 0.45 µm for water, and Millipore Type FH, 0.5 µm for organics, available from Millipore Corp., 80 Ashby Rd., Bedford, MA 01730, or equivalent, has been found suitable for use.

[9] Millipore stainless steel XX300/200, available from Millipore Corp., 80 Ashby Rd., Bedford, MA 01730, or equivalent, has been found suitable for use.

[10] Nucleopore 180406, available from Costar Corp., 1 Alewife Center, Cambridge, MA 02140, or equivalent, has been found suitable for use.

[11] Luer-Lok connectors are available from most laboratory suppliers.

[12] Hamilton HV3-3, available from Hamilton Co., P.O. Box 10030, Reno, NV 89502, or equivalent, has been found suitable for use.

[13] Consult HPLC manufacturer's operation manuals for specific instructions relating to the equipment.

[14] Beckman Ultrasphere ODS, available from Beckman Instruments, 2500 Harbor Blvd., Fullerton, CA 92634, has been found suitable for use.

TABLE 2 Retention Times for Method Analytes Retention Time[A]

Analyte	Primary [B]	Confirmation [C]	Confirmation [D]
	Minutes		
Aldicarb sulfoxide	6.80	17.5	
Aldicarb sulfone	7.77	12.2	
Oxamyl	8.20	14.6	
Methomyl	8.94	14.8	
3-Hydroxycarbofuran	13.65	19	
Aldicarb	16.35	21.4	
Baygon (Propoxur)	18.86	24.4	
Carbofuran	19.17	23.4	
Carbaryl	20.29	25.4	
Methiocarb	24.74	28.6	
BDMC	25.28		...

[A] Columns and analytical conditions are described in 7.5.2, 7.5.3.
[B] Beckman Ultasphere ODS.
[C] Supelco LC-1.
[D] Waters Carbamate Analysis Column using ternary gradient conditions.

linear gradient from 15:85 methanol: water to 100 % methanol in 32 min. Data presented in this test method were obtained using this column.[15]

7.5.3 *Column 2 (Alternative Column),* 250 mm long by 4.6 mm inside diameter, stainless steel, packed with 5 µm silica beads coated with trimethylsilyl.[16] Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol: water to 100 % methanol in 32 min.

7.5.4 *Column 3 (Alternative Column, used for EPA 531.2 validation),* 150 mm long by 3.9 mm inside diameter, stainless steel, packed with 5 mm C_{18}.[17] Mobile phase is a ternary methanol, acetonitrile, water gradient over 24 min. See Annex A1.

7.5.5 *Post Column Reactor,* capable of mixing reagents into the mobile phase. The reactor should be constructed using PTFE tubing and should be equipped with pumps to deliver 0.1 mL/min to 1.0 mL/min of each reagent; mixing tees; and two 1.0 mL delay coils, with one thermostated at 90 °C.[18,17]

7.5.6 *Fluorescence Detector,* capable of excitation at 230 nm and detection of emission energies greater than 418 nm,[19] or variable wavelength fluorescence detector capable of 340 nm excitation, 465 nm emission with a 18 nm band width, and 16 mL flow cell.[17]

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 reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[20] 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. For trace analysis using organic solvents for liquid-liquid extraction or elution from solid sorbents, solvents specified as distilled-in-glass, nano-grade, or pesticide-grade frequently have lower levels of interfering impurities. In all cases, sufficient reagent blanks must be processed with the samples to ensure that all of the compounds of interest are not present as blanks due to reagents or glassware.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification D1193. It must be shown that this water does not contain contaminants at concentrations sufficient to interfere with the analysis. The reagent water used to generate the validation data in this test method was distilled water.[21]

8.3 *Buffer Solutions:*

8.3.1 *Monochloroacetic Acid (pH 3) ($ClCH_3CO_2H$) Buffer Solution*—Prepare by mixing 156 mL of monochloroacetic acid ($ClCH_3CO_2H$) solution (236.2 g/L) and 100 mL of potassium acetate (KCH_3CO_2) solution (245.4 g/L).

8.3.2 *Buffered Water,* to prepare 1 L, mix 10 mL of monochloroacetic acid buffer (pH 3) and 990 mL of water.

8.4 *Helium,* for degassing solutions and solvents.

8.5 *HPLC Mobile Phase:*

8.5.1 *Water,* HPLC grade,[22] or equivalent Type I Reagent Water.

[15] Newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.
[16] Supelco LC-1, available from Supelco, Inc., Supelco Park, Bellefonte, PA 16823, has been found suitable for use.
[17] Waters Carbamate Analysis Column, available from Waters Corp., Milford, MA, 01757, has been found suitable for use.
[18] ABI URS 051 and URA 100, available from ABI Analytical, Inc., 170 Williams Drive, Ramsey, NJ 07446, or equivalent, has been found suitable for use.
[19] A Schoffel Model 970 fluorescence detector was used to generate the validation data presented in this test method. Now available from Kratos Division of ABI Analytical, Inc., 170 Williams Drive, Ramsey, NJ 07446.
[20] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.
[21] Available from the Magnetic Springs Water Co., 1801 Lone Eagle St., Columbus, OH 43228.
[22] Available from Burdick and Jackson. Distributed by Scientific Products, 1430 Waukegan Road, McGraw Park, IL 60085-6787.

TABLE 3 Instrument Quality Control Standard

Test	Analyte	Concentration, µg/L	Requirements
Sensitivity	3-Hydroxycarbofuran	2	Detection of analyte; *S/N* > 3
Chromatographic performance	aldicarb sulfoxide	100	0.90 < PGF [A] < 1.1

[A] PGF = peak Gaussian factor

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where:

$W(1/2)$ = peak width at half height, and
$W(1/10)$ = peak width at tenth height.

8.5.2 *Methanol,* HPLC grade. Filter and degas before use.

8.5.3 *Acetonitrile,* HPLC grade. Filter and degass before use.

8.6 *Internal Standard Solution*—Prepare an internal standard solution by weighing approximately 0.0010 g of pure BDMC (4-Bromo-3,5-Dimethylphenyl N-Methylcarbamate, 98 % purity)[23] to two significant figures. Dissolve the BDMC in methanol and dilute to volume in a 10 mL volumetric flask. Transfer the internal standard solution to a TFE-fluorocarbon-sealed screw-cap bottle and store it at room temperature. The addition of 5 µL of the internal standard solution to 50 mL of sample results in a final internal standard concentration of 10 µg/L. Replace the solution when ongoing quality control indicates a problem.

NOTE 1—BDMC has been shown to be an effective internal standard for the method analytes (**1**), but other compounds may be used if the quality control requirements in Section 11 are met.

8.7 *Laboratory Performance Check Solution*—Prepare the concentrate by adding 20 µL of the 3-hydroxycarbofuran stock standard solution (8.11), 1.0 mL of the aldicarb sulfoxide stock standard solution (8.11), and 1 mL of the internal standard fortification solution (8.7) to a 10 mL volumetric flask (Table 3). Dilute to volume with methanol. Mix concentrate thoroughly. Prepare a check solution by placing 100 µL of the concentrate solution into a 100 mL volumetric flask. Dilute to volume with buffered water. Transfer to a TFE-fluorocarbon-sealed screw-cap bottle and store it at room temperature. The solution should be replaced when ongoing quality control indicates a problem.

8.8 *Methanol,* distilled-in-glass quality or equivalent.

8.9 *Post Column Derivatization Solutions:*

8.9.1 *Sodium Hydroxide* (2 g/L)—Dissolve 2.0 g of sodium hydroxide (NaOH) in water. Dilute to 1.0 L with water. Filter and degas just before use.

8.9.2 *2-Mercaptoethanol* (1 + 1)—Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap and store in hood. (**Warning—**Work in a hood due to reagent volatility and odor.)

8.9.3 *Sodium Borate Solution* (19.1 g/L)—Dissolve 19.1 g of sodium borate ($Na_2B_4O_7 \times 10H_2O$) in water. Dilute to 1.0 L with water. The sodium borate will dissolve completely at room temperature if prepared one day before use.

8.9.4 *OPA Reaction Solution*—Dissolve 100 mg ± 10 mg of o-phthalaldehyde (melting point range from 55 °C to 58 °C) in 10 mL of methanol. Add to 1.0 L of sodium borate solution (19.1 g/L). Mix, filter, and degas with helium. Add 100 µL of 2-mercaptoethanol (1 + 1) and mix. Make up fresh solutions daily.

8.10 Sodium thiosulfate ($Na_2S_2O_3$).

8.11 *Stock Solutions, Standard* (1.00 µg/µL)—Stock standard solutions may either be purchased as certified solutions or prepared from pure standard materials by using the following procedure:

8.11.1 Prepare stock standard solutions by weighing approximately 0.0100 g of pure material. Dissolve the material in methanol and dilute to volume in a 10 mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If the compound purity is certified at 96 % or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by either the manufacturer or an independent source.

8.11.2 Transfer the stock standard solution into TFE-fluorocarbon-sealed screw-cap vials. Store it at room temperature and protect it from light.

8.11.3 Stock standard solutions should be replaced after two months, or sooner, if comparison with laboratory-fortified blanks, or quality-control samples indicate a problem.

9. Sample Collection and Handling

9.1 Collect the samples in accordance with Guide D1192, Practices D3370, or Practices D3694.

9.2 Additionally, grab samples must be collected in glass containers. Follow conventional sampling practices (**6**); however, the bottle must not be prerinsed with sample before collection.

10. Preservation of Samples

10.1 *Sample Preservation/pH Adjustment*—Oxamyl, 3-hydroxycarbofuran, aldicarb sulfoxide, and carbaryl can all degrade rapidly in neutral and basic waters held at room temperature (**7, 8**). This short-term degradation is of concern during the periods of time that samples are being shipped and that processed samples are held at room temperature in autosampler trays. Samples targeted for the analysis of these three analytes must be preserved at a pH of 3, as shown as follows. The pH adjustment also minimizes analyte biodegradation.

10.1.1 Add 1.8 mL of monochloroacetic acid buffer solution (pH 3) to the 60 mL sample bottle. Add buffer to the sample bottle either at the sampling site or in the laboratory before shipping to the sampling site.

[23] Available from Aldrich Chemical Co., Inc., 1001 West Saint Paul Ave., Milwaukee, WI 53233.

10.1.2 If residual chlorine is present, add 80 mg of sodium thiosulfate per litre of sample to the sample bottle prior to collecting the sample.

10.1.3 After the sample is collected in a bottle containing buffer, seal the sample bottle and shake it vigorously for 1 min.

10.1.4 Samples must be iced or refrigerated at 4 °C from the time of collection until storage; they must be stored at −10 °C until analyzed. Preservation study results indicate that test method analytes are stable in water samples for at least 28 days when adjusted to pH 3 and stored at −10 °C. However, analyte stability may be affected by the matrix; the analyst should therefore verify that the preservation technique is applicable to the samples under study.

11. Calibration

11.1 Establish HPLC operating parameters equivalent to those indicated in 7.5. Calibrate the HPLC system using either the internal (11.2) or the external (11.3) standard technique.

11.2 *Internal Standard Calibration Procedure*—The analyst must select one or more internal standards similar in analytical behavior to the analytes of interest. In addition, the analyst must demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. BDMC has been identified as a suitable internal standard.

11.2.1 Prepare calibration standards at a minimum of three (recommended, five) concentration levels for each analyte of interest by adding volumes of one or more of the stock standards to a volumetric flask. Add a known constant amount of one or more internal standards to each calibration standard, and dilute to volume with buffered water. The lowest standard should represent analyte concentration near, but above, their respective estimated detection limit (EDL) (Table 1). The remaining standards should bracket the analyte concentrations expected in the sample extracts, or they should define the working range of the detector.

11.2.2 Analyze each calibration standard in accordance with the procedure in 13.2. Tabulate the peak height or area responses against the concentration for each compound and internal standard.

11.2.3 Calculate response factors (RF) for each analyte, surrogate, and internal standard using Eq 1 as follows:

$$RF = \frac{(A_s)\,(C_{is})}{(A_{is})\,(C_s)} \quad (1)$$

where:

A_s = response for the analyte to be measured,
A_{is} = response for the internal standard,
C_{is} = concentration of the internal standard, µg/L, and
C_s = concentration of the analyte to be measured, µg/L.

11.2.4 If the RF value over the working range is constant (20 % RSD or less) use the average response factor for calculations. Alternatively, use the results to plot a calibration curve of response ratios (A_s/A_{is}) versus C_s.

11.2.5 Verify the working calibration curve or RF on each working shift by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ±20 %, repeat the test using a fresh calibration standard. If the repetition also fails, generate a new calibration curve for that analyte using freshly prepared standards.

11.2.6 Single-point calibration is a viable alternative to a calibration curve. Prepare single-point standards from the secondary dilution standards. Prepare the single-point standards at a concentration deviating from the sample extract response by no more than 20 %.

11.2.7 Verify calibration standards periodically (recommended at least quarterly) by analyzing a standard prepared from reference material obtained from an independent source. The results from these analyses must be within the limits used to check calibration routinely.

11.3 *External Standard Calibration Procedure:*

11.3.1 Prepare calibration standards at a minimum of three (recommended five) concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. Dilute to volume with buffered water. The lowest standard should represent analyte concentrations near, but above, the respective EDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or they should define the working range of the detector.

11.3.2 Beginning with the standard of lowest concentration, analyze each calibration standard in accordance with 13.2, and tabulate the response (peak height or area) versus the concentration in the standard. Use the results to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range <20 % relative standard deviation, assume linearity through the origin and use the average ratio or calibration factor in place of a calibration curve.

11.3.3 Verify the working calibration curve or calibration factor on each working day by measuring a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels in order to verify the concentration curve. For extended analysis periods (longer than 8 h), it is strongly recommended that check standards be interspersed with the samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than ±20 %, repeat the test using a fresh calibration standard. If the results still do not agree, generate a new calibration curve or use a single-point calibration standard in accordance with 11.3.4.

11.3.4 Single-point calibration is a viable alternative to a calibration curve. Prepare single-point standards from the secondary dilution standards. Prepare the single-point standards at a concentration deviating from the sample extract response by no more than 20 %.

11.3.5 Verify the calibration standards periodically, (recommended, at least quarterly), by analyzing a standard prepared from reference material obtained from an independent source. The results from these analyses must be within the limits used to check calibration routinely.