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.

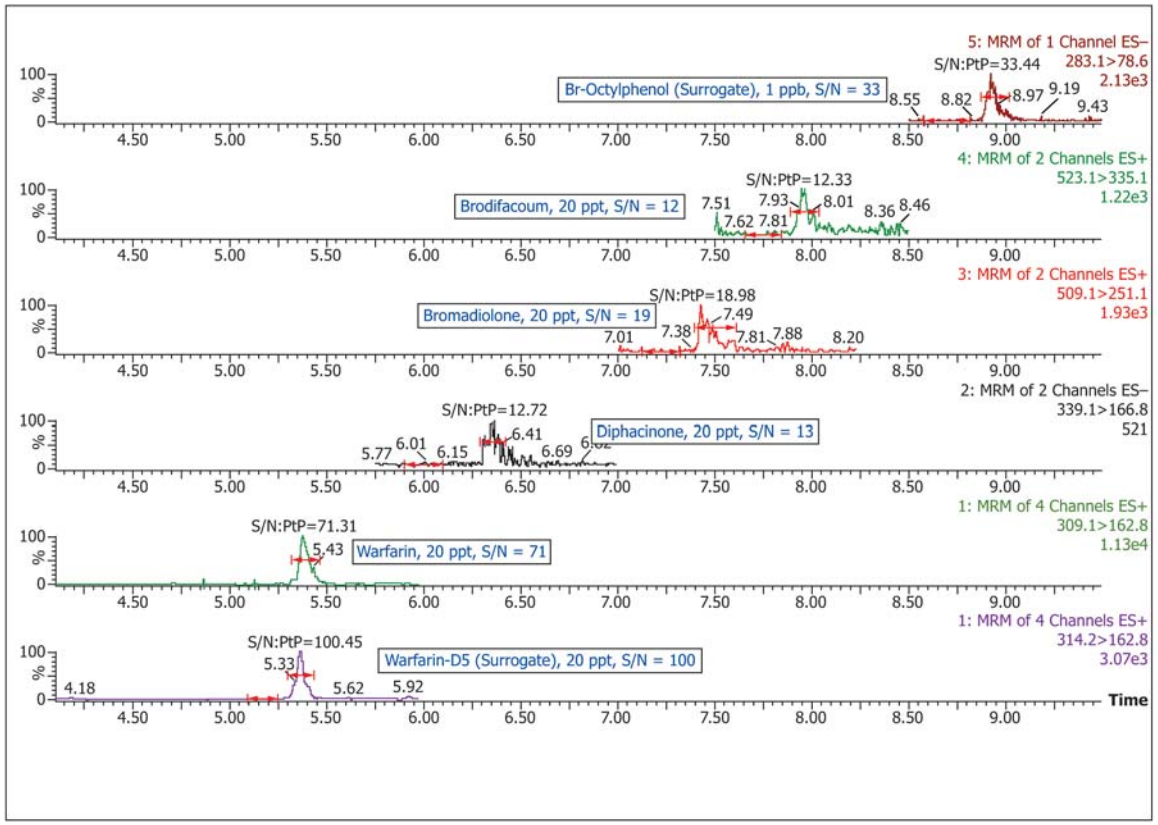

FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios

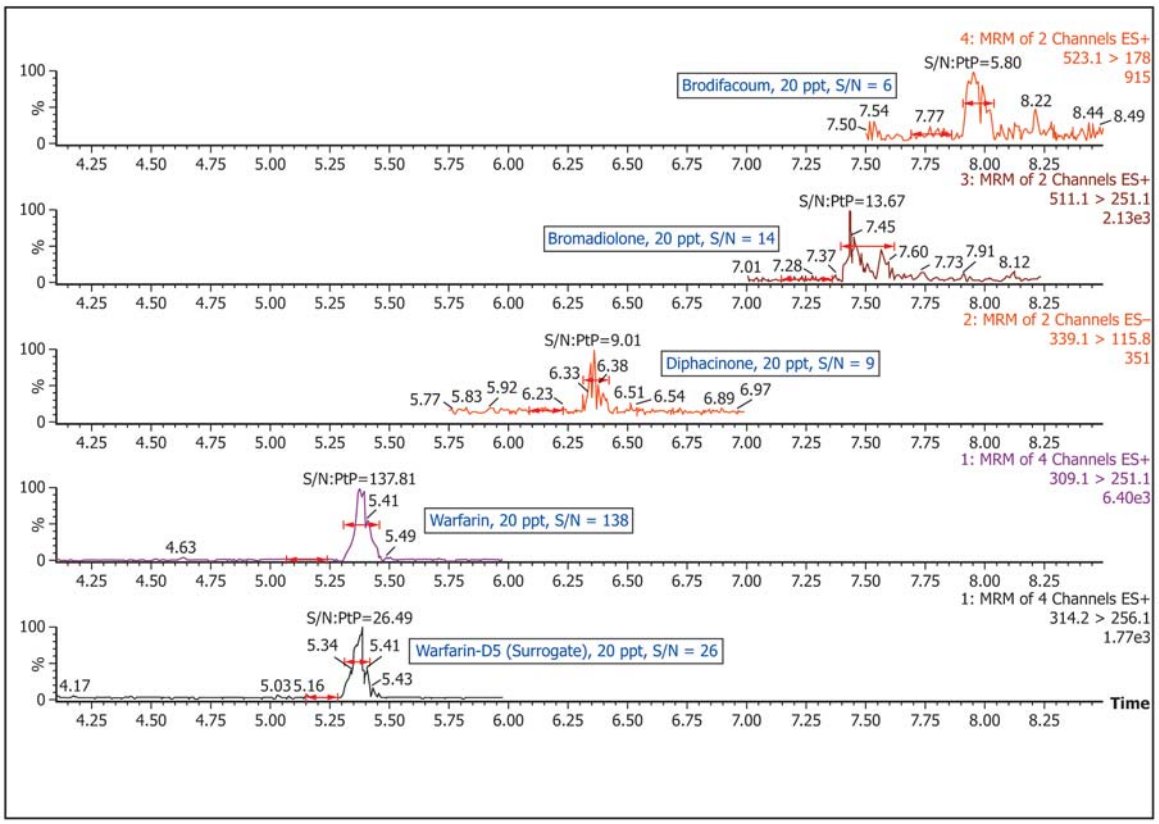

FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios

6.4 Matrix interferences may be caused by contaminants in 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 to analyze samples.[5] This should include a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. 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*[6]—A C18 column was used to develop this test method.

NOTE 1—Any column that can achieve baseline resolution of these analytes may be used. Baseline resolution simplifies data analysis and can reduce the chance of ion suppression, leading to higher limits of detection.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[7] Any 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 syringe-driven filter unit or similar may be used.

7.2.1.1 A 50-mL lock tip glass syringe size is recommended since a 50-mL sample size is used in this test method.

7.2.2 *Filter Unit*[8]—PVDF filter units were used to filter the samples.

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 conform to the Committee on Analytical Reagents of the American Chemical Society.[9] 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 Methanol (CAS # 67-56-1).

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

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

8.7 Ammonium Hydroxide (Concentrated, CAS # 1336-21-6).

8.8 Ascorbic Acid (CAS # 50-81-7).

8.9 Bromadiolone (CAS # 28772-56-7).

8.10 Brodifacoum (CAS # 56073-10-0).

8.11 Diphacinone (CAS # 82-66-6).

8.12 Warfarin (CAS # 81-81-2).

8.13 Warfarin-D_5 (Phenyl-D_5, CAS # (unlabeled) 81-81-2).[10]

8.13.1 *Discussion*—Warfarin-D_5 is used as the electrospray positive analyte surrogate in this standard.

8.14 2-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol (Br-OP).[11]

8.14.1 *Discussion*—Br-OP is used as the electrospray negative analyte 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 Safety Data Sheets (SDS) for all reagents used in this test method.

10. Sampling

10.1 *Sampling*—Grab samples must be collected in 40 mL pre-cleaned amber glass vials with Teflon[12]-lined caps demonstrated to be free of interferences. Surface water samples are collected unpreserved, shipped between 0°C and 6°C, and stored in the laboratory between 0°C and 6°C. Chlorinated drinking water samples are dechlorinated with ascorbic acid; 10 mg of ascorbic acid is added to each 40 mL vial prior to water collection. This test method requires a 40 mL sample size

[5] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC) System (a trademark of the Waters Corporation, Milford, MA), or equivalent, was found suitable for use. All parameters in this test method are based on this system and may vary depending on your instrument.

[6] Waters ACQUITY UPLC (a trademark of the Waters Corporation, Milford, MA) BEH C18, 2.1 × 100 mm, 1.7 µm particle size was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

[7] A Waters Quattro Premier (a trademark of the Waters Corporation, Milford, MA) XE tandem quadrupole mass spectrometer, or equivalent, was found suitable for use. All parameters in this test method are based on this system and may vary depending on your instrument.

[8] A Millex HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog #SLGV033NS; Millex is a trademark of Merck KGAA, Darmstadt, Germany) has been found suitable for use for this test method, any filter unit may be used that meets the performance of this test method may be used.

[9] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. 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 Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[10] A source of Warfarin-D_5 is Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413.

[11] A source of Br-OP is Accustandard, Inc., 125 Market Street, New Haven CT 06513.

[12] Teflon is a trademark of The Chemours Company in Wilmington, DE.

per analysis. Conventional sampling practices should be followed. Refer to Guide D3856 and Practices D3694.

10.1.1 Ammonium acetate was evaluated as an agent to bind free chlorine in drinking water and was found to be ineffective in the preservation of the rodenticides in chlorinated drinking water. Ascorbic acid was effective as a dechlorinating agent in chlorine fortified Chicago tap water, which contained 3.2 ppm free chlorine and was dechlorinated with 10 mg ascorbic acid per 40 mL water sample.[13]

10.2 The samples are collected using 40 mL glass vials. A 40 mL volume is collected directly into the sample collection vial without using any other measuring devices. This is a requirement due to the rodenticides' affinity for surfaces, which will lead to biased low results if transferring between containers. Before collection, the vials must be evaluated to determine a 40 mL sample volume. For example, the vials used in this test method were calibrated before use to determine that filling the vial to approximately 1.6 cm below the rim would result in a 40 mL sample volume. The greatest amount of water held by the 40 mL vials used in this test method was approximately 42 mL. Vials filled to 42 mL in the field would not allow the laboratory to spike the samples before quantitatively transferring to the 50 mL graduated cylinder. It is imperative that the samplers do not overfill the vials.

10.3 *Preservation*—Store samples between 0°C and 6°C from the time of collection until analysis. Analyze the sample within 14 days of collection. Chlorinated drinking water samples are dechlorinated with ascorbic acid; 10 mg of ascorbic acid is added to each 40 mL vial prior to water collection.

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 50 µL volume using a full loop injection. If a 50 µL volume loop is installed in the LC, a "full loop" mode is the preferred technique when performing fast, qualitative analyses. This mode should be used whenever accuracy and precision are the primary concerns. 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 2.

NOTE 2—If your instrument does not have a 50 µL injection capability a different volume may be used. This is a performance-based method and modifications are allowed as long as minimum performance criteria are met.

11.2 *LC Sample Manager Conditions:*

11.2.1 *Wash Solvents*—Weak wash is 4.0 mL of 95 % water/5 % methanol. Strong wash is 2.0 mL of methanol. The strong wash solvent is needed to eliminate carry-over between injections of rodenticide samples. The weak wash is used to remove the strong wash solvent. These rodenticides were shown to carry-over when acetonitrile was used for this analysis. The use of methanol corrected this problem while providing separation and sensitivity. Instrument manufacturer specifications should be followed in order to eliminate sample carry-over.

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent 95% Water/ 5% Methanol, 5 mM NH_4OH	Percent 95% Methanol/ 5% Water, 5 mM NH_4OH
0.0	300	100	0
2.0	300	100	0
6.0	300	20	80
6.1	200	5	95
7.0	200	5	95
8.5	200	0	100
13.0	300	0	100
14.0	300	100	0
16.0	300	100	0

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

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

11.3 *Mass Spectrometer Parameters:*[7]

11.3.1 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 two surrogates and four target compounds that can be acquired in 5 MRM acquisition functions. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this test method are listed below:

The instrument is set in the Electrospray source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.1 Volts
Source Temperature: 120°C
Desolvation Temperature: 375°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.0
High Mass Resolution 1: 14.0
Ion Energy 1: 0.5
Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 0
Low Mass Resolution 2: 14.0
High Mass resolution 2: 14.0
Ion Energy 2: 0.7
Multiplier: 650
Gas Cell Pirani Gauge: 7.0×10^{-3} Torr
Inter-Channel Delay: 0.02 seconds
Inter-Scan Delay: 0.02 seconds
Dwell: 0.1 seconds

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain valid and accurate analytical values through this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

[13] A Hach Pocket Colorimeter II (a trademark of Hach Company in Loveland, CO) was used to measure free chlorine.

TABLE 3 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Analyte	ESI	Primary/ Confirmatory	Retention Time (min)	Cone Voltage (Volts)	Collision Energy (eV)	SRM Mass Transition (Parent > Product)	Primary/ Confirmatory SRM Area Ratio
Bromadiolone	Positive	Primary	7.45 and	30	22	509.1 > 251.1	1.0
		Confirmatory	7.59	30	22	511.1 > 251.1	
Brodifacoum	Positive	Primary	7.95	42	20	523.1 > 335.1	1.1
		Confirmatory		42	33	523.1 > 178	
Diphacinone	Negative	Primary	6.36	32	25	339.1 > 166.8	3.0
		Confirmatory		32	47	339.1 > 115.8	
Warfarin	Positive	Primary	5.38	26	14	309.1 > 162.8	1.7
		Confirmatory		26	20	309.1 > 251.1	
Warfarin-D_5 (Surrogate)	Positive	Primary	5.37	26	14	314.2 > 162.8	1.7
		Confirmatory		26	19	314.2 > 256.1	
Br-OP (Surrogate)	Negative	Primary	8.92	35	25	283.1 > 78.6	N/A

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze 7 calibration standards containing the 7 concentration levels of the rodenticides and surrogates prior to analysis as shown in Table 4. A calibration stock standard solution is prepared from standard materials or they are purchased as certified solutions. Stock standard solution A containing bromadiolone, brodifacoum, diphacinone, warfarin, warfarin-D_5 (surrogate), and 2-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol (surrogate) is prepared at Level 7 concentration, and aliquots of that solution are diluted to prepare Levels 1 through 6. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations.

12.2.1 Prepare stock standard solution A (Level 7) by adding to a 100 mL volumetric flask individual methanol solutions of the following: 50 µL of bromadiolone, brodifacoum, diphacinone, warfarin, warfarin-D_5 (surrogate) each at 4.0 mg/L and 50 µL of 2-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol (surrogate) at 0.2 g/L, dilute to 100 mL with 80 % water/20 % methanol. The preparation of the Level 7 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, solubility at that concentration will have to be ensured.

12.2.2 Aliquots of solution A are then diluted with 80 % water/20 % methanol to prepare the desired calibration levels in 2 mL amber glass LC vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used monitoring the primary and confirmatory SRM transition of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the primary SRM transition. The ratios of the primary/confirmatory SRM transition area counts are given in Table 3 and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio must be within 35 % of the individual labs accepted primary/confirmatory SRM transition area ratio. The primary SRM transition of each analyte is used for quantitation and the confirmatory SRM transition for confirmation. This gives additional confirmation by isolating the parent ion, forming two product ions via fragmentation, and relating it to the retention time in the calibration standard.

12.2.4 The calibration software manual should be consulted to properly use the software. The quantitation method is set as an external calibration using the peak areas in ppt or ppb units, as long as the analyst is consistent. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.5 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards, other than the high or low point, causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the low or high point is excluded, minimally a five point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.6 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. Minimally a six point curve is acceptable using a quadratic fit. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

12.2.6.1 An initial seven point curve over the calibration range is encouraged in the event the low or high point must be excluded to obtain a coefficient of determination >0.99. In this event, the reporting range must be modified to reflect this change.

12.2.7 The retention time window of the SRM transitions must be within 5 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis, and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

TABLE 4 Concentrations of Calibration Standards (ppt)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7
Bromadiolone	100	200	500	750	1000	1500	2000
Brodifacoum	100	200	500	750	1000	1500	2000
Diphacinone	100	200	500	750	1000	1500	2000
Warfarin	100	200	500	750	1000	1500	2000
Warfarin-D_5 (Surrogate)	100	200	500	750	1000	1500	2000
Br-OP (Surrogate)	5000	10 000	25 000	37 500	50 000	75 000	100 000

12.2.8 A midpoint calibration check standard must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated. This end calibration check should be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard must have a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria, the problem must be corrected, and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standard and notices that the sample evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates, the results may be reported unqualified.

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability.

12.3.1 Analyze at least four replicates of a sample solution containing bromadiolone, brodifacoum, diphacinone, warfarin, warfarin-D_5 (surrogate) and Br-OP (surrogate) at a concentration in the calibration range of Levels 3 to 5. A 750 ppt spike for bromadiolone, brodifacoum, diphacinone and warfarin, 1000 ppt spike for warfarin-D_5 (surrogate), and 50 000 ppt spike for Br-OP (surrogate) were used to set the QC acceptance criteria in this test method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 were generated from a single-laboratory. Data from reagent, surface, and drinking water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. References on how to generate QC acceptance criteria are Practices D2777, D5847, E2554, or Method 8000B in U.S. EPA publication SW-846.

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate standard solution containing warfarin-D_5 and Br-OP is added to all samples. A stock surrogate spiking solution is prepared in methanol at 800 ppb for warfarin-D_5 and 40 ppm for Br-OP. Spiking 50 µL of this spiking solution into a 40 mL water sample results in a concentration of 1000 ppt for warfarin-D_5 and 50 000 ppt for Br-OP in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method. Surrogate spiking solutions are routinely replaced every 30 days if not previously discarded for quality control failure.

12.5 *Method Blank:*

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of the rodenticides found in the blank must be below the DVL. If the concentrations of the rodenticides are found above this level, analysis of samples is halted until the contamination is eliminated and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.6 *Laboratory Control Sample (LCS):*

12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the rodenticides at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing bromadiolone, brodifacoum, diphacinone and warfarin each at 300 ppb. Spike 100 µL of this stock solution into 40 mL of water to yield a concentration of 750 ppt for the rodenticides in the sample. The LCS result must fall within the limits in Table 5. If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. Matrix spiking solutions are routinely replaced every 30 days if not previously discarded for quality control failure.

12.7 *Matrix Spike (MS):*

12.7.1 Check for interferences in the specific matrix being tested by performing a MS on at least one sample from each

TABLE 5 QC Acceptance Criteria

Analyte	Test Conc. (ng/L)	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
Bromadiolone	750	70	130	13	70	130
Brodifacoum	750	51	130	25	47	130
Diphacinone	750	70	130	10	70	130
Warfarin	750	70	130	10	70	130
Warfarin-D_5 (Surrogate)[A]	1000	70	130	10	70	130
Br-OP (Surrogate)[B]	50 000	64	130	13	61	130

[A] Warfarin-D_5 is used as the surrogate for Bromadiolone, Brodifacoum and Warfarin only.
[B] Br-OP is used as the surrogate for Diphacinone only.

batch of 20 or fewer samples by spiking a sample with a known concentration of rodenticides and following the test method. Prepare a stock matrix spiking solution in methanol containing bromadiolone, brodifacoum, diphacinone and warfarin each at 300 ppb. Spike 100 µL of this stock solution into 40 mL of water to yield a concentration of 750 ppt for the rodenticides in the sample.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 7 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.7.3 Calculate the percent recovery of the spike (P) using Eq 1:

$$P = 100\,\frac{\left|A(V_s+V) - BV_s\right|}{CV} \quad (1)$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = percent recovery.

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, a matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 were generated by a single-laboratory using surface and drinking water samples from the data in the Precision and Bias Section 16. The matrix variation between the different waters may have a tendency to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard.

12.7.5.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria are Practices D5847, D2777, E2554, or Method 8000B in U.S. EPA publication SW-846.

TABLE 6 MS/MSD QC Acceptance Criteria

Analyte	Test Conc. (ng/L)	MS/MSD		
		Recovery (%)		Precision
		Lower Limit	Upper Limit	Maximum RPD (%)
Bromadiolone	750	26	130	61
Brodifacoum	750	28	130	30
Diphacinone	750	38	155	57
Warfarin	750	70	134	26
Warfarin-D_5 (Surrogate)	1000	70	130	20
Br-OP (Surrogate)	50 000	53	157	22

12.8 *Duplicate:*

12.8.1 Check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. Compare to the RPD limit in Table 6.

$$RPD = \frac{\left|MSR - MSDR\right|}{(MSR+MSDR)\div 2} \times 100 \quad (2)$$

where:

RPD = relative percent difference,
MSR = matrix spike recovery, and
$MSDR$ = matrix spike duplicate recovery.

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 The water samples are stored in the laboratory between 0°C and 6°C. The samples must be analyzed within 14 days of collection. If the samples are above 6°C when received or during storage or not analyzed within 14 days of collection, the data is qualified estimated and noted in the case narrative that accompanies the data. All samples are prepared in the lab using Class A glassware.

13.2 The sample vial is spiked with the appropriate surrogates and target compounds in the lab, for matrix spike samples, as explained in Section 12 in the laboratory before any sample transfer. The sample is shaken ensuring a homogeneous solution. The entire sample is then poured into a 50 mL graduated cylinder, and the sample volume is recorded to the 0.1 mL. The sample volume should be 40.0 ± 1.0 mL after subtracting the spike volume. The vial is then washed 3 times with 3 mL portions of methanol transferring each portion to the graduated cylinder containing the water sample. The final volume of the graduated cylinder is then brought to 50 mL with methanol. If the sample collected was more than 40 mL the volume of methanol used will vary slightly. The methanolic water sample in the graduated cylinder is then mixed using a glass stirring rod.

13.2.1 The entire 50 mL methanolic water sample is filtered through the filtration device described in 7.2 into a 60 mL pre-cleaned amber glass vial with a Teflon[12]-lined cap demonstrated to be free of interferences. A new filter unit is used for each sample. The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of acetone, then 3 volumes of methanol, and a final rinse with water.

13.3 An aliquot of that filtered sample is placed into 2 mL amber glass LC vials for analysis.

13.4 Once a passing calibration curve is generated, the analysis of samples may begin. An order of analysis may be: method blank(s), laboratory control sample(s), sample(s), duplicate(s), matrix spike sample(s) followed by an end calibration check standard.

13.5 A spiked water sample displayed low matrix spike recoveries or less than the reporting limit for brodifacoum. This occurred in Miami, FL drinking water, and the data is shown in the Precision and Bias Section 16. If this is observed, another sample must be prepared in order to analyze for bromadiolone and brodifacoum. Concentrated NH_4OH is added to the 50 mL sample in the graduated cylinder prior to mixing and filtering to bring the pH to ≥9. One drop of concentrated NH_4OH was shown to be effective to adjust the pH to ≥9; this pH adjustment will have to be checked to determine the amount of NH_4OH that is required in the particular matrix. The recoveries of these analytes were shown to improve greatly with pH adjustment in this Miami, FL matrix. These particles may bind to tannins in the sample resulting in low recoveries. Under basic conditions, these analytes may be released from the tannin complexes.[14] A separate batch with all applicable quality control (laboratory control spikes, matrix spikes and method blanks) for the analysis of bromadiolone and brodifacoum must be prepared, analyzed and reported for all samples in that particular matrix, with warfarin-D_5 surrogate recovery, and noted in the narrative the reason for the re-analysis.

[14] Martin, M. M., and Martin, J. S., 'Surfactants: their role in preventing the precipitation of proteins by tannins in insect guts," *Oecologia*, Vol 61, No. 3, 1984, pp. 342–345.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the rodenticides and surrogates, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. External calibration curves are used to calculate the amounts of rodenticides and surrogates. Calculate the concentration in ng/L (ppt) for each analyte. The individual rodenticides may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the midpoint of the calibration range and re-analyzed.

14.2 The use of isotopically labeled bromadiolone, brodifacoum, and diphacinone is cost prohibitive for the analysis. Only two surrogates were chosen in this test method to reduce the cost of analysis while maintaining quality. Br-OP surrogate is acquired in the electrospray negative mode and is used as a surrogate for diphacinone only. Warfarin-D_5 surrogate is acquired in the electrospray positive mode and is used as a surrogate for bromadiolone, brodifacoum and warfarin. Despite that the structure of warfarin-D_5 is similar to bromadiolone and brodifacoum, the need to monitor matrix spike recoveries in a particular matrix is still important. This was demonstrated with the analysis of Miami, FL drinking water shown in Section 16.

14.3 The analysis of rodenticides includes a sample dilution step with the addition of methanol to ensure analyte solubility. This dilution factor must be accounted for in the reported concentration. For example, the concentrations in Table 4 are used to set up the quantitation method. The curves generated would be based on an undiluted sample. The diluted 50.0 mL sample (V_f) results in an uncorrected concentration of 600 ppt (C_u) from the generated calibration curve. The corrected concentration is 750 ppt (C_f) in the initial 40 mL sample (V_i). See Eq 3.

$$\frac{V_f}{V_i}(C_u) = C_f \tag{3}$$

where:

V_f = final volume,
V_i = initial volume,
C_u = uncorrected concentration, and
C_f = final concentration (corrected for dilution).

15. Report

15.1 Determine the results in units of ng/L (ppt) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Precision and Bias

16.1 Standard Test Methods under the jurisdiction of the ASTM committee D19 may be published for a maximum of five years to the completion of a full collaborative study validation. Such standards are deemed to have met all other D19 qualifying requirements but have not completed the

TABLE 7 Single-Laboratory Recovery Data in Reagent Water

Precision and Accuracy Samples	Measured ppt from 750 ppt Rodenticide Spikes (50 000 ppt for Br-OP, 1000 ppt for Warfarin-D_5)					
	Warfarin	Diphacinone	Bromadiolone	Brodifacoum	Br-OP	Warfarin-D_5
1	838	725	825	625	43 225	1000
2	825	763	750	500	40 975	963
3	788	775	850	588	45 588	975
4	800	738	825	700	44 375	1013
5	788	750	813	600	41 463	988
6	813	725	788	563	39 325	1013
7	788	725	725	525	39 163	988
Average Recovery:	805	743	796	586	42 016	991
Average % Recovery:	107	99	106	78	84	99
Standard Deviation:	20	20	45	66	2468	19
% Relative SD	3	3	6	11	6	2

required validation studies to fully characterize the performance of the methods across multiple laboratories and matrices. Publication of standards that have not been fully validated is done to make current technology accessible to users of standards, and to solicit additional input from the user community. The determination of precision and bias was conducted through U.S. EPA and generated applicable data to determine the precision and bias as described in Practice D2777.

16.2 This test method was tested by CRL on reagent water. The samples were spiked with target compounds to obtain a 750 ppt concentration of each rodenticide, a 1000 ppt concentration of warfarin-D_5 (surrogate), and 50 000 ppt concentration of Br-OP (surrogate) described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the surrogates and target compounds.

16.3 This test method was tested by CRL on Chicago River water. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 8 contains the recoveries for the surrogates and target compounds.

16.4 This test method was tested by CRL on Lake Michigan water. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 9 contains the recoveries for the surrogates and target compounds.

16.5 This test method was tested by CRL on Chicago drinking water. The free chlorine residual was determined to be 150 ppb in the native tap water sample before dechlorinating with ascorbic acid. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 10 contains the recoveries for the surrogates and target compounds.

16.6 This test method was tested by CRL on chlorine fortified Chicago drinking water. The drinking water was fortified with Clorox[15] bleach to 3.2 ppm free chlorine. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 11 contains the recoveries for the surrogates and target compounds.

16.7 This test method was tested by CRL on Madison, WI, drinking water. The free chlorine residual was determined to be 330 ppb in the native tap water sample before dechlorinating with ascorbic acid. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 12 contains the recoveries for the surrogates and target compounds.

16.8 This test method was tested by CRL on Miami, FL, drinking water. The free chlorine residual was determined to be 240 ppb in the native tap water sample before dechlorinating with ascorbic acid. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 13 contains the recoveries for the surrogates and target compounds. The recovery for brodifacoum in this sample was below the reporting limit for five out of six spiking concentrations and at the reporting limit for one. A new sample was obtained and prepared in the same manner as stated in Section 13 with the addition of NH_4 OH as stated in 13.5. The results from the addition of NH_4OH to the Miami, FL drinking water sample are shown in 16.9.

16.9 This test method was tested by CRL on Miami, FL, drinking water with the addition of NH_4OH as described in 13.5. The free chlorine residual was determined to be 240 ppb in the native tap water sample before dechlorinating with ascorbic acid. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 14 contains the recoveries for the surrogates and target compounds. The recoveries for brodifacoum and bromadiolone improved.

16.10 This test method was tested by CRL on Florida Everglades water. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 15 contains the recoveries for the surrogates and target compounds.

[15] Clorox is a trademark of The Clorox Company in Oakland, CA.

TABLE 8 Single-Laboratory Recovery Data in Chicago River Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							900	90
Sample 1	1	150	125	83	138	92	925	93
Sample 2		180	163	90	138	76	963	96
Sample 3	2	750	725	97	625	83	950	95
Sample 4		900	913	101	775	86	975	98
Sample 5	3	1500	1513	101	1350	90	950	95
Sample 6		1800	1800	100	1725	96	963	96
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							45 400	91
Sample 1	1	150	113	75	125	83	54 438	109
Sample 2		180	125	69	125	69	47 138	94
Sample 3	2	750	563	75	600	80	45 713	91
Sample 4		900	613	68	650	72	46 963	94
Sample 5	3	1500	1025	68	1175	78	45 088	90
Sample 6		1800	1288	72	1400	78	51 150	102

TABLE 9 Single-Laboratory Recovery Data in Lake Michigan Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							988	99
Sample 1	1	150	125	83	125	83	988	99
Sample 2		180	163	90	150	83	988	99
Sample 3	2	750	788	105	700	93	975	98
Sample 4		900	900	100	813	90	950	95
Sample 5	3	1500	1538	103	1425	95	1013	101
Sample 6		1800	1800	100	1638	91	963	96
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							46 563	93
Sample 1	1	150	100	67	138	92	56 250	113
Sample 2		180	125	69	138	76	44 963	90
Sample 3	2	750	513	68	625	83	41 275	83
Sample 4		900	650	72	738	82	45 800	92
Sample 5	3	1500	1063	71	1188	79	48 350	97
Sample 6		1800	1163	65	1350	75	42 813	86

TABLE 10 Single-Laboratory Recovery Data in Chicago Drinking Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							1013	101
Sample 1	1	150	300	200	200	133	1000	100
Sample 2		180	163	90	163	90	975	98
Sample 3	2	750	813	108	663	88	1025	103
Sample 4		900	1388	154	1738	193	975	98
Sample 5	3	1500	1675	112	1738	116	975	98
Sample 6		1800	1863	103	1713	95	975	98
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							49 800	100
Sample 1	1	150	125	83	100	67	52 588	105
Sample 2		180	113	63	88	49	54 413	109
Sample 3	2	750	488	65	400	53	43 288	87
Sample 4		900	1363	151	763	85	46 900	94
Sample 5	3	1500	1075	72	825	55	4 5775	92
Sample 6		1800	1200	67	1100	61	48 688	97

16.11 This test method was tested by CRL on Florida Everglades water with the addition of NH_4OH as described in 13.5. The samples were spiked with target compounds across the calibration range as Youden pairs and surrogates as described in Section 12. Table 16 contains the recoveries for the surrogates and target compounds.

TABLE 11 Single-Laboratory Recovery Data in Chlorine Fortified Chicago Drinking Water to 3.2 ppm Free Chlorine

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							988	99
Sample 1	1	150	238	158	238	158	975	98
Sample 2		180	200	111	188	104	963	96
Sample 3	2	750	900	120	1000	133	963	96
Sample 4		900	938	104	800	89	988	99
Sample 5	3	1500	1788	119	1450	97	938	94
Sample 6		1800	1850	103	1688	94	938	94
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							50 600	101
Sample 1	1	150	163	108	63	42	56 525	113
Sample 2		180	125	69	50	28	51 563	103
Sample 3	2	750	625	83	288	38	53 875	108
Sample 4		900	575	64	263	29	44 025	88
Sample 5	3	1500	950	63	325	22	57 225	114
Sample 6		1800	1163	65	613	34	55 675	111

TABLE 12 Single-Laboratory Recovery Data in Madison, WI Drinking Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							888	89
Sample 1	1	150	125	83	150	100	963	96
Sample 2		180	163	90	163	90	938	94
Sample 3	2	750	775	103	650	87	950	95
Sample 4		900	863	96	738	82	963	96
Sample 5	3	1500	1450	97	1300	87	950	95
Sample 6		1800	1738	97	1663	92	938	94
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							52 263	105
Sample 1	1	150	150	100	75	50	78 038	156
Sample 2		180	138	76	75	42	52 500	105
Sample 3	2	750	588	78	413	55	65 213	130
Sample 4		900	825	92	613	68	79 800	160
Sample 5	3	1500	1250	83	1013	68	74 513	149
Sample 6		1800	1513	84	1313	73	77 975	156

TABLE 13 Single-Laboratory Recovery Data in Miami, FL Drinking Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							913	91
Sample 1	1	150	125	83	163	108	925	93
Sample 2		180	163	90	175	97	913	91
Sample 3	2	750	713	95	688	92	913	91
Sample 4		900	838	93	775	86	913	91
Sample 5	3	1500	1475	98	1300	87	963	96
Sample 6		1800	1663	92	1563	87	900	90
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							59 475	119
Sample 1	1	150	88	58	< RL	-	60 063	120
Sample 2		180	113	63	< RL	-	56 475	113
Sample 3	2	750	375	50	< RL	-	59 363	119
Sample 4		900	388	43	< RL	-	61 000	122
Sample 5	3	1500	863	58	125	8	57 525	115
Sample 6		1800	975	54	< RL	-	64 675	129

17. Quality Control

17.1 A crucial part of a test method is quality control. A laboratory should follow their in-house QA/QC procedures and should meet or exceed the criteria given in this test method. The quality-control criteria are given in the various test method sections. Section 10 contains the sampling and preservation

TABLE 14 Single-Laboratory Recovery Data in Miami, FL Drinking Water, Addition of NH_4OH

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							1038	104
Sample 1	1	150	150	100	150	100	1038	104
Sample 2		180	175	97	150	83	1038	104
Sample 3	2	750	775	103	775	103	1038	104
Sample 4		900	888	99	900	100	1025	103
Sample 5	3	1500	1475	98	1563	104	1038	104
Sample 6		1800	1813	101	1900	106	1025	103
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							96 838	194
Sample 1	1	150	125	83	138	92	83 763	168
Sample 2		180	163	90	138	76	77 888	156
Sample 3	2	750	625	83	625	83	77 388	155
Sample 4		900	775	86	775	86	87 725	175
Sample 5	3	1500	1100	73	1125	75	76 250	153
Sample 6		1800	1400	78	1588	88	84 563	169

TABLE 15 Single-Laboratory Recovery Data in Florida Everglades Water

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							1013	101
Sample 1	1	150	150	100	138	92	1025	103
Sample 2		180	175	97	163	90	1013	101
Sample 3	2	750	738	98	638	85	1025	103
Sample 4		900	913	101	825	92	1038	104
Sample 5	3	1500	1538	103	1388	93	1000	100
Sample 6		1800	1850	103	1638	91	1025	103
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							36 525	73
Sample 1	1	150	100	67	113	75	44 038	88
Sample 2		180	113	63	125	69	37 325	75
Sample 3	2	750	450	60	513	68	35 750	72
Sample 4		900	538	60	600	67	37 325	75
Sample 5	3	1500	913	61	1025	68	38 800	78
Sample 6		1800	1088	60	1325	74	36 838	74

TABLE 16 Single-Laboratory Recovery Data in Florida Everglades Water, Addition of NH_4OH

Sample	Youden Pair	Target Compound Spike (ppt)	Warfarin Measured (ppt)	Warfarin % Recovery	Diphacinone Measured (ppt)	Diphacinone % Recovery	Warfarin-D_5 Measured (ppt) (1000 ppt Spike)	Warfarin-D_5 % Recovery
Blank							1013	101
Sample 1	1	150	150	100	138	92	1025	103
Sample 2		180	175	97	163	90	1025	103
Sample 3	2	750	738	98	738	98	1000	100
Sample 4		900	888	99	850	94	1025	103
Sample 5	3	1500	1475	98	1488	99	1025	103
Sample 6		1800	1775	99	1700	94	1000	100
Sample	Youden Pair	Target Compound Spike (ppt)	Bromadiolone Measured (ppt)	Bromadiolone % Recovery	Brodifacoum Measured (ppt)	Brodifacoum % Recovery	Br-OP Measured (ppt) (50 000 ppt Spike)	Br-OP % Recovery
Blank							52 038	104
Sample 1	1	150	100	67	138	92	51 875	104
Sample 2		180	138	76	175	97	58 250	117
Sample 3	2	750	563	75	650	87	60 513	121
Sample 4		900	638	71	738	82	51 438	103
Sample 5	3	1500	1100	73	1263	84	56 963	114
Sample 6		1800	1313	73	1425	79	54 575	109

requirements and Section 12 contains the majority of quality control requirements when following this test method. Section 12 includes requirements for calibration, precision and bias study to demonstrate laboratory capability, initial demonstration of performance, surrogate, method blank, reporting limit check, laboratory control, matrix spike and duplicate sample

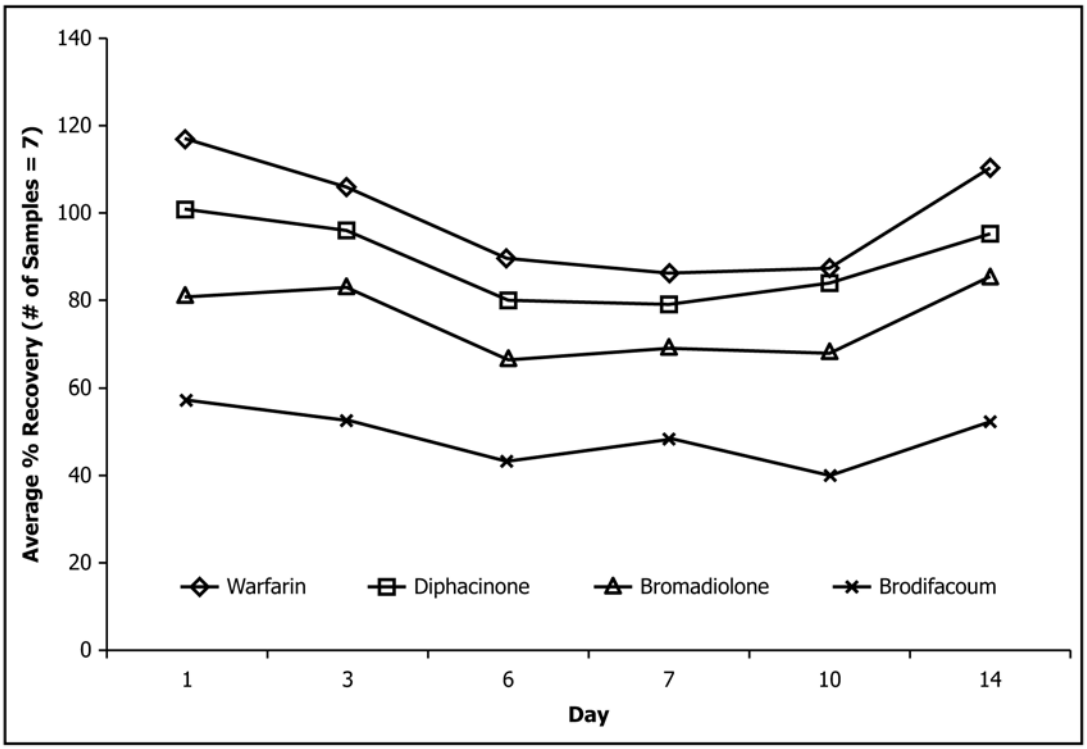

FIG. X1.1 Fourteen Day Holding Time Study

requirements. An IRM should be incorporated into the analysis periodically to verify that standard concentrations are comparable between sources. The IRM criteria should be based upon the laboratories QA/QC policies and the individual data quality objectives.

18. Keywords

18.1 liquid chromatography; mass spectrometry; rodenticides; water

APPENDIX

(Nonmandatory Information)

X1. HOLDING TIME STUDY

X1.1 A holding time study was performed by CRL on chlorine fortified Chicago drinking water. The drinking water was fortified with Clorox[15] bleach to 3.2 ppm free chlorine. After the addition of ascorbic acid, the free chlorine concentration was less than the detection limit (100 ppb) of the chlorine meter. Seven samples were spiked with target compounds to obtain a 750 ppt concentration of each rodenticide as described in Section 12. Fig. X1.1 contains the average percent recoveries over a 14 day period for the target compounds. Holding time is dependent upon your individual matrix and will vary. ASTM Standard Practice D4841 may be used to conduct a holding time study on your individual matrix.