Designation: D7644 – 16

Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D7644; 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 bromadiolone, brodifacoum, diphacinone and warfarin (referred to collectively as rodenticides in this test method) in 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 test method. This test method adheres to multiple reaction monitoring (MRM) mass spectrometry.

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

1.2.1 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 of the primary single reaction monitoring (SRM) transitions, and Fig. 2 displays the confirmatory SRM transitions at the DVLs for the rodenticides.

1.2.2 The reporting limit was calculated from the concentration of the Level 1 calibration standard, as shown in Table 4, accounting for the dilution of a 40 mL water sample up to a final volume of 50 mL with methanol to ensure analyte solubility.

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

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.*

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

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

D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water

D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic 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 Using Control Chart Techniques

2.2 *Other Documents:*[3]

U.S. EPA publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods

3. Terminology

3.1 *Definitions:*

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

3.2 *Definitions of Terms Specific to This Standard:*

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

3.2.2 *independent reference material, IRM, n*—a material of known purity and concentration obtained either from the

[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.

Current edition approved Feb. 1, 2016. Published May 2016. Originally approved in 2010. Last previous edition approved in 2010 as D7644 – 10ε2. DOI: 10.1520/D7644-16.

[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 (ng/L)	Reporting Range (ng/L)
Bromadiolone	20	125-2500
Brodifacoum	20	125-2500
Diphacinone	20	125-2500
Warfarin	20	125-2500

National Institute of Standards and Technology (NIST) or other reputable supplier. The IRM shall be obtained from a different lot of material than is used for calibration.

3.2.3 *reporting limit, RL, n*—the concentration of the lowest-level calibration standard used for quantification accounting for the sample dilution.

3.2.3.1 *Discussion*—In this test method, a 40 mL sample aliquot is diluted to a 50 mL final volume after thoroughly rinsing the collection vial with methanol for quantitative transfer. In this case, the lowest calibration level of 100 ppt would allow a reporting limit of 125 ppt to be achieved.

3.2.4 *rodenticides, n*—in this test method, bromadiolone, brodifacoum, diphacinone, and warfarin collectively.

3.3 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *IC, n*—Initial Calibration

3.3.3 *LC, n*—Liquid Chromatography

3.3.4 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.5 *MeOH, n*—Methanol

3.3.6 *mM, n*—millimolar, 1×10^{-3} moles/L

3.3.7 *MRM, n*—Multiple Reaction Monitoring

3.3.8 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.9 *NA, adj*—Not Available

3.3.10 *ND, n*—non-detect

3.3.11 *P&A, n*—Precision and Accuracy

3.3.12 *PPB, n*—parts per billion

3.3.13 *PPT, n*—parts per trillion

3.3.14 *QA, adj*—Quality Assurance

3.3.15 *QC, adj*—Quality Control

3.3.16 *RL, n*—Reporting Limit

3.3.17 *RSD, n*—Relative Standard Deviation

3.3.18 *RT, n*—Retention Time

3.3.19 *SDS, n*—Safety Data Sheets

3.3.20 *SRM, n*—Single Reaction Monitoring

3.3.21 *SS, n*—Surrogate Standard

3.3.22 *TC, n*—Target Compound

3.3.23 *μM, n*—micromolar, 1×10^{-6} moles/L

3.3.24 *VOA, n*—Volatile Organic Analysis

4. Summary of Test Method

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

4.2 For rodenticide analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 14 days of collection. In the lab, the samples are spiked with surrogates, quantitatively transferred to a graduated cylinder using three methanol rinses, filtered using a syringe driven filter unit, and analyzed directly by LC/MS/MS.

4.3 Bromadiolone, brodifacoum, diphacinone, warfarin, warfarin-D_5 (surrogate) and 2-bromo-4-(1,1,3,3-tetramethylbutyl)phenol (brominated octylphenol, Br-OP, surrogate) are identified by retention time and two SRM transitions. The target analytes and surrogates are quantitated using the primary SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of bromadiolone, brodifacoum, diphacinone, warfarin, and surrogate recoveries.

5. Significance and Use

5.1 This test method has been developed by U.S. EPA Region 5 Chicago Regional Laboratory (CRL).

5.2 Bromadiolone, brodifacoum, diphacinone and warfarin are rodenticides for controlling mice, rats, and other rodents that pose a threat to public health, critical habitats, native plants and animals, crops, food and water supplies. These rodenticides also present human and environmental safety concerns. Warfarin and diphacinone are first-generation anticoagulants, while bromadiolone and brodifacoum are second-generation. The anticoagulants interfere with blood clotting, and death can result from excessive bleeding. The second-generation anticoagulants are especially hazardous for several reasons. They are highly toxic and persist a long time in body tissues. The second-generation anticoagulants are designed to be toxic in a single feeding, but time-to-death occurs in several days. This allows rodents to feed multiple times before death, leading to carcasses containing residues that may be many times the lethal dose.[4]

5.3 This test method has been investigated for use with reagent, surface, and drinking water for the selected rodenticides.

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 detergent and rinsed in hot water followed by distilled water. 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 followed by methanol.

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

[4] Additional information about rodenticides is available from United States Environmental Protection Agency (EPA), http://www.epa.gov.

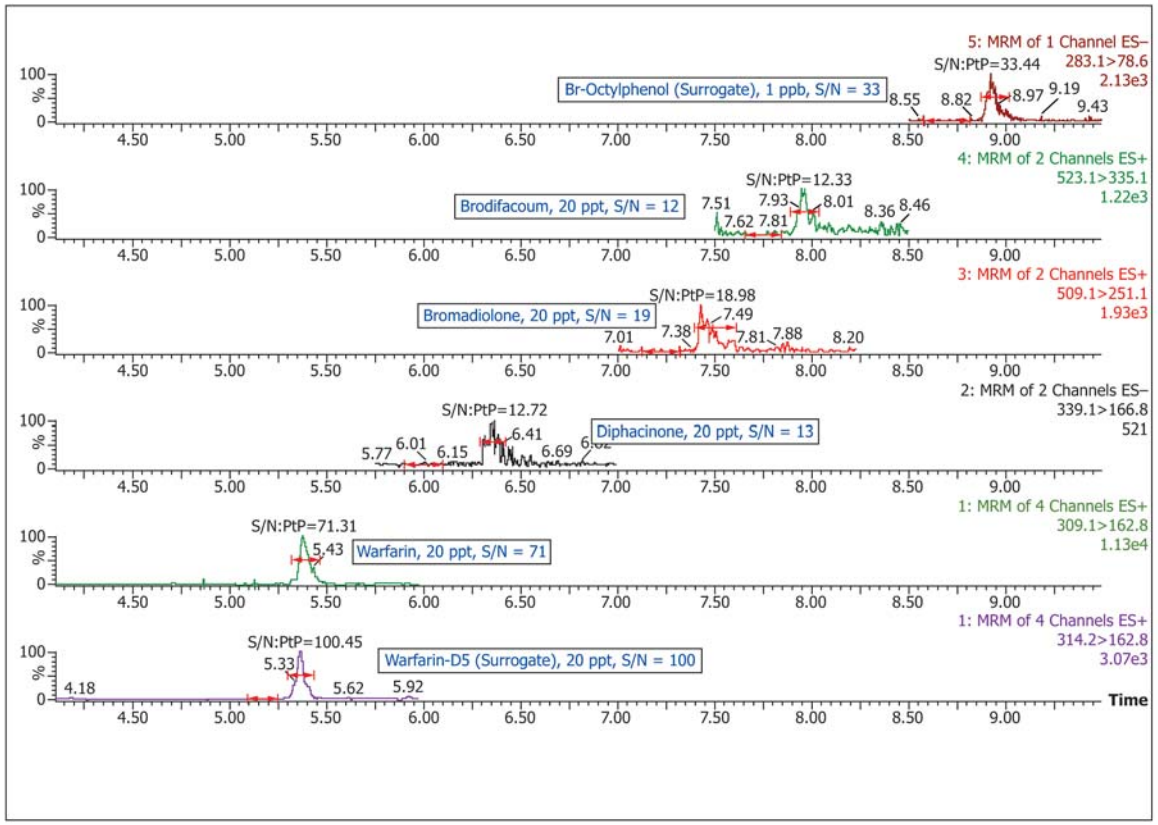

FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

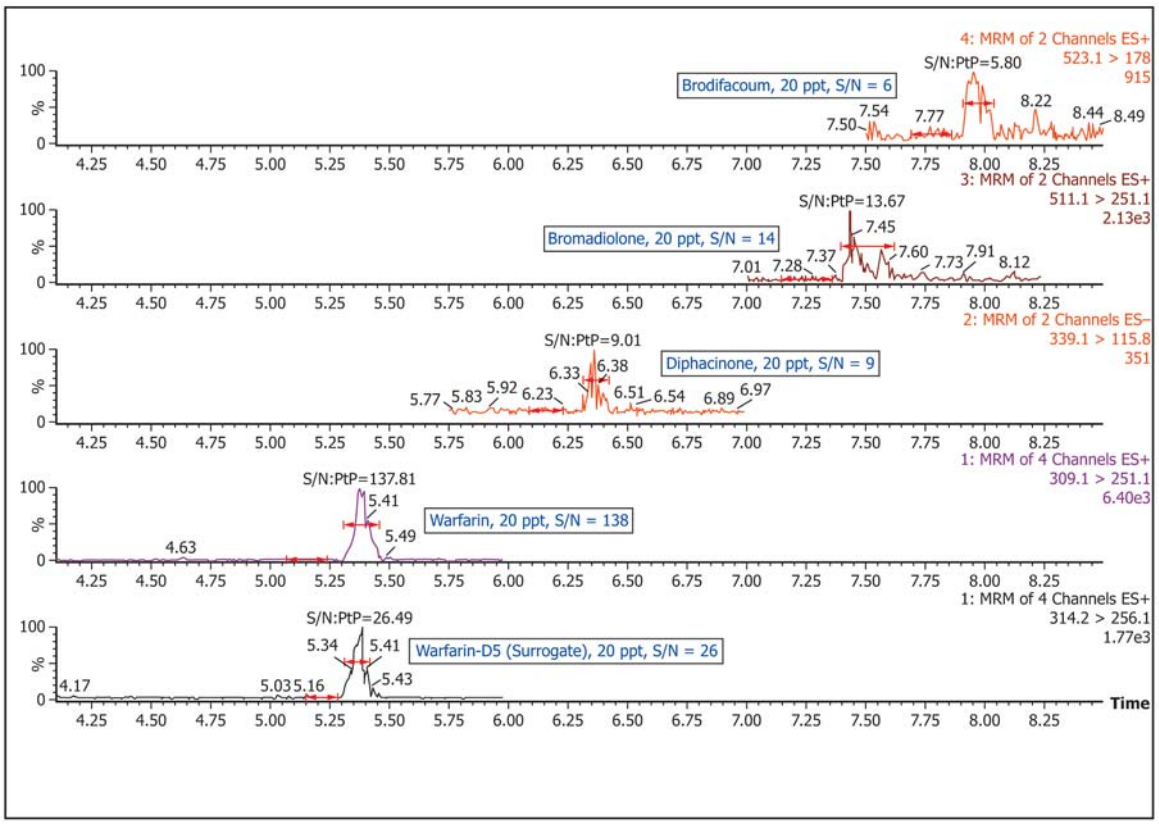

FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios