METHOD 532. DETERMINATION OF PHENYLUREA COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

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METHOD 532

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1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method for the determination of phenylurea pesticides in drinking waters. This method is applicable to phenylurea compounds that are efficiently extracted from the water using a C_{18} solid phase cartridge or disk. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds in reagent water and finished ground and surface waters:

Analyte	Chemical Abstracts Service Registry Number
Diflubenzuron	35367-38-5
Diuron	330-54-1
Fluometuron	2164-17-2
Linuron	330-55-2
Propanil	709-98-8
Siduron	1982-49-6
Tebuthiuron	34014-18-1
Thidiazuron	51707-55-2

1.2 MDLs are compound, instrument, and matrix dependent. The MDL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.(1) Experimentally determined MDLs for the above listed analytes are provided in Section 17, Table 3. The MDL differs from, and is usually lower than (but never above), the minimum reporting limit (MRL) (Sect. 3.16). The concentration range for target analytes in this method was evaluated between 1.0 ug/L and 30 ug/L for a 500 mL sample. Precision and accuracy data and sample holding time data are presented in Section 17, Tables 4 - 9.

1.3 This method is restricted to use by or under the supervision of analysts skilled in solid phase extraction (SPE), and HPLC analysis.

2. SUMMARY OF METHOD

2.1 A 500 mL water sample is passed through a SPE cartridge or disk containing a chemically bonded C_{18} organic phase to extract the phenylurea pesticides and surrogate compounds. The analytes and surrogates are eluted from the solid phase with methanol, and the extract is concentrated to a final volume of 1 mL. Components are then chromatographically separated by injecting an aliquot of the extract into an HPLC system equipped with a C_{18} column and detected using a UV/Vis detector. Identification of target and surrogate analytes and quantitation is accomplished by comparison of retention times and analyte responses using external standard procedures. Sample extracts with positive results are solvent exchanged and confirmed using a second, dissimilar HPLC column that is also calibrated using external standard procedures.

3. DEFINITIONS

3.1 EXTRACTION BATCH – A set of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices and solvents, surrogate solution, and fortifying solutions. Required QC samples include: Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Matrix, and either a Field Duplicate or Laboratory Fortified Matrix Duplicate.

3.2 ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check standards (CCC). Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.3 SURROGATE ANALYTE (SUR) – A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

3.4 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, sample preservatives, and surrogates that are used in the extraction batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.5 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation

compounds are added. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMD) – A second aliquot of the Field Sample used to prepare the LFM which is fortified, extracted, and analyzed identically. The LFMD is used instead of the Field Duplicate to access method precision and accuracy when the occurrence of target analytes is low.

3.8 LABORATORY DUPLICATES (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.9 FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage.

3.10 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing method analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution or stock standard solution and the surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing one or more of the method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.14 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check standard integrity.

3.15 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.4). Accurate quantitation is not expected at this level. [(1)]

3.16 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest continuing calibration standard for that analyte, and can only be used if acceptable quality control criteria for this standard are met.

3.17 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.18 PEAK GAUSSIAN FACTOR (PGF) – The peak gaussian factor is calculated using the equation in Section 10.2.3.1. It provides a quantitative measure of peak asymmetry. A perfectly symmetric peak would have a PGF of 1. Poor peak symmetry can result in imprecise quantitation, degraded resolution and poor retention reproducibility. For this reason, columns and conditions that produce symmetric peaks are required.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 hours. Volumetric glassware should not be heated above 120 °C.

4.2 Method interferences may also be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for each analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3. **Subtracting blank values from sample results is not permitted.**

4.2.1 An extraneous peak was noted that elutes very near fluometuron on the confirmation column that can cause problems with quantitation if the chromatography is not fully optimized. No interferences were observed for the primary column analysis.

4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. Water samples high in total organic carbon may have an elevated baseline and/or interfering peaks.

4.4 Solid phase cartridges and disks and their associated extraction devices have been observed to be a source of interferences in other EPA organic methods. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of solid phase extraction devices should be monitored to ensure that contamination will not preclude analyte identification or quantitation.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining an awareness of OSHA regulations regarding the safe handling of the chemicals used in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[2-4]

5.2 Pure standard materials and stock standards of these compounds should be handled with suitable protection to skin and eyes, and care should be taken not to breath the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS – 500 mL amber or clear glass bottles fitted with PTFE (polytetrafluoroethylene) lined screw caps.

6.2 VIALS – Screw cap or crimp top glass autosampler vials with PTFE faced septa, amber or clear.

6.3 VOLUMETRIC FLASKS – Class A, suggested sizes include 1,5,and 10 mL.

6.4 GRADUATED CYLINDERS –Suggested sizes include 5,10, and 500 mL.

6.5 MICRO SYRINGES – Various sizes.

6.6 ANALYTICAL BALANCE – Capable of accurately weighing to the nearest 0.0001 g.

6.7 DISPOSABLE SYRINGES – 1 mL (B-D cat.#: 309602 or equivalent) size, used to filter sample extracts before analysis.

6.8 FILTERS – Disposable filters to filter sample extracts before analysis (Gelman 0.45 um Nylon Acrodisk cat.#: 4426 or equivalent).

6.9 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

6.9.1 EXTRACTION CARTRIDGES – 6 mL, packed with 500 mg (40 um d_p) silica bonded with C_{18} (Varian cat.#: 1210-2052 or equivalent).

6.9.2 SAMPLE RESERVOIRS – (VWR cat.#: JT7120-3 or equivalent) These are attached to the cartridges and water samples are poured into them, although they hold only 75 mL at one time. An alternative is a transfer tube system (Supelco "Visiprep"; cat. #: 57275 or equivalent) which transfers the sample directly from the sample container to the SPE cartridge.

6.9.3 VACUUM EXTRACTION MANIFOLD – With flow/vacuum control (Supelco cat.#: 57044 or equivalent). The use of replaceable needles or valve liners may be used to prevent cross contamination.

6.9.4 REMOTE VACUUM GAUGE/BLEED ASSEMBLY – To monitor and adjust vacuum pressure delivered to the vacuum manifold (Supelco cat.#: 57161-U or equivalent).

6.9.5 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for elution of the sample from the cartridge after extraction.

6.9.6 An automatic or robotic system designed for use with SPE cartridges may be used if all quality control requirements discussed in Section 9 are met. Automated systems may use either vacuum or positive pressure to process samples and solvents through the cartridge. All extraction and elution steps must be the same as the manual procedure. Extraction or elution steps may not be changed or omitted to accommodate the use of the automated system.

6.10 SOLID PHASE EXTRACTION (SPE) APPARATUS USING DISKS

6.10.1 EXTRACTION DISKS – 47 mm diameter, manufactured with a C_{18} bonded sorbent phase (Varian cat.#: 1214-5004 or equivalent). Larger disks may be used as long as the QC performance criteria outlined in Section 9 are met.

6.10.2 SPE DISK EXTRACTION GLASSWARE – Funnel, PTFE coated support screen, PTFE gasket, base, and clamp used to support SPE disks and contain samples during extraction. May be purchased as a set (Fisher cat# K971100-0047 or equivalent) or separately.

6.10.2 VACUUM EXTRACTION MANIFOLD – Designed to accommodate extraction glassware (Varian cat. #: 1214-6001 or equivalent).

6.10.3 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for collection of the eluent that drips from the disk extraction base.

6.10.4 An automated or robotic system may be used as specified in Section 6.9.6.

6.11 EXTRACT CONCENTRATION SYSTEM – To concentrate extracts in 15 mL conical tubes, the bottoms of which are submersed in a 40°C water bath, under a steady steam of nitrogen to the desired volume (Meyer N-Evap, Model III, Organomation Associates, Inc. or equivalent).

6.12 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a minimum vacuum of approximately 25 cm (10 in.) of mercury for cartridges. A greater vacuum of approximately 66 cm (26 in.) of mercury may be used with disks.

6.13 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) INSTRUMENTATION

6.13.1 HPLC SYSTEM – Capable of reproducibly injecting 20 or 10 uL aliquots, and performing binary linear gradients at a constant flow rate near 1.5 mL/min.

6.13.2 HPLC DETECTOR – A UV detector capable of collecting data at 240-245 nm. For the development of this method, a photodiode array detector was used. A LC/MS system may also be used.

6.13.3 PRIMARY COLUMN – An HPLC column (4.6 × 150 mm) packed with 3.5 um d_p C_{18} solid phase particles (Waters cat. # WAT200632). Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

6.13.4 CONFIRMATION COLUMN – An HPLC column (4.6 × 150mm) packed with 5 d_p cyanopropyl stationary phase (Supelco "Supelcosil LC-CN" cat. # 58221-U). The secondary column must be chemically dissimilar to the primary column and must yield a different elution order for some compounds which will result in dissimilar retention times compared to the primary column.

6.13.5 HPLC DATA SYSTEM - A computerized data system is recommended for data acquisition and manipulation. The Waters Millennium software system was used to generate all primary column data contained in the Section 17 tables.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes or interfering compounds at or above 1/3 the MRL for each compound of interest.

7.1.2 ACETONITRILE – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.3 METHANOL – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.4 ACETONE – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.5 PHOSPHATE BUFFER SOLUTION, 25 mM – Used for HPLC mobile phase. Add 100 mL 0.5 M potassium phosphate stock solution (Sect. 7.1.5.1) and 100 mL of 0.5 M phosphoric acid stock solution (Sect. 7.1.5.2) to reagent water and dilute to a final volume of 4 L . The pH should be about 2.4 and should be confirmed with a pH meter. Filter using a 0.45 um nylon filter.

7.1.5.1 POTASSIUM PHOSPHATE STOCK SOLUTION (0.5 M) – Weigh 68 g KH_2PO_4 (Monobasic Potassium Phosphate) and dilute to 1 L using reagent water.

7.1.5.2 PHOSPHORIC ACID STOCK SOLUTION (0.5M) – 34.0 mL of phosphoric acid (85%, HPLC grade in reagent water) diluted to 1 L with reagent water.

7.1.6 SAMPLE PRESERVATION REAGENTS

7.1.6.1 CUPRIC SULFATE, $CuSO_4¢5H_2O$ (ACS Grade or equivalent) – Added as a biocide to guard against potential degradation of method analytes by microorganisms (Sect. 8.1.2).

7.1.6.2 TRIZMA PRESET CRYSTALS, pH 7.0 (Sigma cat# T 3503 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]. Alternatively, a mix of the two components with a weight ratio of 15.5/1; Tris HCL/Tris may be used. These blends are targeted to produce a pH of 7.0 at 25°C in reagent water. Tris functions as a buffer, binds free chlorine in chlorinated finished waters, and prevents the formation of a copper-based precipitate.

7.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should used standard QC practices to determine when their standards need to be replaced.**

7.2.1 SURROGATE ANALYTES (SUR), MONURON (CAS #150-68-5) and CARBAZOLE (CAS# 86-74-8) – Monuron and carbazole were chosen as surrogates. Monuron is a phenylurea no longer in use in the U.S. that elutes early in the chromatogram. Carbazole elutes late in the chromatogram (Figure 1) and has been used as a surrogate in other EPA drinking water methods. Alternate surrogates may be selected if there is a problem with matrix interferences or chromatography. However, if an alternate surrogate is used, it must have similar chemical properties (structure, solubility, C_{18} retention, etc.) to the phenylureas, be chromatographically resolved from all target analytes and matrix interferences, and be highly unlikely to be found in any sample.

7.2.1.1 SUR STOCK SOLUTION (5 to 7 mg/mL) – Accurately weigh

approximately 25 to 35 mg of the neat SUR to the nearest 0.1 mg into a tared, 5 mL volumetric flask. Dilute to the mark with the appropriate solvent: methanol should be used for monuron and acetonitrile for carbazole. Prepare each compound individually, as they will be combined in the SUR primary dilution standard. Stock solutions have been shown to be stable for 6 months when stored at -10°C or less. Laboratories should use standard QC practices to determine when their standards need to be replaced.

7.2.1.2 SUR PRIMARY DILUTION STANDARD (500 ug/mL) – Prepare the SUR Primary Dilution Standard (PDS) by dilution of the SUR stock standards. Add enough of each of the SUR stock standards to a volumetric flask partially filled with methanol to make a 500 ug/mL solution when filled to the mark with methanol. The PDS has been shown to be stable for 3 months when stored at -10°C or less.

7.2.2 ANALYTE STOCK STANDARD SOLUTION – Prepare analyte stock standard solutions for all compounds in methanol except thidiazuron and diflubenzuron. Thidiazuron and diflubenzuron should be prepared in acetone due to their limited solubility in methanol. Acetone elutes early in the chromatogram and should not interfere with compound quantitation as long as its volume is minimized as specified in this method. Method analytes may be obtained as neat materials or as ampulized solutions. Stock solutions have been shown to be stable for 6 months when stored at -10°C or less.

7.2.2.1 For analytes in their pure form that are soluble in methanol, prepare stock solutions by accurately weighing 25 to 35 mg of pure material to the nearest 0.1 mg in a 5 mL volumetric flask. Dilute to volume with methanol.

7.2.2.2 Thidiazuron and diflubenzuron should be dissolved in acetone. Accurately weigh neat material to the nearest 0.1 mg into volumetric flasks, but using smaller amounts than those used for other target analytes, approximately 10 to 12 mg. Thidiazuron is especially difficult to dissolve, 10 mg of pure material should dissolve in a 10 mL final volume of acetone. Sonnication may be used to help dissolve these compounds.

7.2.3 ANALYTE PRIMARY DILUTION STANDARD (PDS, 200 ug/mL and 10 ug/mL) – Prepare the Analyte PDS by dilution of the stock standards (Sect. 7.2.2). Add enough of each stock standard to a volumetric flask partially filled with methanol to make a 200 ug/mL solution when filled to the mark with

methanol. Once prepared, a dilution of the 200 ug/mL solution may be used to prepare a 10 ug/mL solution used for low concentration spiking. The PDSs can be used to prepare calibration and fortification solutions. Analyte PDSs have been shown to be stable for 3 months when stored at -10°C or less.

7.2.4 CALIBRATION SOLUTIONS – At least 5 calibration concentrations will be required to prepare the initial calibration curve (Sect. 10.2). Prepare at least 5 Calibration Solutions over the concentration range of interest, approximately 0.5-15 ug/mL, from dilutions of the analyte PDS in methanol. The lowest concentration of calibration standard must be at or below the MRL, which will depend on system sensitivity. In this method, 500 mL of an aqueous sample is concentrated to a 1 mL final extract volume. The calibration standards for the development of this method were prepared as specified below.

PREPARATION OF CALIBRATION (CAL) CURVE STANDARDS

CAL Level	PDS Conc. (ug/mL)	Volume PDS Standard (uL)	Final Volume of CAL Standard (mL)	Final Conc. of CAL Standard (ug/mL)	Equivalent Conc. in 500 mL sample (ug/L)
1	10	25	1	0.25	0.50
2	10	50	1	0.50	1.00
3	200	5.0	1	1.00	2.00
4	200	25	1	5.00	10.0
5	200	50	1	10.0	20.0
6	200	75	1	15.0	30.0

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [5] using 500 mL amber or clear glass bottles fitted with PTFE lined screw caps.

8.1.2 Prior to shipment to the field, 0.25 g of cupric sulfate and 2.5 g of Trizma crystals (Sect. 7.1.6) must be added to each bottle for each 500 mL of sample collected. Alternately, the Tris buffer may be prepared by adding 2.35 g of Tris HCl and 0.15 g Tris to the sample bottle in addition to the 0.25 g of cupric

sulfate. Cupric sulfate acts as a biocide to inhibit bacteriological decay of method analytes. Trizma functions as a buffering reagent, binds the free chlorine, and helps to prevent the formation of a precipitate. Add these materials as dry solids to the sample bottle. The stability of these materials in concentrated aqueous solution has not been verified.

8.2 SAMPLE COLLECTION

8.2.1 When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample from a representative area, and carefully fill sample bottles from the container. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2.3 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.4 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 minute. Keep samples sealed from collection time until extraction.

8.3 SAMPLE SHIPMENT AND STORAGE – All samples should be iced during shipment and must not exceed 10° C during the first 48 hours after collection. Samples should be confirmed to be at or below 10 °C when they are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but should not be frozen.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study of all method analytes indicated that all compounds are stable for 14 days in water samples that are collected, dechlorinated, preserved, shipped and stored as described in Sections 8.2 and 8.3. Samples must be extracted within 14 days. Sample extracts may be stored in methanol at 0°C or less for up to 21 days after extraction. Samples that are exchanged into reagent water/acetonitrile (60/40) for confirmational analysis may be stored 7 days at 0° or less; however, the combined extract holding time may not exceed 21 days.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability, the determination of the MDL, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards (CCC), a Laboratory Fortified Blank (LFB), a Laboratory Fortified Sample Matrix (LFM), and either a Laboratory Fortified Sample Matrix Duplicate (LFMD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 10 and 11. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections and summarized in Section 17, Table 10.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solid phase extraction (SPE) cartridges or disks is used, it must be demonstrated that a laboratory reagent blank is reasonably free of contamination and that the criteria in Section 9.4 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare, extract, and analyze 4-7 replicate LFBs fortified at 5 to 10 ug/L, or near the mid-range of the initial calibration curve, according to the procedure described in Section 11. Sample preservatives as described in Section 8.1.2 must also be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY – Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.4 MDL DETERMINATION – Prepare, extract and analyze at least 7 replicate LFBs at a concentration estimated to be near the MDL over at least 3 days (both extraction and analysis should be conducted over at least 3 days) using the procedure described in Section 11.. This fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level. The appropriate concentration will be dependent upon the sensitivity of the HPLC system being used. Sample preservatives as described in Section 8.1.2 must be added to these samples. Calculate the MDL using the equation

$$MDL = St_{(n-1,\ 1-\ alpha\ =\ 0.99)}$$

where

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations. This is a statistical determination based on precision only. [1] If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet method precision and accuracy criteria.

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify HPLC columns, HPLC detector, HPLC conditions, evaporation techniques, and surrogate standards, but each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.3 Minimum Reporting Level (MRL) – The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL may be established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal to noise ratio of five. **Although the lowest calibration standard for an analyte may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard**.

9.4 LABORATORY REAGENT BLANK (LRB) – A LRB is required with each extraction batch (Sect. 3.1) of samples to determine any background system contamination. If within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes must be below $^1/_3$ the MRL. If the target analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.5 CONTINUING CALIBRATION CHECK (CCC) – A CCC is a standard prepared with all compounds of interest which is analyzed during an analysis batch to ensure the stability of the instrument initial calibration. See Section 10.3 for concentration requirements, frequency requirements, and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANKS – A LFB is required with each extraction batch. The fortified concentration of the LFB should be rotated between, low, medium and high concentrations from day to day. The low concentration LFB must be as near as practical to, but no more than two times the MRL. Similarly, the high concentration should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the LFB for the low level fortification must be 50-150% of the true value. The concentration determined for the medium and high LFBs must be 70-130% of the true value. If LFB results do not meet these criteria for target analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.7 SURROGATE RECOVERY – The surrogate standards are fortified into the aqueous portion of all samples, LRBs, and LFMs and LFMDs prior to extraction. They are also added to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.7.1 When surrogate recovery from a sample, blank, or CCC is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.7.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.7.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.7.1, recalibration is in order per Section 10.2. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect/surrogate recovery.

9.8 LABORATORY FORTIFIED SAMPLE MATRIX AND DUPLICATE (LFM AND LFMD) – Analysis of LFMs are required in each extraction batch and are used to determine that the sample matrix does not adversely affect method accuracy. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFM, or LMFD, must be prepared, extracted, and analyzed from a duplicate of the field sample used to prepare the LFM to assess method precision. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate (Sect. 9.8). If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should

be established for each. Over time, LFM data should be documented for all routine sample sources for the laboratory.

9.8.1 Within each extraction batch, a minimum of one field sample is fortified as a LFM for every 20 samples extracted. The LFM is prepared by spiking a sample with an appropriate amount of the appropriate Analyte PDS (Sect. 7.2.3). Select a spiking concentration at least twice the matrix background concentration, if known. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate spiking levels.

9.8.2 Calculate the percent recovery (R) for each analyte using the equation

$$R = \frac{(A - B)}{C} * 100$$

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample, and
C = fortification concentration.

9.8.3 Analyte recoveries may exhibit a matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%, except for thidiazuron which should be recovered at 60 - 120%. For LFM fortification at the MRL, 50 to 150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.8.4 If a LFMD is analyzed instead of a Field Duplicate, calculate the relative percent difference (RPD) for duplicate LFMs (LFM and LFMD) using the equation

$$RPD = \frac{LFM - LFMD}{(LFM + LFMD)/2} * (100)$$

RPDs for duplicate LFMs should fall in the range of ± 30% for samples fortified at or above their native concentration. Greater variability may be observed when LFMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.9 FIELD DUPLICATES (FD1 AND FD2) – Within each extraction batch, a minimum of one field duplicate (FD) or LFMD (Sect. 9.8) must be analyzed. FDs serve as a check the precision associated with sample collection, preservation, and storage, as well as laboratory procedures. If target analytes are not routinely observed in field samples, a LFMD should be analyzed to substitute for this requirement. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate.

9.9.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2)using the equation

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} * (100)$$

RPDs for duplicates should fall in the range of ± 30%. Greater variability may be observed when analyte concentrations are near the MRL. At the MRL, RPDs should fall in the range of ± 50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.10 QUALITY CONTROL SAMPLES (QCS) – Each time that new standards are prepared or a new calibration curve is run, analyze a QCS from a source different from the source of the calibration standards. The QCS may be injected as a calibration standard, or fortified into reagent water and analyzed as a LFB. If the QCS is analyzed as a continuing calibration check, then the acceptance criteria are the same as for the CCC. If the QCS is analyzed as a LFB, then the acceptance criteria are the same as for an LFB. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10. CALIBRATION AND STANDARDIZATION

10.1 After initial calibration is successful, a Continuing Calibration Check is required at the beginning and end of each analysis batch, and after every tenth sample (Sect. 10.3). Initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2 INITIAL CALIBRATION

10.2.1 Establish HPLC operating parameters equivalent to the suggested conditions in Section 17, Table 1. The system is calibrated using the external standard technique. For this method, a PDA detector was used and the analyte absorbance at 240 or 245 nm was used in order to maximize target compound signal relative to the background interferences. Other HPLC conditions may be used as long as all QC requirements in Section 9 are met.

10.2.2 Prepare a set of at least 5 calibration standards as described in Section 7.2.4. The lowest concentration of calibration standard must be at or below the MRL, which will depend on system sensitivity.

10.2.3 INJECTION VOLUME – Optimum injection volume for the primary column may vary between HPLC instruments when a sample is dissolved in an organic solvent such as methanol. Prior to establishing the initial calibration, the injection volume must first be determined. Peak asymmetry on the primary column was occasionally noted on one of the two instruments used to develop this method. This asymmetry only occurred with 20 uL injections. This phenomena is attributed to the difference in elutropic strength between the initial mobile phase composition (phosphate buffer/acetonitrile; 60/40) and the extract solvent composition (100% methanol). In all cases, the asymmetry was eliminated by reducing the injection volume to 10 uL. Prior to establishing the initial calibration curve, acceptable chromatographic performance is determined by calculating the Peak Gaussian Factor (PGF).

10.2.3.1 Section 17 (Table 3) lists MDLs on the primary column using two injection sizes: 20 uL and 10 uL. Inject a 20 uL aliquot of the medium level calibration standard on the primary column using the suggested conditions listed in Section 17, Table 1. Determine the PGF for the analyte fluometuron using the equation

$$PGF = \frac{1.83 \times W_{0.5}}{W_{0.1}}$$

where,
$W_{0.5}$ is the peak width at half height, and
$W_{0.1}$ is the peak width at tenth height.

If fluometuron has not been included in the calibration standards, one of the surrogate compounds may be substituted.

NOTE: Values for $W_{0.5}$ and $W_{0.1}$ can be attained via most data acquisition software packages. If these values are manually measured, the analyst should limit the retention time window to enlarge the peak of interest allowing accurate determination of the PGF. Inaccurate measurements may result when using a chromatogram of the entire analysis run.

10.2.3.2 If the PGF is in the range of 0.90 to 1.10, the peak shape is considered acceptable, and a 20 uL injection may be used. If not, injection volume should be reduced, and the PGF redetermined as described in Section 10.2.3.1.

10.2.4 Generate a calibration curve for each analyte by plotting the peak response (area is recommended) against analyte concentration. Both instruments used during method development yielded linear curves for the target analytes over the concentration range of interest. However, data may be fit with either a linear regression (response vs concentration) or quadratic fit (response vs concentration). Alternately, if the ratio of the analyte peak area to concentration (or response factor) is relatively constant (RSD < 30%) an average response factor may be used to calculate analyte concentration. Siduron separates into two isomers (labeled A & B in Sect. 17, Figure 1). The responses of the two isomers should be added together before plotting against the concentration.

10.2.5 Repeat steps 10.2.1 through 10.2.4 for the confirmation column. Laboratories may choose to wait to establish an initial calibration curve for the confirmation column until they have samples with positive results that require confirmation; however, this step must be successfully completed prior to confirming sample results.

10.3 CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. (In this context, a "sample" is considered to be a Field sample. LRBs, LFBs, LFMs, LFMDs and CCCs

are not counted as samples.) The beginning CCC each day should be at or below the MRL in order to verify instrument sensitivity prior to any analyses. Subsequent CCCs should alternate between a medium and high concentration standard.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ±50% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken which may require recalibration. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored.

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper preparation of laboratory glassware and sample containers (Sect. 4.1), and sample collection and storage (Sect. 8). This section details the procedures for sample preparation, solid phase extraction (SPE) using cartridges or disks, and extract analysis.

11.2 SAMPLE PREPARATION

11.2.1 Samples are preserved, collected and stored as presented in Section 8. All field and QC samples must contain the preservatives listed in Section 8.1.2, including the LRB and LFB. Determine sample volume. The sample volume may be measured directly in a graduated cylinder to the nearest 10 mL. To minimize the need to use a different graduated cylinder for each sample, an indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 10 g. After extraction, proceed to Section 11.3.5 for final volume determination. The LRB and LFB may be prepared by measuring 500 mL of reagent water into an erlenmeyer flask.

11.2.2 Add an aliquot of the SUR PDS (Sect. 7.2.1.2) to all samples and mix by swirling the sample. Addition of 10 uL of a 500 ug/mL SUR PDS to a 500 mL sample will result in a concentration of 10 ug/L.

11.2.3 If the sample is a LFB or LFM, add the necessary amount of analyte PDS. Swirl each sample to ensure all components are properly mixed.

11.2.4 Proceed with sample extraction. Refer to Section 11.3 if SPE cartridges are being used. Refer to Section 11.4 if SPE disks are being used.

11.3 CARTRIDGE SPE PROCEDURE – Proper conditioning of the solid phase can have a marked effect on method precision and accuracy. This section describes the SPE procedure using the equipment outlined in Section 6.9 in its simplest, least expensive mode without the use of the alternate transfer system or robotics systems (Sect. 6.9.2 and 6.9.6) This configuration was used to collect data presented in Section 17.

11.3.1 CARTRIDGE CONDITIONING – Once the conditioning of the cartridge is started, the cartridge must not be allowed to go dry until the last portion of the sample is filtered through it. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. However, if the cartridge goes dry during sample extraction, the analyte and surrogate recoveries may be affected. If this happens the analyst should make note of this as this sample may require re-extraction due to low surrogate recoveries.

11.3.1.1 CONDITIONING WITH METHANOL – Assemble the extraction cartridges into the vacuum manifold. Rinse each cartridge with two, 5 mL aliquots of methanol, allowing the sorbent to soak in the methanol for about 30 seconds by turning off the vacuum temporarily during the first rinsing. Do not allow the methanol level to go below the top of the cartridge packing.

11.3.1.2 CONDITIONING WITH REAGENT WATER – Follow the methanol rinse with two, 5 mL aliquots of reagent water being careful not to allow the water level to go below the cartridge packing. Turn off the vacuum. Add approximately 5 mL additional reagent water to the cartridge, and attach a reservoir (or transfer tube - Sect. 6.9.2) before adding sample to the cartridge.

11.3.2 CARTRIDGE EXTRACTION – Prepare samples, including QC samples, as specified in Section 11.2. The samples may be added to the cartridge using either a large reservoir attached to the cartridge or using a transfer tube from the sample bottle to the cartridge.

11.3.2.1 SAMPLE ADDITION USING RESERVOIRS – Attach a reservoir to the conditioned cartridge. Fill the reservoir (Sect. 6.9.2) with sample and then turn on the vacuum. Adjust the vacuum so that the

approximate flow rate is about 20 mL/min (minus 9-10 in Hg.). Care must be taken to add additional aliquots of sample to the reservoir to keep the cartridge packing from going dry before all the sample has been extracted. Rinse the sample container with reagent water and add to the reservoir after the last addition of sample, but before the cartridge goes dry. After all of the sample has passed through the SPE cartridge, detach the reservoir and draw air through the cartridge for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.3.2.3 SAMPLE ADDITION USING TRANSFER TUBES – If the sample transfer tubes are employed, make sure the transfer tube is attached to the conditioned cartridge before turning on the vacuum. Adjust the vacuum to a similar flow rate of approximately 20 mL/min (minus 9-10 in Hg). Rinse down the sample container with reagent water as it approaches dryness. After all of the sample has passed through the SPE cartridge, detach the transfer tube and draw air through the cartridge for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.3.3 CARTRIDGE ELUTION – Lift the extraction manifold top and insert collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridge. Add approximately 3 mL of methanol to the top of each cartridge. Pull enough of the methanol into the cartridge at low vacuum to soak the sorbent. Turn off the vacuum and vent the system. Allow the sorbent to soak in methanol for approximately 30 seconds. Start a low vacuum (minus 2-4 in Hg) and pull the methanol through in a dropwise fashion into the collection tube. Repeat this elution a second time with approximately 2 mL of methanol, and then a third time with approximately 1 mL.

11.3.4 EXTRACT CONCENTRATION – Concentrate the extract to about 0.5 mL in a warm water bath (at about 40^{0C}) under a gentle steam of nitrogen. Transfer to a 1 mL volumetric flask, rinsing the collection tube with small amounts of methanol. Adjust to volume with methanol. Filter the sample using a 1 mL syringe and filter (Sects. 6.7 & 6.8) into an appropriate autosampler vial.

11.3.5 SAMPLE VOLUME DETERMINATION – If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 10 mL. If using weight to determine volume, weigh the empty bottle to the nearest 10 g and determine the sample

weight by subtraction of the empty bottle from the original weight (Sect. 11.2.1). In either case, the sample volume will be used in the final calculations of analyte concentration (Sect. 12.4).

11.4 DISK SPE PROCEDURE – Proper conditioning of the solid phase can have a marked affect on method precision and accuracy. This section describes the SPE procedure using the equipment outlined in Section 6.10 in its simplest, least expensive mode without the use of robotics systems (Sect. 6.10.4). This configuration was used to collect data presented in Section 17.

11.4.1 DISK CONDITIONING – Once the conditioning of the disk is started, the disk must not be allowed to go dry until the last portion of the sample is filtered through it. If the disk goes dry during the conditioning phase, the conditioning must be started over. However, if the disk goes dry during sample extraction, the analyte and surrogate recoveries may be affected. If this happens the analyst should make note of this as this sample may require re-extraction due to low surrogate recoveries.

11.4.1.1 CONDITIONING WITH METHANOL – Assemble the extraction glassware in the vacuum manifold, placing the disks on a support screen between the funnel and base. Rinse each disk with two, 10 mL aliquots of methanol to the funnel, allowing the sorbent to soak for about 30 seconds by pulling approximately 1mL through the disk and turning off the vacuum temporarily during the first rinsing. Draw the methanol through the disk until it is 3-5 mm above the disk surface, adding more methanol if needed to keep the methanol from going below this level.

11.4.1.2 CONDITIONING WITH WATER – Follow the methanol rinse with two, 10 mL aliquots of reagent water being careful to keep the water level at 3-5 mm above the disk surface. Turn off the vacuum.

11.4.2 DISK EXTRACTION – Prepare samples, including QC samples, as specified in Section 11.2. Fill the extraction funnel containing the conditioned disk with sample and turn on the vacuum. Care must be taken to add additional aliquots of sample to the funnel to keep the disk from going dry before all the sample has been extracted. Rinse the sample container with reagent water and add to the funnel after the last addition of sample, but before the disk goes dry. After all of the sample has passed through the SPE disk, draw air through the disk for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.4.3 DISK ELUTION – Detach the glassware base from the manifold without disassembling the funnel from the base. Dry the underside of the base. Insert collection tubes into the manifold to collect the extracts as they are eluted from the disk. The collection tubes must fit around the drip tip of the base to ensure the collection of all of the eluent. Reattach the base to the manifold. Add approximately 5 mL of methanol to the top of each disk. Pull enough of the methanol into the disk to soak the sorbent. Turn off the vacuum and vent the system. Allow the sorbent to soak in methanol for approximately 30 seconds. Start the vacuum and pull the methanol through. Attempt to pull the methanol through in a dropwise fashion into the collection tube by slowly turning the valve (which controls the flow through the funnel) until the methanol starts eluting into the collection tube. Repeat this elution a second time with approximately 4 mL of methanol, and then a third time with approximately 3 mL.

11.4.4 EXTRACT CONCENTRATION – Proceed with extract concentration as in Section 11.3.4.

11.4.5 SAMPLE VOLUME DETERMINATION – Proceed with sample volume determination as in Section 11.3.5.

11.5 SOLVENT EXCHANGE FOR CONFIRMATION ANALYSIS – Samples that will be confirmed must be exchanged into reagent water/acetonitrile (60/40). To accomplish this, transfer the remaining 980 uL of the extract to a 1 mL volumetric (or other appropriate collection tube). Mark the sample volume. Take the extract to dryness in a warm water bath (at ~ 40^0C) under a gentle steam of nitrogen. Reconstitute the residue with a mixture of reagent water/acetonitrile (60/40) to the mark made before the extract was taken to dryness. Care must be taken to redissolve the film as thoroughly as possible. Use of a vortex mixer is recommended. Transfer to an appropriate autosampler vial.

Note: Recovery experiments have been conducted to investigate the effect of drying time on compound recoveries by allowing the extract to sit an additional 1, 2, or 4 hours in the bath after being taken to dryness. These studies indicate that: 1) the best recoveries are obtained when extracts are reconstituted immediately after the methanol is removed; 2) recoveries decrease with increasing time in the bath; and 3) carbazole recovery is the most sensitive to the additional time. In our experiments, carbazole had acceptable recovery criteria (Sect. 12.3) at 2 additional hours of drying, but had exceeded the acceptable range after 4 hours of additional time.

11.6 ANALYSIS OF SAMPLE EXTRACTS

11.6.1 Establish operating conditions as summarized in Table 1 of Section 17 for the

HPLC system. Confirm that retention times, compound separation and resolution on the primary and secondary columns are similar to those listed in Tables 1 and 2 and Figures 1 and 2, respectively.

11.6.2 Determine the optimal injection volume. Establish a valid initial calibration following the procedures outlined in Section 10.2 using the optimum injection size. Complete the IDC requirements described in Section 9.2.

11.6.3 Establish an appropriate retention time window for each target and surrogate to identify them in the QC and field samples. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the HPLC over the course of time. Plus or minus three times the standard deviation of the retention time for each compound while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

11.6.4 Check system calibration by analyzing a CCC (Sect. 10.3) and begin to inject aliquots of field and QC samples using the same injection volume and conditions used to analyze the initial calibration.

11.6.5 The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with methanol. Acceptable surrogate performance (Sect. 9.7) should be determined from the undiluted sample extract. If confirmation analyses require dilution, this must be done using reagent water/acetonitrile (Sect. 11.5) Incorporate the dilution factor into final concentration calculations. Any dilutions will also affect analyte MRL.

12. DATA ANALYSIS AND CALCULATION

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to retention time of an analyte peak in a calibration standard or the laboratory fortified blank. Surrogate retention times should be confirmed to be within acceptance limits (Sect. 11.6.3) even if no target compounds are detected.

12.2 Calculate the analyte concentrations using the initial calibration curve generated as described in Section 10.2. Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (Sect. 11.6.5).

12.3 Positive results should be confirmed on the confirmation column (Sect. 17, Table 2) that has been initially calibrated, and confirmed to still be in calibration by analyzing appropriate CCCs (Sect. 10.3) prior to the confirmation analysis. Quantitated values for the targets and surrogates on the confirmation column should be 50 - 150% of the primary column result. If so, report the more accurate primary column result. If not, report the lower of the 2 values and mark the results as suspect/confirmation to inform the data user that the results are suspect due to lack of confirmation. If values are taken from the confirmation column, both surrogates must meet recovery acceptance criteria.
Note: The PDA spectra of these compounds do not have sufficient resolution to definitively identify each target compound in this method.

12.4 Adjust the calculated concentrations of the detected analytes to reflect the initial sample volume and any dilutions performed.

12.5 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

12.6 Analyte concentrations are reported in ug/L. Calculations should use all available digits of precision, but final concentrations should be rounded to an appropriate number of significant figures.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND MDLs – Method detection limits (MDLs) are presented in Table 3 and were calculated using the formula present in Section 9.2.4. Tables for these data are presented in Section 17. Single laboratory precision and accuracy data are presented for three water matrices: reagent water (Table 5); chlorinated, "finished" surface water (Table 6); and chlorinated, "finished" ground water (Table 7).

13.2 COMPOUND STABILITY – Thidiazuron and diflubenzuron are extremely sensitive to free chlorine. LFM recoveries were found to drop to 0% for thidiazuron and about 5% for diflubenzuron in the time required to extract samples in the presence of free chlorine. Other analytes are sensitive to free chlorine over time, even within the recommended sample holding time of 14 days. For example, LFM recoveries for diuron drop to about 12% on day 14 in a finished surface water with a residual free chlorine concentration of 0.8 mg/L. This illustrates the importance of proper sample preservation (Sect. 8). During method development, it was experimentally determined that the 2.5 g Trizma preset crystals (Sect. 7.1.6.2) was sufficient to dechlorinate a 500 mL sample with up to approximately 10 mg/L free chlorine as measured colorimetrically. This level is about 2.5 times the maximum allowable free chlorine residual in municipal tap waters promulgated in Stage I of the Disinfectant/Disinfection By-product Rule[6] and so should be sufficient to

reduce free chlorine in samples from public water systems.

13.3 ANALYTE STABILITY STUDIES

13.3.1 FIELD SAMPLES – Chlorinated finished surface water samples, fortified with method analytes at 10 ug/L, were preserved and stored as required in Section 8. The average of triplicate analyses, conducted on days 0, 2, 7, and 14, are presented in Section 17, Table 8. These data document the 14-day sample holding time.

13.3.2 EXTRACTS – Extracts from the day 0 extract holding time study described above were stored below 0 °C and analyzed on days 0, 8, 14, and 21. The data presented in Section 17, Table 9, document the 21-day extract holding time.

14. POLLUTION PREVENTION

14.1 This method utilizes solid phase extraction technology to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, D.C., 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE PRIMARY COLUMN

Peak Number (Figure 1)	Analyte	Retention Time (min.)
1	Tebuthiuron	2.03
2	Thidiazuron	2.48
3	Monuron (SUR)	2.80
4	Fluometuron	4.45
5	Diuron	5.17
6	Propanil	8.53
7	Siduron A	8.91
8	Siduron B	9.76
9	Linuron	11.0
10	Carbazole (SUR)	12.8
11	Diflubenzuron	13.9

Primary Column: Symmetry 4.6 × 150 mm packed with 3.5 um C_{18} stationary phase.

Conditions:

Solvent A	25 mM phosphate buffer
Solvent B	acetonitrile
40% B	0-9.5 minutes
linear gradient 40-50% B	9.5-10.0 minutes
linear gradient 50-60% B	10.0-14.0 minutes
linear gradient 60-40%B	14.0-15.0 minutes
Flow rate	1.5 mL/min
Wavelength	245 nm
Equilibration time prior to next injection 15 minutes.	

TABLE 2. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE CONFIRMATION COLUMN

Peak Number (Figure 1)	Analyte	Retention Time (min.)
1	Tebuthiuron	2.56
2	Monuron (SUR)	3.98
3	Thidiazuron	4.93
4	Fluometuron	5.94
5	Diuron	7.67
6	Siduron A	9.53
7	Propanil	10.1
8	Siduron B	10.8
9	Linuron	12.2
10	Carbazole (SUR)	14.3
11	Diflubenzuron	15.2

Confirmation Column: Supelcosil 4.6 × 150 mm packed with 5 um cyanopropyl stationary phase.

Conditions:

Solvent A	25 mM phosphate buffer
Solvent B	acetonitrile
linear gradient 20% B	0-11.0 minutes, 1.5 mL/min. flow rate
linear gradient 20-40% B	11.0-12.0 minutes, 1.5 mL/min flow rate
40% B	hold to 16 minutes at 1.5 mL/min. flow rate, step to 2.0 mL/min flow rate at 16 min. and hold to 20 min.
linear gradient 40-20% B	20.0-20.1 minutes, 2.0 mL/min. flow rate
Wavelength	240 nm
Equilibration time prior to next injection 15 minutes.	

TABLE 3. METHOD DETECTION LIMITS FOR 2 INJECTION VOLUMES IN REAGENT WATER WITH CARTRIDGE AND DISK EXTRACTION TECHNIQUES ON BOTH THE PRIMARY AND CONFIRMATION COLUMNS

Table 3A. Cartridge Extraction, Primary Column				
Analyte	**Spiking Conc. (ug/L)**		**MDL[a] (ug/L)**	
	20 uL inj.	10 uL inj.	20 uL inj.	10 uL inj.
Tebuthiuron	0.050	0.100	0.032	0.071
Thidiazuron	0.100	0.100	0.035	0.047
Fluometuron	0.050	0.100	0.013	0.027
Diuron	0.050	0.100	0.010	0.026
Propanil	0.050	0.300	0.023	0.084
Siduron A&B	0.100	0.600	0.024	0.091
Linuron	0.050	0.300	0.062	0.067
Diflubenzuron	0.050	0.100	0.014	0.033

Table 3B. Disk Extractions, Primary Column, 20 uL Injection		
Analyte	**Spiking Conc. (ug/L)**	**MDL[a] (ug/L)**
Tebuthiuron	0.050	0.046
Thidiazuron	0.100	0.047
Fluometuron	0.050	0.028
Diuron	0.050	0.018
Propanil	0.100	0.071
Siduron A&B	0.100	0.067
Linuron	0.100	0.032
Diflubenzuron	0.050	0.035

[a] Method detection limit samples extracted over 3 days for 7 replicates and analyzed using conditions described in Table 1.

Table 3C. Cartridge Extraction, Confirmation Column, 20 uL Injection		
Analyte	**Spiking Conc. (ug/L)**	**MDL[b] (ug/L)**
Tebuthiuron	0.300	0.145
Thidiazuron	0.300	0.143
Fluometuron	0.300	0.065[c]
Diuron	0.300	0.056
Propanil	0.300	0.066
Siduron A&B	0.300	0.136
Linuron	0.300	0.085
Diflubenzuron	0.300	0.126

[b] Method detection limit samples extracted over 3 days for 7 replicates and analyzed using conditions described in Table 2.

[c] MDLs for fluometuron were reinjected due to an interfering peak which tended to coelute with fluometuron.

TABLE 4. PRECISION, ACCURACY AND SIGNAL-TO-NOISE COMPARISON FOR TWO HPLC SYSTEMS FOR LOW LEVEL SPIKES IN REAGENT WATER EXTRACTED WITH CARTRIDGES AND ANALYZED USING THE PRIMARY COLUMN

Table 4. Precision, Accuracy and S/N Comparison, 10 uL Injection, Primary Column						
Analyte	HPLC System #1[a]			HPLC System #2[b]		
	Concentration = 1.0 ug/L(n=7)			Concentration = 1.0 ug/L(n=7)		
	Mean % Rec.[c]	RSD (%)	S/N Ratio[d]	Mean % Rec.	RSD (%)	S/N Ratio[c]
Tebuthiuron	100	1.8	84	105	2.7	17
Thidiazuron	103	2.4	33	102	1.9	36
Fluometuron	99	2.2	43	104	2.3	35
Diuron	102	1.8	44	104	3.8	34
Propanil	96	3.6	28	100	6.4	16
Siduron A&B[a]	100	3.0	16	109	11	40
Linuron	108	1.6	12	103	4.1	21
Diflubenzuron	100	2.0	22	98	3.3	11
Monuron (SUR)[a]	105	2.8	NC	94	2.3	NC
Carbazole (SUR)[a]	97	2.7	NC	104	2.7	NC

NC: Not Calculated

[a]HPLC System 1 was equipped with a photodiode array detector that employed a 1 cm path length.

[b]HPLC System 2 was equipped with a photodiode array detector that employed a 5 cm path length.

[c]Seven replicates of the low level reagent water spikes were processed through the cartridge extraction procedure and injected on both instruments.

[d]Signal-to-noise ratios were calculated for each peak by dividing the peak height for each compound by the peak-to-peak noise for each peak, which was determined for each component from the method blank over a period of time equal to the full peak width.

TABLE 5. PRECISION AND ACCURACY DATA IN REAGENT WATER

Table 5A. Cartridge Extraction, Primary Column, 20 uL Injection				
Analyte	**Concentration = 1 ug/L (n=7)**		**Concentration = 30 ug/L (n=7)**	
	Mean Recovery (%)	**Relative Standard Deviation (%)**	**Mean Recovery (%)**	**Relative Standard Deviation (%)**
Tebuthiuron	107	2.4	97.9	1.4
Thidiazuron	106	1.1	96.7	1.6
Fluometuron	106	0.9	97.6	1.5
Diuron	107	1.0	97.5	0.0
Propanil	105	1.7	97.2	1.4
Siduron A&B	106	1.9	97.8	1.6
Linuron	104	1.6	97.4	1.5
Diflubenzuron	107	1.2	96.0	1.4
Monuron (SUR)[a]	100	1.8	100.	2.0
Carbazole (SUR)[a]	96.7	1.0	96.1	1.5

Table 5B. Disk Extractions, Primary Column, 20 uL Injection				
Analyte	**Concentration = 1 ug/L (n=7)**		**Concentration = 30 ug/L(n=7)**	
	Mean Recovery (%)	**Relative Standard Deviation (%)**	**Mean Recovery (%)**	**Relative Standard Deviation (%)**
Tebuthiuron	104	2.2	101	2.3
Thidiazuron	99.8	4.8	101	2.4
Fluometuron	100	4.2	101	2.3
Diuron	104	5.9	101	3.2
Propanil	101	2.7	101	2.3
Siduron A&B	110	5.0	103	2.3
Linuron	99.2	4.8	99.0	2.5
Diflubenzuron	102	4.2	100	2.5
Monuron (SUR)[a]	102	2.6	99.0	2.8
Carbazole (SUR)[a]	96.9	3.3	95.1	3.0

[a]Surrogate concentration in all samples is 10 ug/L. Chromatographic conditions are described in Table

1.

Table 5C. Cartridge Extraction, Confirmation Column, 20 uL Injection				
Analyte	**Concentration = 1 ug/L (n=7)**		**Concentration = 30 ug/L (n=7)**	
	Mean % Recovery	**Relative Standard Deviation (%)**	**Mean % Recovery**	**Relative Standard Deviation (%)**
Tebuthiuron	100	4.9	103	4.8
Thidiazuron	93.6	5.1	101	4.8
Fluometuron	87.6	12.4	97.0	5.7
Diuron	96.9	3.9	102	2.7
Propanil	88.0	5.9	103	4.9
Siduron A&B[a]	91.0	7.0	103	5.0
Linuron	79.8	13.3	99.8	5.6
Diflubenzuron	83.7	6.9	80.4	18.6
Monuron (SUR)[b]	105	4.3	102	4.5
Carbazole (SUR)[b]	93.8	6.0	83.6	7.6

[a]Total siduron concentration is twice the concentration of the other target analytes: 2.0 and 60.0 ug/L.

[b]Surrogate concentration in all samples is 10 ug/L. Conditions described in Table 2.

TABLE 6. PRECISION AND ACCURACY[a] OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED SURFACE WATER USING CARTRIDGES

Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Tebuthiuron	108	1.6	96	1.7
Thidiazuron	81	2.9	84	1.9
Fluometuron	109	1.0	96	1.5
Diuron	110	1.7	96	1.6
Propanil	104	1.8	96	1.5
Siduron A&B	102	1.8	96	1.6
Linuron	102	1.3	96	1.6
Diflubenzuron	109	1.5	95	1.7
Monuron (SUR)[b]	103	2.3	97	2.3
Carbazole (SUR)[b]	98	2.7	94	1.9

[a]All data collected using a 20 uL injection volume on the primary column and conditions described in Table 1.

[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 7. PRECISION AND ACCURACY[a] OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED GROUND WATER USING CARTRIDGES

Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation	Mean % Recovery	Relative Standard Deviation
Tebuthiuron	109	1.5	99	1.2
Thidiazuron	95	2.9	94	1.2
Fluometuron	103	1.9	97	1.9
Diuron	106	2.1	99	1.1
Propanil	106	1.9	99	1.1
Siduron A&B	106	4.2	99	1.2
Linuron	101	2.7	98	1.9
Diflubenzuron	104	2.6	98	1.0
Monuron (SUR)[b]	100	1.3	99	1.0
Carbazole (SUR)[b]	95	1.3	89	4.7

[a]All data collected using a 20 uL injection volume on the primary column and conditions described in Table 1.

[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 8. SAMPLE HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 10 ug/L, WITH CUPRIC SULFATE AND TRIZMA (Sect. 8.1.2)

Analyte	Day 0 % Recovery	Day 2 % Recovery	Day 7 % Recovery	Day 14 % Recovery
Tebuthiuron	93	95	98	96
Thidiazuron	72	76	84	77
Fluometuron	93	95	98	97
Diuron	94	97	99	97
Propanil	93	97	98	98
Siduron A&B	92	96	98	98
Linuron	93	97	98	99
Diflubenzuron	93	95	98	97
Monuron (SUR)[b]	96	101	103	102
Carbazole (SUR)[b]	88	96	92	95

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery] × 100) for replicate analyses were all less than 7.0%.
Samples analyzed using a 20 uL injection volume and conditions described in Table 1.
[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 9. EXTRACT HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 10 ug/L, WITH CUPRIC SULFATE AND TRIZMA (Sect. 8.1.2)

Analyte	Initial Injection[b] % Recovery	Day 8 Reinjection % Recovery	Day 14 Reinjection % Recovery	Day 21 Reinjection % Recovery
Tebuthiuron	93	95	93	103
Thidiazuron	72	73	71	80
Fluometuron	93	95	93	107
Diuron	94	96	94	99
Propanil	93	96	93	105
Siduron A&B	92	94	93	106
Linuron	93	97	93	102
Diflubenzuron	93	95	93	107
Monuron (SUR)	96	98	96	94
Carbazole (SUR)	88	90	89	91

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery] × 100) for replicate analyses were all less than 5.0%.
[b]Same as day 0 sample hold time analysis.
Extract stability study consisted of the analysis of day 0 extracts stored at < 0^{O} C and reinjected. Samples were analyzed using a 20 uL injection volume and conditions described in Table 1.

TABLE 10. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all target analytes are below ½ the intended MRL or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantitation of method analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at 10 ug/L (or mid cal).	RSD must be $\leq$ 20%.
Sect. 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery for replicates used in IDP.	Mean recovery $\pm$ 20% of true value.
Sect. 9.2.4	Method Detection Limit (MDL) Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the MDL. Analyze the replicates through all steps of the analysis. Calculate the MDL using the equation in Sect. 9.2.4.	Data from MDL replicates are not required to meet method precision and accuracy criteria. If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.
Sect. 10.2.3	Peak Gaussian Factor (PGF)	Calculated prior to establishing the initial calibration.	A PGF range of 0.90 to 1.10 is acceptable.

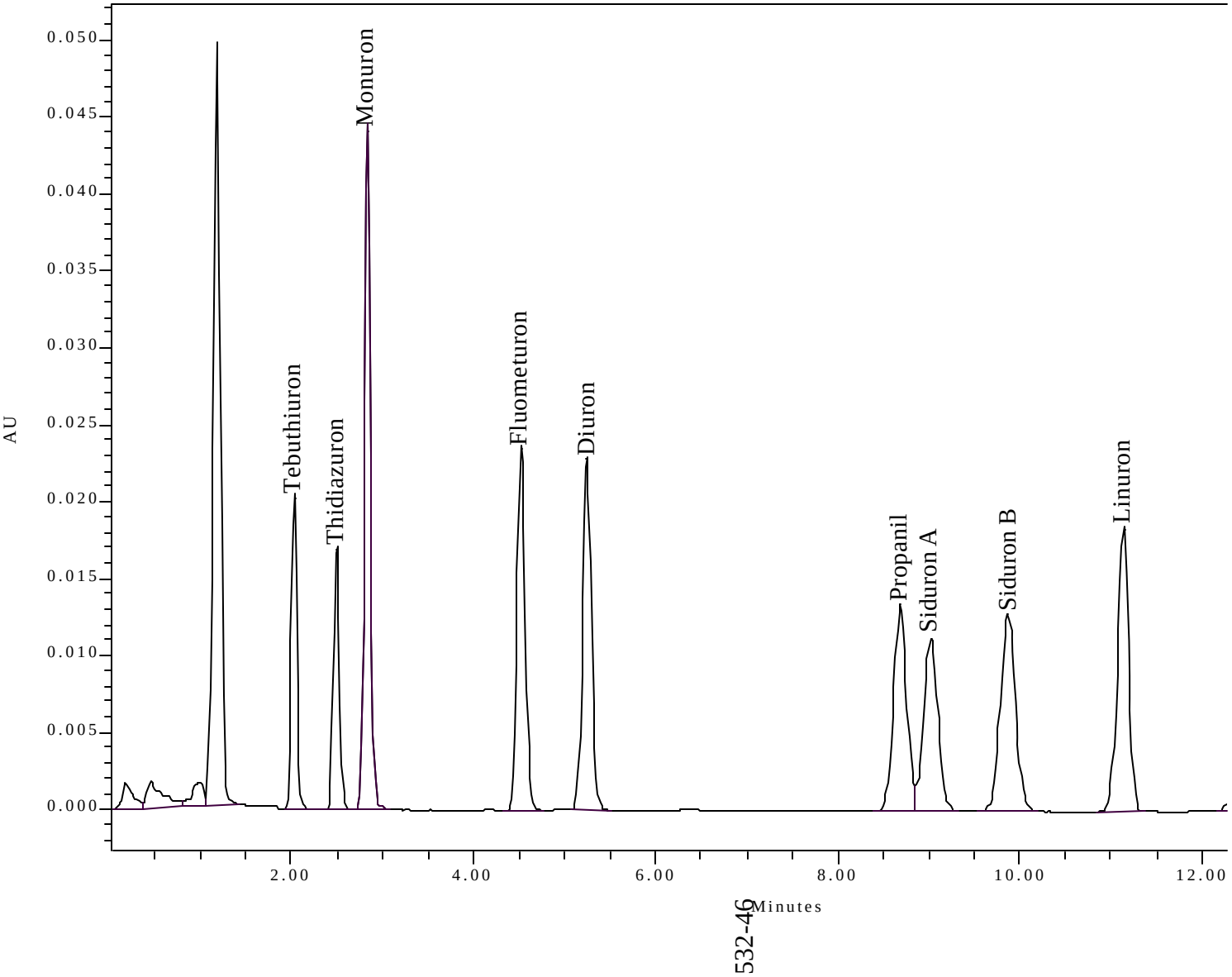
TABLE 11. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.4	Sample and Extract Holding Times	Properly preserved samples must be shipped at or below 10^0C and may be held in the lab at or below 6^0C for 14 days. Extracts in methanol may be stored at or below 0^0C for up to 21 days after extraction. Samples exchanged into 60/40 reagent water/acetonitrile may be held at or below 0^0C for 7 days, with the combined extract hold time not to exceed 21 days.	Do not report data for samples or extracts that have not been properly preserved or stored, or that have exceeded their holding time.
Sect. 9.4	Laboratory Reagent Blank (LRB)	Include LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free from interferences. Each analysis batch (Sect. 3.2) must include either a LRB or an instrument blank after the initial low level CCC injection.	Demonstrate that all target analytes are below 1/3 the intended MRL or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantitation of method analytes.
Sect. 9.7	Surrogate Standards	Surrogate standards are added to all calibration standards and samples, including QC samples.	Surrogate recovery must be 70-130% of the true value.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.8	Laboratory Fortified Sample Matrix (LFM)	With each extraction batch (Sect. 3.1), a minimum of one LFM is extracted and analyzed. A duplicate LFM, or LFMD, should be extracted when occurrence of target analytes is low. Laboratory duplicate analysis is not required for extraction batches containing a LFMD.	Recoveries not within 70-130% of the fortified amount may indicate a matrix effect, with the exception of thidiazuron which should have recoveries of 60-120%. If a LFMD is analyzed instead of a Laboratory Duplicate , target RPDs should be $\pm$ 30%.
Sect. 9.9	Field Duplicates (LD1 and LD2)	Extract and analyze at least one duplicate with each extraction batch (20 samples or less). A Laboratory Fortified Sample Matrix Duplicate may be substituted for a Field Duplicate when the occurrence of target analytes is low.	RPDs should be $\pm$ 30%.
Sect. 9.10	Quality Control Sample	Analyze either as a CCC or a LFB. Analyze at least quarterly, with each initial calibration, or when preparing new standards.	A QCS analyzed as a CCC will have the same acceptance criteria as a CCC, while a QCS analyzed as a LFB will have the same criteria as a LFB.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 10.2	Initial Calibration	Use external standard calibration technique to generate a calibration curve with five standards that span the approximate range of 1 to 30 ug/L sample concentration. Calibration curve fit options are discussed in Sect. 10.2.4. Analyze a QCS.	QCS must be ± 30% of true value. When each calibration standard is calculated as an unknown using the calibration curve, the results must be 70-130% of the true value for all but the lowest standard. The lowest standard must be 50-150% of the true value. The lowest CAL standard concentration must be as low or lower than the intended MRL.
Sect. 10.2.3	Peak Gaussian Factor (PGF)	Calculated prior to establishing the initial calibration.	A PGF range of 0.90 to 1.10 is acceptable.
Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte must be 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be 50-150% of the true value.
Sect. 12.3	Confirmation Column Results	Positive results should be confirmed using a chemically dissimilar column.	Quantitated target values should be ±50% of the primary result. Primary column results should be reported. If reporting confirmation results, both surrogates must meet ± 30% criteria.

Figure 1. Primar



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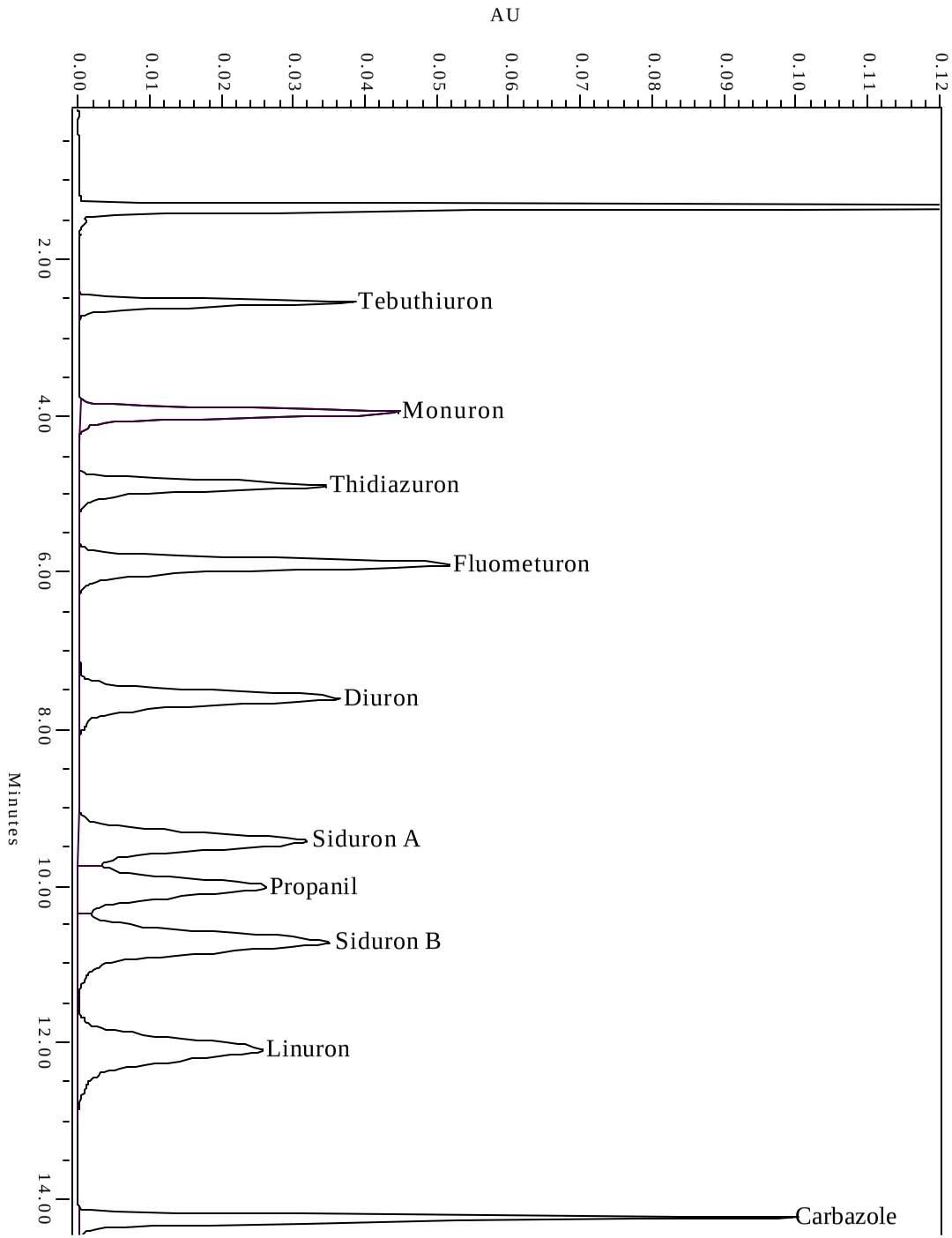

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Figure 2. Con