Designation: D7599 – 09

Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D7599; 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 procedure covers the determination of diethanolamine, triethanolamine, *N*-methyldiethanolamine and *N*-ethyldiethanolamine (referred to collectively as ethanolamines in this test method) in surface water by direct injection using liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS). These analytes are qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 This test method has been developed in support of the National Homeland Security Research Center, US EPA by Region 5 Chicago Regional Laboratory.

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

1.4 The Detection Verification Level (DVL) and Reporting Range for the ethanolamines are listed in Table 1.

1.4.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Fig. 1 displays the signal/noise ratios at the DVLs and at higher concentrations for *N*-methyldiethanolamine.

1.4.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 2 for diethanolamine, triethanolamine, and *N*-ethyldiethanolamine and Level 2 for *N*-methyldiethanolamine. The reporting limit for *N*-methyldiethanolamine is set at 50 µg/L due to poor sensitivity at a 5 µg/L concentration which did not meet the DVL criteria. The DVL for *N*-methyldiethanolamine is at 10 µg/L, which forces a raised reporting limit (chromatograms are shown in Fig. 1). However, the multi-laboratory validation required a spike of all target analytes at 25 µg/L. The mean recovery for *N*-methyldiethanolamine at this level was 88 % as shown in Table 3. If your instrument's sensitivity can meet the requirements in this test method, *N*-methyldiethanolamine may have a 25 µg/L reporting limit.

1.5 *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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water
D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method in a Single Laboratory Using a Control Sample Program

2.2 *Other Documents:*

EPA publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *detection verification level (DVL), n*—a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the reporting limit (RL).

3.1.2 *reporting limit (RL), n*—the concentration of the lowest-level calibration standard used for quantification.

[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] 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.

[3] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm.

TABLE 1 Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
Diethanolamine	5	25-500
Triethanolamine	5	25-500
N-Ethyldiethanolamine	5	25-500
N-Methyldiethanolamine	10	50-500

3.1.3 *ethanolamines*, *n*—in this test method, diethanolamine, triethanolamine, *N*-methyldiethanolamine and *N*-ethyldiethanolamine collectively.

3.2 *Abbreviations:*

3.2.1 *ND*—non-detect

4. Summary of Test Methods

4.1 This is a performance-based method and modifications are allowed to improve performance.

4.2 For ethanolamines analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 7 days of collection. In the lab, the samples are spiked with surrogate, filtered using a syringe-driven Millex HV PVDF filter unit and analyzed directly by LC/MS/MS.

4.3 Diethanolamine, triethanolamine, *N*-methyldiethanolamine and *N*-ethyldiethanolamine and diethanolamine-D_8 (surrogate) are identified by retention time and one SRM transition. The target analytes and surrogate are quantitated using the SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of diethanolamine, triethanolamine, *N*-methyldiethanolamine and *N*-ethyldiethanolamine and the diethanolamine-D_8 surrogate recovery.

5. Significance and Use

5.1 *N*-Ethyldiethanolamine, *N*-methyldiethanolamine and triethanolamine are Schedule 3 compounds under the Chemical Weapons Convention (CWC). Schedule 3 chemicals include those that have been produced, stockpiled or used as a chemical weapon, poses otherwise a risk to the object and purpose of the CWC because they possess such lethal or incapacitating toxicity as well as other properties that might enable it to be used as a chemical weapon, poses otherwise a risk to the object and purpose of the CWC by virtue of it's importance in the production of one or more chemicals listed in Schedules 1 or 2, or it may be produced in large commercial quantities for purposes not prohibited under the CWC.[4] Ethanolamines have a broad spectrum of applications. They are used to produce adhesives, agricultural products, cement grinding aids, concrete additives, detergents, specialty cleaners, personal care products, gas treatments, metalwork, oil well chemicals, packaging and printing inks, photographic chemicals, rubber, textile finishing, urethane coatings, textile lubricants, polishes, pesticides, and pharmaceuticals. Ethanolamines are readily dissolved in water, biodegradable and the bio-concentration potential is low.[5]

5.2 This method has been investigated for use with reagent and surface water.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with a detergent, rinsed in hot water followed by distilled water. Detergents containing ethanolamines must not be used to clean glassware. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with acetone, then methanol.

6.3 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

7. Apparatus

7.1 *LC/MS/MS System*

7.1.1 *Liquid Chromatography (LC) System*—A complete LC system is needed in order to analyze samples.[6] A system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes and requirements of the standard may be used.

7.1.2 *Analytical Column-Waters*—Atlantis HILIC Silica, 100 mm × 2.1 mm, 3 µm particle size, or equivalent.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[7] A system that is capable of performing at the requirements in this standard may be used.

7.2 *Filtration Device*

7.2.1 *Hypodermic syringe*—A lock tip glass syringe capable of holding a Millex HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) or similar may be used.

7.2.1.1 A 25-mL lock tip glass syringe size is recommended since a 25-mL sample size is used in this test method.

7.2.2 *Filter*—Millex HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) or similar may be used.

8. Reagents and Materials

8.1 *Purity of Reagents*—High-performance liquid chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall

[4] Additional information about CWC and ethanolamines are available on the Internet at http://www.opcw.org (2009).

[5] Additional information can be found on the Dow Chemical Company website at http://www.dow.com/amines/prod/index.htm (2009).

[6] A Waters Alliance High Performance Liquid Chromatography (HPLC) System was used to develop this test method. The multi-laboratory study included Agilent and Waters LC systems.

[7] A Waters Quattro micro API mass spectrometer was used to develop this test method. The multi-laboratory study included Applied Biosystems, Varian and Waters mass spectrometers.

FIG. 1 Example SRM Chromatograms Signal/Noise Ratios

TABLE 2 Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7
Diethanolamine	25	50	75	150	250	350	500
Triethanolamine	25	50	75	150	250	350	500
N-Ethyldiethanolamine	25	50	75	150	250	350	500
N-Methyldiethanolamine	25	50	75	150	250	350	500
Diethanolamine-D_8 (Surrogate)	25	50	75	150	250	350	500

conform to the Committee on Analytical Reagents of the American Chemical Society.[8] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Acetone (CAS # 67-64-1).

8.7 Ammonium acetate (CAS # 631-61-8).

8.8 Diethanolamine (CAS # 111-42-2).

8.9 Triethanolamine (CAS # 102-71-6).

8.10 *N*-Ethyldiethanolamine (CAS # 139-87-7).

8.11 *N*-Methyldiethanolamine (CAS # 105-59-9).

8.12 Bis(2-hydroxyethyl)-D_8-amine; (Diethanolamine-D_8), where the ethylene moieties contain all ^2H (CAS # 103691-51-6).

8.12.1 Diethanolamine-D_8 is used as a surrogate in this standard.

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling*—Grab samples must be collected in ≥25-mL pre-cleaned amber glass bottles with Teflon-lined caps demonstrated to be free of interferences. This test method requires a 25-mL sample size per analysis. Conventional sampling practices should be followed. Refer to Guide D3856 and Practices D3694.

10.2 *Preservation*—Store samples between 0°C and 6°C from the time of collection until analysis. Analyze the sample within 7 days of collection.

[8] *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 *Annual 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.

TABLE 3 Multi-Laboratory Recovery Data in Reagent Water

Analyte	Spike Conc. (ppb)	# Results	# Labs	Bias			Precision			
				Mean Recovery (%)	Min Recovery (%)	Max Recovery (%)	Overall SD (%)	Pooled within-lab SD (%)	Overall RSD (%)	Pooled within-lab RSD (%)
Diethanolamine	25	24	6	96.34	51.00	156.96	31.31	10.96	32.50	9.49
Diethanolamine	50	24	6	101.41	54.00	154.80	29.54	7.97	29.13	7.91
Diethanolamine	200	24	6	101.57	61.00	138.00	20.98	10.50	20.66	10.85
Diethanolamine	425	24	6	102.06	70.00	138.82	17.98	5.90	17.61	5.70
Triethanolamine	25	24	6	87.70	35.96	157.20	27.00	25.18	30.79	27.48
Triethanolamine	50	24	6	94.95	67.00	121.66	16.39	9.57	17.26	9.66
Triethanolamine	200	22	6	105.00	79.50	132.00	14.06	11.81	13.39	11.52
Triethanolamine	425	24	6	96.94	40.00	144.94	27.56	4.41	28.43	5.76
N-Ethyldiethanolamine	25	24	6	90.61	31.00	132.00	39.42	7.47	43.51	10.42
N-Ethyldiethanolamine	50	23	6	111.88	49.00	146.00	28.71	7.19	25.66	7.56
N-Ethyldiethanolamine	200	24	6	106.20	60.00	134.00	23.09	11.96	21.74	12.23
N-Ethyldiethanolamine	425	24	6	99.67	51.00	130.00	23.07	4.68	23.15	6.01
N-Methyldiethanolamine	25	24	6	88.43	41.72	133.60	25.24	13.29	28.55	16.70
N-Methyldiethanolamine	50	24	6	102.28	56.00	153.80	25.85	8.73	25.27	8.22
N-Methyldiethanolamine	200	24	6	101.02	59.00	136.50	20.07	9.51	19.87	9.54
N-Methyldiethanolamine	425	24	6	94.75	63.00	115.76	15.02	3.34	15.85	3.72
Diethanolamine-D_8 (Surrogate)	200	96	6	103.02	60.00	151.95	21.13	9.40	20.51	9.25

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions*[6]:

11.1.1 Injection volumes of all calibration standards and samples are 25 µL. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in Table 4.

11.1.2 *Temperatures*—Column, 30°C; Sample compartment, 15°C.

11.1.3 *Seal Wash*—Solvent: 50 % Acetonitrile/50 % Water; Time: 5 minutes.

11.1.4 *Needle Wash*—Solvent: 50 % Acetonitrile/50 % Water; Normal wash, approximately 13 second wash time.

11.1.5 *Autosampler Purge*—Three loop volumes.

11.1.6 Specific instrument manufacturer wash/purge specifications should be followed in order to eliminate sample carry-over in the analysis of ethanolamines.

11.2 *Mass Spectrometer Parameters*[7]:

11.2.1 In order to acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This standard contains only one surrogate and four target compounds which are located in the same multiple reaction monitoring (MRM) experiment window. Variable parameters regarding retention times, SRM Transitions and cone and collision energies are shown in Table 5.

The instrument is set in the Electrospray (+) positive setting.
Capillary Voltage: 0.5 kV
Cone: Variable depending on analyte (Table 5)
Extractor: 2 Volts
RF Lens: 0.2 Volts
Source Temperature: 120°C
Desolvation Temperature: 300°C
Desolvation Gas Flow: 500 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.5
High Mass Resolution 1: 14.5
Ion Energy 1: 0.5
Entrance Energy: –1
Collision Energy: Variable depending on analyte (Table 5)
Exit Energy: 2
Low Mass Resolution 2: 15
High Mass resolution 2: 15
Ion Energy 2: 0.5
Multiplier: 650
Gas Cell Pirani Gauge: 3.3×10^{-3} Torr
Inter-Channel Delay: 0.02 seconds
Inter-Scan Delay: 0.1 seconds
Repeats: 1
Span: 0 Daltons
Dwell: 0.1 Seconds

TABLE 4 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent CH_3CN	Percent Water	Percent 200 mmolar Ammonium Acetate
0	400	95	0	5
1	400	95	0	5
2	400	90	0	10
4	300	90	0	10
10	300	60	30	10
13	300	60	30	10
15	300	40	50	10
18	300	30	60	10
20	300	30	60	10
25	300	95	0	5
27	300	95	0	5

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order that analytical values obtained using this test method are valid and accurate within the confidence limits of the test method, the following procedures must be followed when performing the test method.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze seven calibration standards containing the seven concentration levels of the ethanolamines and diethanolamine-D_8 surrogate prior to analysis as shown in Table 2. A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock standard solution A (Level 7) containing diethanolamine, triethanolamine, *N*-methyldiethanolamine and *N*-ethyldiethanolamine and diethanolamine-D_8 is prepared at Level 7 concentration and aliquots of that solution are diluted