Designation: D7644 – 23

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

1. Scope

1.1 This test method 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *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:*[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).

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

3.2.2 *independent reference material, IRM, n*—a material of known purity and concentration obtained either from the National Institute of Standards and Technology (NIST) or other reputable supplier; the IRM must 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 above freezing 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 min to

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[4] Additional information about rodenticides is available from United States Environmental Protection Agency (EPA), http://www.epa.gov.
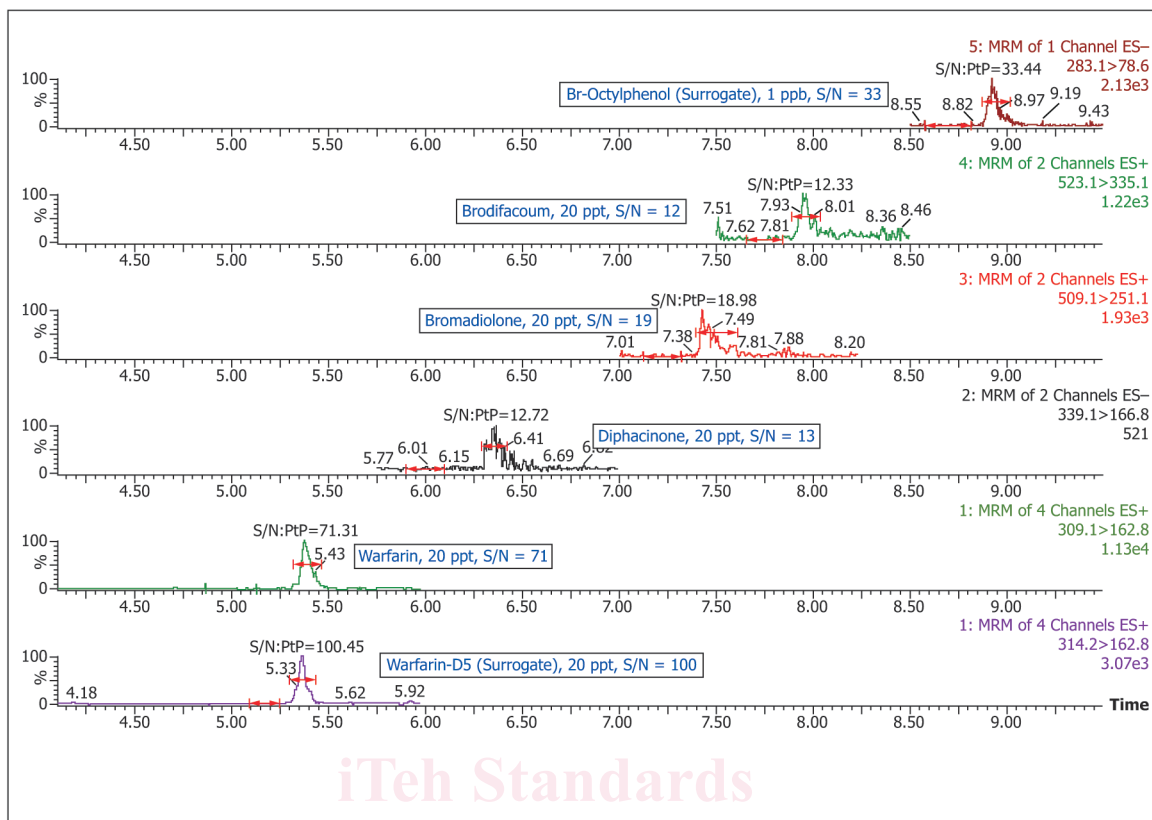
FIG. 1 Example Primary SRM Chromatograms Signal/Noise Ratios
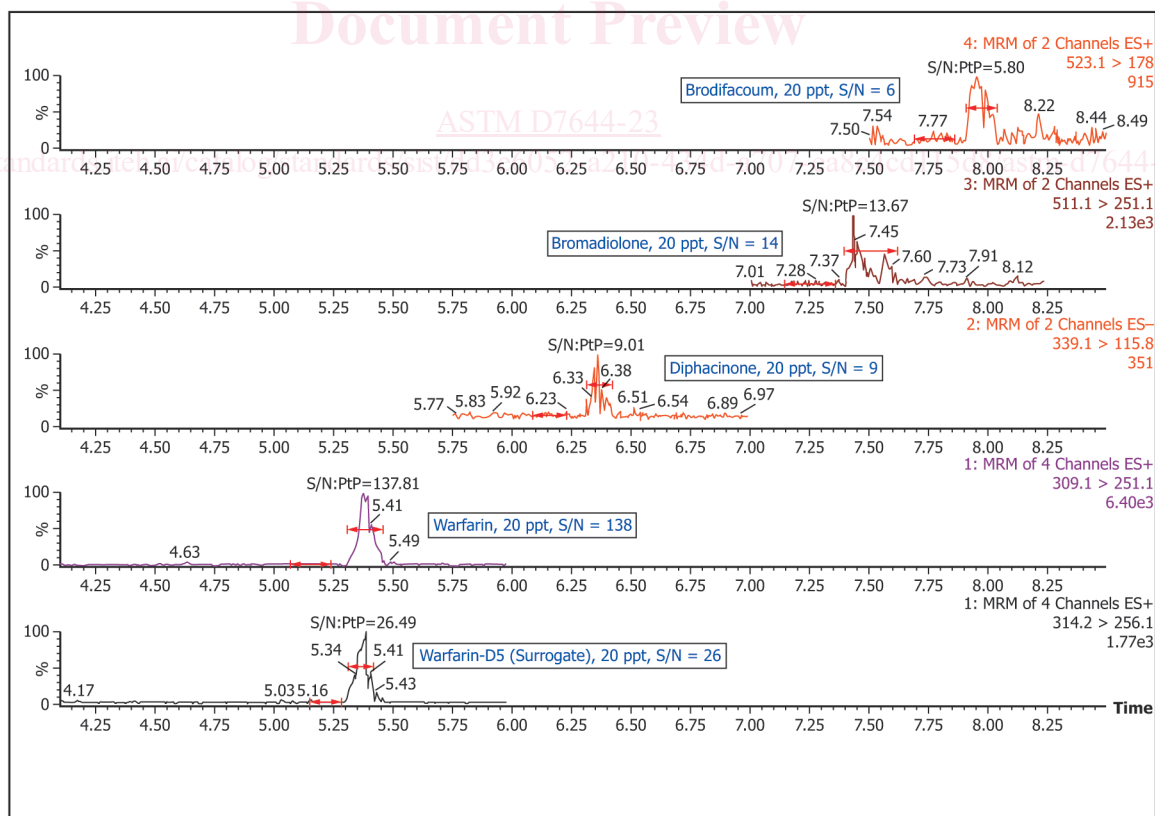

FIG. 2 Example Confirmatory SRM Chromatograms Signal/Noise Ratios

30 min. 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.

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. 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*[5]—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. 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*[6]—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 must be used in all tests. Unless indicated otherwise, it is intended that all reagents must conform to the Committee on Analytical Reagents of the American Chemical Society.[7] 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 must 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).[8]

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).[9]

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 inert-lined caps demonstrated to be free of interferences. Surface water samples are collected unpreserved, shipped between above freezing and 6 °C, and stored in the laboratory between above freezing and 6 °C. Chlorinated drinking water samples are dechlorinated

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[5] A Waters ACQUITY UPLC (a trademark of the Waters Corporation, Milford, MA) BEH C18, 2.1 × 100 mm, 1.7 µm particle size was used, if you are aware of an alternative column that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[6] A Millex HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog #SLGV033NS; Millex is a trademark of Merck KGAA, Darmstadt, Germany) was used, if you are aware of an alternative filter unit that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[7] *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.

[8] A source of Warfarin-D_5 is Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413. If you are aware of an alternative source that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[9] A source of Br-OP is Accustandard, Inc., 125 Market Street, New Haven CT 06513. If you are aware of an alternative source that meets the performance of the standard, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

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 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.[10]

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 above freezing 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:*

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 the 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

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent 95 % Water/ 5 % Methanol, 5 mM NH$_4$OH	Percent 95 % Methanol/ 5 % Water, 5 mM NH$_4$OH
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

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.

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

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

11.3 *Mass Spectrometer Parameters:*

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 the 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/h
Cone Gas Flow: 25 L/h
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 s
Inter-Scan Delay: 0.02 s
Dwell: 0.1 s

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.

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[10] A Pocket Chlorine meter 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 h 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. Curves should be evaluated using relative error or relative standard error.[11]

12.2.5 Linear calibration may be used if 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. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

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[11] Management and Technical Requirements for Laboratories Performing Environmental Analysis; Module 4: Quality Systems for Chemical Testing; The NELAC Institute, 2017.

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

12.2.6 Quadratic calibration may be used if 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. Each calibration point used to generate the curve must have a calculated percent deviation less than 25 % from the generated curve.

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.

12.2.8 A midpoint calibration check standard must be analyzed at the end of each batch of 20 samples or within 24 h 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.

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.