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METHOD 535. MEASUREMENT OF CHLOROACETANILIDE AND OTHER ACETAMIDE HERBICIDE DEGRADATES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

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METHOD 535

MEASUREMENT OF CHLOROACETANILIDE AND OTHER ACETAMIDE HERBICIDE DEGRADATES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1 This is a liquid chromatographic (LC)/tandem mass spectrometric (MS/MS) method applicable to the determination of the ethanesulfonic acid (ESA) and oxanilic acid (OA) degradates of the chloroacetanilide and other acetamide herbicides in finished drinking waters. The single laboratory Lowest Concentration Minimum Reporting Level (LCMRL) has also been determined in reagent water.[1] The following compounds can be determined using this method:

Analyte	Chemical Abstracts Service (CAS) Registry Number
Acetochlor ESA	187022-11-3
Acetochlor OA	184992-44-4
Alachlor ESA	142363-53-9
Alachlor OA	171262-17-2
Dimethenamid ESA	205939-58-8
Dimethenamid OA	—
Flufenacet ESA	—
Flufenacet OA	—
Metolachlor ESA	171118-09-5
Metolachlor OA	152019-73-3
Propachlor ESA	—
Propachlor OA	—

1.2 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended

use of this method. The lowest concentration MRL (LCMRL) is a single laboratory determination of the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50 and 150% recovery. The procedure used to determine the LCMRL is described elsewhere.[1] Single laboratory LCMRLs for analytes in this method range from 0.016 - 0.11 µg/L for the ion trap instrument and 0.22 - 0.55 µg/L for the triple quadrupole instrument, and are listed in Table 5.

1.3 Laboratories using this method are not required to determine the LCMRL for this method, but must demonstrate that their laboratory MRL for this method meets the requirements described in Section 9.2.4.

1.4 Detection limit (DL) is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[2] The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance. Determining the DL for analytes in this method is optional (Sect. 9.2.5). DLs for target analytes range from 0.0060 - 0.043 µg/L for the ion trap instrument and 0.057 - 0.11 µg/L for the triple quadrupole instrument, and are listed in Table 5.

1.5 This method is intended for use by or under the supervision of analysts skilled in solid phase extractions, LC/MS/MS analysis and the interpretation of LC/MS/MS chromatograms and mass spectra.

2. SUMMARY OF METHOD

2.1 Analytes and surrogate are extracted and concentrated by passing a 250-mL water sample through a solid phase extraction (SPE) cartridge containing 0.5 g of nonporous graphitized carbon. The compounds are eluted from the solid phase with a small quantity of methanol containing 10 mM ammonium acetate. The methanol extract is then concentrated to dryness and reconstituted with 1 mL of water containing 5 mM ammonium acetate. The analytes are chromatographically separated by injecting an aqueous aliquot (100 µL) into an LC system equipped with a reversed phase (C_{18}) column. After elution from the column, the analytes are detected by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The concentration of each identified component is measured by an internal standard procedure, i.e. relating the product ion response of that compound to the product ion response of the compound that is used as an internal standard. A surrogate analyte, whose concentration is known in every sample, is measured with the same internal standard calibration procedure.

3. DEFINITIONS

3.1 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 (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of field samples.

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

3.3 COLLISIONALLY ACTIVATED DISSOCIATION (CAD) – The process of converting the precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

3.4 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the target analytes, internal standard(s) and surrogate(s), which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5 DETECTION LIMIT (DL) – 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. 9.2.5), and accurate quantitation is not expected at this level.[2]

3.6 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, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

3.7 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, as well as laboratory procedures.

3.8 INTERNAL STANDARD (IS) – A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses

of other target analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.9 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the target analytes and all the preservation compounds are added in the laboratory. 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.

3.10 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of a preserved field sample to which known quantities of the target analytes are added in the laboratory. The LFSM is processed and 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 sample extraction and the measured values in the LFSM corrected for background concentrations.

3.11 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the field sample used to prepare the LFSM. The LFSMD is fortified, extracted, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the occurrence of target analytes are low.

3.12 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 and reagents, sample preservatives, internal standard, and surrogates that are used in the analysis batch. The LRB is used to determine if target analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.13 LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50 and 150% recovery.[1]

3.14 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.

3.15 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 calibration standard for that analyte and can only be used if acceptable

quality control criteria for this standard are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.4.

3.16 PRECURSOR ION – For the purpose of this method, the precursor ion is the deprotonated molecule ($[M-H]^-$) of the target analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*.

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

3.18 PRODUCT ION – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisionally activated dissociation of the precursor ion.

3.19 QUALITY CONTROL SAMPLE (QCS) – A solution of target 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 laboratory performance with externally prepared test materials.

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

3.21 SURROGATE ANALYTE (SUR) – A pure analyte, which chemically resembles target analytes and is extremely unlikely to be found in any sample. This analyte is added to a sample aliquot in known amount(s) before 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.

4. INTERFERENCES

4.1 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. After analysis of a sample containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample. In the case of automated analysis, the analyst may not be aware of high concentration samples until after an entire batch is analyzed. In this situation, the analyst should carefully review data from samples analyzed immediately after high concentration samples, and reanalyze them if necessary.

4.2 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 of the water. Humic and/or fulvic material is co-extracted by this method and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the carbon SPE. For this method, total organic carbon (TOC) is a good indicator of humic content of the sample. See Section 13.4 for more details.

4.3 The carbon solid phase cartridges did not produce any interferences during method development. However, brands and lots of carbon cartridges should be tested to ensure that contamination does not preclude analyte identification and quantitation. The analysis of laboratory reagent blanks can provide important information regarding the presence or absence of such interferences.

4.4 Method interferences may 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 target 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.**

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 a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis.

5.2 Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.[3-5]

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

6.1 GLASSWARE – 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.

6.2 SAMPLE CONTAINERS – Amber glass bottles (250 mL or larger) fitted with PTFE (polytetrafluoroethylene) lined screw caps.

6.3 VIALS – Amber glass autosampler vials with PTFE faced septa for storing standards and extracts.

6.4 VOLUMETRIC FLASKS – Class A, various sizes used for preparation of standards.

6.5 GRADUATED CYLINDERS – Various sizes.

6.6 MICRO SYRINGES – Various sizes.

6.7 BALANCE – Analytical, capable of accurately weighing to 0.0001 g.

6.8 DISPOSABLE PIPETTES – 1 mL, used to transfer sample extracts to autosampler vials.

6.9 CONICAL COLLECTION TUBES –15 mL, or other glassware suitable for collection of the eluent from the solid phase cartridge after extraction.

6.10 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

6.10.1 SPE CARTRIDGES – 0.5 g, 6 mL SPE cartridges containing a nonporous graphitized carbon sorbent phase (Supelclean ENVI-Carb SPE cartridges, Supelco Catalog No. 57904 or equivalent).

6.10.2 VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold (Supelco Cat. No. 57030 and 57275 or equivalent) for cartridge extractions, or an automatic/robotic sample preparation (Zymark Autotrace SPE Workstation or equivalent) system designed for use with solid phase extraction cartridges, may be used if all quality control requirements discussed in Section 9 are met. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system.

6.10.3 SAMPLE DELIVERY SYSTEM – Use of a transfer tube system (Supelco "Visiprep"; cat. #: 57275 or equivalent), which transfers the sample directly from the sample container to the SPE cartridge, is recommended. Sample reservoirs, which attach to the cartridge, may be used, although they hold only 75 mL at one time.

6.10.4 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 25 cm (10 in) of mercury.

6.11 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by blowdown with nitrogen using a water bath set at 60-65 °C (Meyer N-EVAP, model 111, Organomation Associates, Inc. or equivalent). Other types of evaporation/concentration equipment may be used as long as all QC requirements in Section 9 are met.

6.12 LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER(MS/MS)/DATA SYSTEM

6.12.1 LC SYSTEM – Capable of reproducibly injecting up to 100-µL aliquots, and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method, e.g., 0.4 mL/min. The use of a column heater is required. During method development, the column was heated to 65 °C to achieve adequate separation of alachlor ESA and acetochlor ESA, although other temperatures may be used as long as the resolution requirement in Section 9.10 is met. The LC must be capable of pumping the water/methanol mobile phase without the use of a vacuum degasser. Vacuum degassers will volatilize the ammonium acetate mobile phase causing the analyte peaks to shift to earlier retention times over the course of the analysis batch. Sparging the mobile phase prior to use for a short time period (approximately 5 minutes) is acceptable.

6.12.2 LC/Tandem Mass Spectrometer – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.4 mL/min. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.18) for the target analytes within specified retention time segments. A minimum of 20 scans across the chromatographic peak is recommended to ensure adequate precision. Data is demonstrated in Tables 5-9 using an ion trap mass spectrometer (Finnigan LCQ Deca) and a triple quadrupole mass spectrometer (Micromass Quattro Micro).

6.12.3 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.12.4 ANALYTICAL COLUMN – An LC C_{18} column (2.1 x 100 mm) packed with 5 µm d_p C_{18} solid phase particles (Agilent # : 79916OD-552 or equivalent) was used on the triple quadrupole instrument. For the ion trap instrument, an LC C_{18} column (2.1 x 200 mm) packed with 5 µm d_p C_{18} solid phase particles (Agilent # : 79916OD-572 or equivalent) was used to achieve more separation between target analytes. Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used. The C_{18} columns employed in the method development were used for over 10 months with no measurable degradation due to the elevated column temperatures (60 to 70 °C).

7. REAGENTS AND STANDARDS

7.1 GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals should be used. 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 greater than 1/3 the MRL for each compound of interest.

7.1.2 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher Optima grade or equivalent).

7.1.3 AMMONIUM ACETATE ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade or equivalent).

7.1.4 10 mM AMMONIUM ACETATE/METHANOL – To prepare 1 L, add 0.7708 g ammonium acetate to 1L of methanol. During method development, this solution was shown to be stable for at least 18 days.

7.1.5 5 mM AMMONIUM ACETATE/REAGENT WATER – To prepare 1 L, add 0.3854 g ammonium acetate to 1 L of reagent water (pH~6.5). This solution is prone to volatility losses and should be replaced at least every 48 hours.

7.1.6 AMMONIUM CHLORIDE (NH_4Cl, CAS#: 12125-02-9) – Acts as a sample dechlorinating agent (Sect. 8.1.1).

7.1.7 NITROGEN – Used for the following purposes.

7.1.7.1 Nitrogen aids in aerosol generation of the ESI liquid spray and used as collision gas in some MS/MS instruments. The nitrogen used should meet or exceed instrument manufacturer's specifications.

7.1.7.2 Nitrogen is used to concentrate sample extracts, Ultra High Purity or equivalent.

7.1.8 ARGON (OPTIONAL) – Can be used as collision gas in some MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications.

7.2 STANDARD SOLUTIONS – Solution concentrations listed in this Section were used to develop this method and are included as an example. If compounds used to prepare solutions are 96% pure or greater, the weight may be used without correction for purity to calculate the concentration of the stock standard. 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 use standard QC practices to determine when Standard Solutions described in this section need to be replaced.**

7.2.1 INTERNAL STANDARD ANALYTE (IS) STANDARD SOLUTION – BUTACHLOR ESA – This IS was not commercially available when the method was developed. Thus, the IS was synthesized by the procedure listed in Appendix A of this method. Commercially available standards may be used, if available. Alternate internal standards may be used provided they meet the definition of an IS (Sect. 3.8) and they meet the QC requirements in Section 9.6.

7.2.1.1 IS STOCK SOLUTION – If preparing from neat material, accurately weigh approximately 5 mg of the neat IS to the nearest 0.1 mg into a tared, 1-mL volumetric flask. Dilute to the mark with methanol. Stock solutions have been shown to be stable for 6 months when stored at 4 °C or less.

7.2.1.2 IS PRIMARY DILUTION STANDARD (IS PDS) – Prepare the IS PDS by adding enough of the IS stock standard to a volumetric flask partially filled with methanol to make a final concentration near 10 µg/mL when filled to the mark with methanol. The IS

PDS has been shown to be stable for 6 months when stored at 4 °C or less.

7.2.2 SURROGATE ANALYTE (SUR) STANDARD SOLUTION, DIMETHACHLOR ESA – This SUR was not commercially available when the method was developed. Thus, the SUR was synthesized by the procedure listed in Appendix B of this method. Commercially available standards may be used, if available. Alternate surrogates may be used provided they meet the definition of a surrogate (Sect. 3.21) and they meet the QC requirements in Section 9.7.

7.2.2.1 SUR STOCK SOLUTION – If preparing from neat material, accurately weigh approximately 5 mg of the neat SUR to the nearest 0.1 mg into a tared, 1-mL volumetric flask. Dilute to the mark with methanol. Stock solutions have been shown to be stable for 6 months when stored at 4 °C or less.

7.2.2.2 SUR PRIMARY DILUTION STANDARD (SUR PDS) – Prepare the SUR PDS by adding enough of the SUR stock standard to a volumetric flask partially filled with methanol to make a final concentration near 24 µg/mL when filled to the mark with methanol. The SUR PDS has been shown to be stable for 12 months when stored at 4 °C or less.

7.2.3 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat standards. Prepare the Analyte Stock and Primary Dilutions Standards as described below.

7.2.3.1 ANALYTE STOCK STANDARD SOLUTION – If preparing from neat material, accurately weigh approximately 5 mg of pure material to the nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methanol. Repeat for each target analyte.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION (10-40 µg/mL) – The analyte PDS contains all the target analytes of interest in methanol at various concentrations. The ESI and MS/MS response varies by compound; therefore, a mix of concentrations may be needed in the analyte PDS. See Tables in Section 17 for suggested concentrations for each analyte. During method development, the analyte PDS was prepared such that approximately the same instrument response was obtained for all the analytes. The analyte PDS is prepared by dilution of the Analyte Stock Standard solution and is used to prepare the

calibration standards, and fortify the LFBs, the LFSMs, the LFSMDs and FDs with the target analytes. The analyte PDS has been shown to be stable for 6 months when stored at 4 °C or less.

7.2.4 CALIBRATION STANDARDS (CAL) – At least five calibration concentrations are required to prepare the initial calibration curve spanning a 20-fold concentration range (Sect. 10.2). Larger concentration ranges will require more calibration points. Prepare the calibration standards over the concentration range of interest from dilutions of the analyte PDS in 5mM ammonium acetate/reagent water (Sect. 7.1.5). The target analyte concentrations found in Tables 5-9 can be used as a starting point for determining the calibration range. The IS and SUR are added to the calibration standards at a constant concentration. During method development, the concentration of the SUR was 120 µg/L in the standard (0.48 µg/L in the sample) and the IS was 80 µg/L. The lowest concentration calibration standard must be at or below the MRL, which may depend on system sensitivity. The CAL standards may be also be used as CCCs. If stored, the aqueous standards must be stored refrigerated in the same manner as the samples. During method development, the calibration standards were shown to be stable for at least two months. Longer storage times are acceptable provided appropriate QC measures are documented demonstrating the calibration standard stability.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Prior to shipment to the field, ammonium chloride (Sect. 7.1.6) must be added as a dry solid to each amber bottle fitted with a PTFE lined screw cap. A 250-mL sample bottle or larger is recommended. Add ammonium chloride to yield a sample concentration of 100 mg/L. During method development, 25 to 30 mg of ammonium chloride was added to each 250-mL sample bottle.

8.1.2 Sample bottles must <u>not</u> be prerinsed with sample before collection. Doing so will wash out the preservative added to the bottles prior to shipment.

8.2 SAMPLE COLLECTION

8.2.1 Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 2 min). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a wide-mouth bottle or 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 analysis.

8.3 SAMPLE SHIPMENT AND STORAGE – All samples should be cooled (ice or chemical freeze packs) during shipment and must not exceed 10 °C during the first 48 hours after collection. Samples must 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 and protected from light until analysis. Samples should not be frozen.

NOTE: Samples that are significantly above 10 °C at the time of collection may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice (or chemical freeze packs) to meet the above requirements.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study of all target analytes indicated that all compounds are stable for 14 days in water samples that are collected, dechlorinated, shipped and stored as described in Sections 8.1 - 8.3. Therefore, aqueous samples must be extracted within 14 days. Sample extracts may be stored at 4 °C or less for up to 28 days after sample extraction. Data from holding time studies are shown in Tables 10 and 11.

9. QUALITY CONTROL

9.1 QC requirements include the Initial Demonstration of Capability and ongoing QC requirements that must be met when preparing and analyzing field samples. This

section describes each QC parameter, their required frequency, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Section 17, Tables 12 & 13. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 METHOD MODIFICATIONS – The analyst is permitted to modify LC columns and conditions provided the QC in Section 9 is still met. The analyst is also allowed to modify the internal and surrogate standards. Each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2). The analyst may not modify the LC mobile phase components or LC modifiers (Sect. 13.4.1)

Caution: Matrix enhancement is a significant problem when analyzing SPE extracts by LC/MS/MS. Thus, if the analyst chooses to modify the LC conditions (e.g., flow rate, temperature, gradient), care must be taken ensure that the target analytes do not elute too closely to the major portion of the TOC elution. An LC/MS analysis of a spiked matrix extract will be necessary to evaluate this.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – The IDC must be successfully performed prior to analyzing any field samples. Prior to conducting the IDC, the analyst must first generate an acceptable Initial Calibration following the procedure outlined in Section 10.2.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Before any field samples are analyzed, and any time a new set of reagents or SPE cartridges is used, it must be demonstrated that a laboratory reagent blank is reasonably free of contamination and that the criteria in Section 9.3 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare and analyze 4-7 replicate LFBs fortified at or near the mid-range of the initial calibration curve, according to the procedure described in Section 11. Ammonium chloride, as described in Section 8.1.1, must also be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be $\leq$20% for all target analytes and the surrogate.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY – Using the same set of replicate LFB data generated for Section 9.2.2, calculate the average recovery. The average recovery of the replicate values must be within ±30% of the true value.

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. Establish an Initial Calibration following the procedure outlined in Section 10.2. The lowest calibration standard used to establish the Initial Calibration (as well as the low-level Continuing Calibration Check standard) must be at or below the concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm or validate the MRL following the procedure outlined below.[1]

9.2.4.1 Fortify, extract, and analyze seven replicate Laboratory Fortified Blanks at the proposed MRL concentration. These LFBs must contain ammonium chloride as described in Section 8.1.1. Calculate the mean (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963S$$

where S is the standard deviation, and 3.963 is a constant value for seven replicates.

9.2.4.2 Confirm that the upper and lower limits for the Prediction Interval of Result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below.

The Upper PIR Limit must be $\leq$ 150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} x100 \leq 150\%$$

The Lower PIR Limit must be $\geq$ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\ Concentration} x100 \geq 50\%$$

9.2.4.3 The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above (Sect. 9.2.4.2). If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

9.2.5 DL DETERMINATION *(optional) – While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

Replicate analyses for this procedure should be done over at least 3 days (both the sample extraction and the LC/MS/MS analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs using solutions at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. The fortification amount used to obtain the DLs in Table 5 can be used as a guide, however the appropriate concentration will depend on the sensitivity of the LC/MS/MS system used. Ammonium chloride must be added to these samples as described in Section 8.1.1. Analyze the seven replicates through all steps of Section 11. (NOTE: If an MRL verification data set meets these requirements, a DL may be calculated from the MRL verification data, and no additional analyses are necessary). Calculate the DL using the following equation

$$\text{Detection Limit} = St_{(n-1,\ 1-\ alpha\ =\ 0.99)}$$

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 DL calculations. The DL is a statistical determination of precision only.[2] If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs, and may result in a calculated DL that is higher than the fortified concentration. Therefore, no precision and accuracy criteria are specified.

9.2.6 CALIBRATION CONFIRMATION – If a QCS is available, analyze the QCS as described in Section 9.11 to confirm the accuracy of the standards/calibration curve.

9.3 LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.6) 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 target analytes or contaminants that interfere with the measurement of target analytes must be $\leq$ 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. Any time a new batch of SPE materials is received, or new supplies of other reagents are used, repeat the demonstration of low system background described in Section 9.2.1.

NOTE: Although quantitative data below the MRL may not be reliably accurate for data reporting, such data is useful in determining an MRL cut off for analytes that are typically detected in LRBs. Therefore, blank contaminantion levels may be estimated by extrapolation, when the concentration is below the lowest calibration standard.

9.4 CONTINUING CALIBRATION CHECK (CCC) – CCC Standards are analyzed at the beginning of each analysis batch, after every ten field samples, and at the end of the analysis batch. See Section 10.3 for concentration requirements, frequency requirements, and acceptance criteria.

9.5 LABORATORY FORTIFIED BLANKS (LFB) – With each extraction batch, extract and analyze an LFB containing each analyte of concern. If more than 20 field samples are included in a batch, analyze an LFB for every 20 samples. The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The low concentration LFB must be as near as practical to the MRL. Similarly, the high concentration LFB should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of LFB analyses corresponding to the low fortification concentration for an analyte must be within 50-150% of the true value for all analytes. Results of LFB analyses from medium and high level concentrations must be 70-130% of the true value for all analytes. If the 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.

9.6 INTERNAL STANDARDS (IS) – The analyst must monitor the peak area of the IS in all injections during each analysis batch. The IS response (as indicated by peak area) in each chromatographic run must not deviate by more than ±50% from the average area measured during the initial calibration for the IS. A poor injection, as well as matrix enhancement or suppression, could cause the IS area to exceed these criteria. Inject a second aliquot of the suspect extract to determine whether the failure is due to poor injection.

9.6.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that injection.

9.6.2 If the IS area for the reinjected extract deviates greater than 50% from the initial calibration average, the analyst should check the continuing calibration check standards that ran before and after the sample. If the continuing calibration check fails the criteria of Section 10.3, recalibration is in order per Section 10.2, followed by sample reanalysis. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within holding time. If reextraction is not possible, sample recollection should be ordered if data is supporting a regulatory monitoring requirement. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.7 SURROGATE (SUR) RECOVERY – The SUR PDS is fortified into all calibration standards, samples, LFBs, LFSMs, LFSMDs, FDs, and LRBs. The SUR is also added to the calibration curve and calibration check standards. The SUR is a means of assessing method performance from extraction to final chromatographic measurement.

9.7.1 Surrogate recovery criteria are 70-130% of the fortified amount for the method surrogate. When surrogate recovery from a sample, blank, or CCC does not meet these criteria, check : (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. Correct any problems that are identified. If these steps do not reveal the cause of the problem, reanalyze the extract.

9.7.2 If the 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 reinjecting the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3, recalibration is in order (Sect. 10.2), followed by sample reanalysis. If the calibration standard is acceptable and a duplicate sample is available, extraction and analysis of the duplicate should be performed provided the sample is still within the holding time. If a duplicate sample is not available, sample recollection should be ordered if the data is supporting a regulatory monitoring requirement. If this sample reanalysis also fails the recovery criterion, report all data for that sample as suspect due to surrogate recovery failure or potential sample matrix effect.

9.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (Sect. 9.9), however, infrequent occurrence of target analytes would hinder this assessment. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFSMD, must be prepared, extracted, and analyzed from a duplicate of the field sample. Extraction batches that contain LFSMDs will not require the extraction of a Field Duplicate. 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, LFSM 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 an LFSM for every 20 samples extracted. The LFSM is prepared by spiking a sample with an appropriate amount of the analyte PDS (Sect. 7.2.3.2). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Use historical data and rotate through the designated concentrations when selecting a fortifying concentration, such that fortified concentrations range from near the MRL to the high level standard. LFSM recoveries may fall outside the acceptance limits (Sect. 9.8.3) if an analyte is present in the sample at a concentration that greatly exceeds the spiking concentration.

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 be between 60-140%, except for low-level fortification near or at the MRL (within a factor of two times the MRL concentration), where 50-150% 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 CCCs, 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 DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or LFSMD) – Within each extraction batch, a minimum of one Field Duplicate (FD) or Laboratory Fortified Sample Matrix Duplicate (LFSMD) must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, an LFSMD must be analyzed rather than a FD. Refer to Section 9.8.1 for guidance on spiking concentrations.

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

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

9.9.2 If an LFSMD is analyzed instead of a Field Duplicate, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

$$RPD = \frac{|LFSM - LFSMD|}{(LFSM + LFSMD)/2} * 100$$

9.9.3 RPDs for FDs, or duplicate LFSMs, should fall in the range of 0-30% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of 0-50% 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.

9.10 RESOLUTION CHECK – The resolution of alachlor ESA and acetochlor ESA, in a calibration standard or CCC near or above mid level calibration, must be monitored in each analysis batch. These closely eluting peaks, which are not baseline resolved, must have a resolution (R_s) of 1.0 or greater using the equation

$$R_s = \frac{1.18(t_2 - t_1)}{W_{0.5,1} + W_{0.5,2}}$$

where

t_1 and t_2 = retention times of the first and second adjacent peaks in minutes
$W_{0.5,1}$ and $W_{0.5,2}$ = widths of the adjacent peaks at half height in minutes.

At a minimum, resolution must be monitored once for every 24-hour analysis batch and may be monitored at any time during the 24-hour period. It is recommended that resolution be checked prior to sample analysis, especially if the system in use has a history of resolution problems. If a resolution check fails, the problem must be corrected and all samples analyzed since the most recent successful calculation of the resolution check, including the QC samples, must be reanalyzed.

Note: Alachlor ESA and acetochlor ESA are structural isomers and have nearly identical product ions in MS/MS. There are dissimilar products ions at m/z 176 (alachlor ESA) and m/z 162 (acetochlor ESA); however, their relative intensity is typically less than 20%. If sufficient sensitivity for these dissimilar ions can be achieved, these ions may be used for quantitation and the resolution requirement is not necessary.

9.11 QUALITY CONTROL SAMPLE (QCS) – *The QCS is required only if an alternate source is available for these target analytes.* Each time that new PDSs are prepared, analyze a QCS from an external source. If standards are prepared infrequently, analyze a QCS at least quarterly. The QCS may be injected as a calibration standard, or fortified into reagent water and analyzed as an LFB. If the QCS is analyzed as a calibration check standard, then the acceptance criteria are the same as for the CCC (Sect. 10.3.3). If the QCS is analyzed as an LFB, then the acceptance criteria are the same as for an LFB (Sect. 9.5). If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

10. CALIBRATION AND STANDARDIZATION

10.1 After initial calibration is successful, a Continuing Calibration Check (CCC) 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 ESI-MS/MS TUNE

10.2.1.1 Calibrate the mass scale of the MS with the calibration compounds and procedures prescribed by the manufacturer.

10.2.1.2 Optimize the $[M-H]^-$ for each target analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the mass spectrometer at the LC mobile phase flow rate (approximately 0.4 mL/min). This tune can be done on a mix of the target analytes. The MS parameters (capillary voltage, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. The ESAs and OAs may have different optima requiring some compromise between the optima. See Table 2 for ESI-MS conditions used in method development.

10.2.1.3 Optimize the product ion (Sect. 3.18) for each target by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the mass spectrometer at the LC mobile phase flow rate (approximately 0.4 mL/min). This tune can be done on a mix of the target analytes. The MS/MS parameters (collision gas pressure, collision energy, etc.) are varied until optimal analytes responses are determined. Typically, all the ESAs have very similar MS/MS conditions and all the OAs have similar MS/MS conditions. See Tables 3 and 4 for MS/MS conditions used in method development.

10.2.2 Establish LC operating parameters equivalent to the suggested conditions in Section 17, Table 1.

10.2.3 Inject a mid-level CAL standard under LC/MS conditions to obtain the retention times of each target analyte. Ensure that the LC conditions are adequate to meet the resolution check in Section 9.10 for alachlor ESA and acetochlor ESA. Divide the chromatogram into retention time windows each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$; Sect. 3.16) for the analytes in that window and choose the most abundant product ion. The product ions (also the quantitation ion) chosen during method development are in Tables 3 and 4, although these will be instrument dependent. For maximum sensitivity, small mass windows of ±2 daltons (depending on the instrument) around the product ion mass were used. If sufficient sensitivity exists to meet the MRL, wider mass ranges may be used to obtain more confirmation ions.

10.2.4 Inject a mid-level CAL standard under LC/MS/MS conditions to ensure that each target analyte is observed in its MS/MS window and that there is at least 20 scans across the peak for optimum precision. Ensure that there

are enough scans on either side of the chromatographic peak to account for shifts in retention times due to injection imprecision.

10.2.5 Prepare a set of at least 5 CAL standards as described in Section 7.2.4. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the CAL standards are at a concentration greater than or equal to the MRL.

10.2.6 The LC/MS/MS system is calibrated using the internal standard technique. Concentrations may be calculated through the use of average relative response factor (RRF) or through the use of a calibration curve. Calculate the RRFs using the equation

$$RRF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)} * 100$$

where

A_x = integrated abundance (peak area) of the quantitation ion of the analyte.
A_{is} = integrated abundance (peak area) of the IS quantitation ion.
Q_x = quantity of analyte injected in ng or concentration units.
Q_{is} = quantity of internal standard injected in ng or concentration units.
RRF = relative response factor

Average RRF calibrations may only be used if the RRF values over the calibration range are relatively constant. Average RRF is determined by calculating the mean RRF of a minimum of five calibration concentrations. Average RRF calibrations may only be used if the criteria in Section 10.2.8 are met.

10.2.7 As an alternative to calculating average RRFs, use the LC/MS/MS data system software to generate a linear regression or quadratic calibration curve. Forcing the calibration curve through the origin is not recommended. The triple quadrupole instrument used during method development yielded linear curves for the target analytes over the concentration range of interest. However, the ion trap instrument yielded weighted (1/X) quadratic curves. Data may be fit with either a linear regression (response vs. concentration) or quadratic fit (response vs. concentration) and weighting may be used, if necessary.

10.2.8 When quantitated using the initial calibration curve, each calibration point, except the lowest point, for each analyte should calculate to be within 70-130% of its true value. The lowest calibration point should calculate to be within 50-150% of its true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. It is recommended that corrective action is taken to reanalyze the calibration standards, restrict the range of calibration, or select an alternate method of calibration.

CAUTION: When acquiring MS/MS data, LC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the time window.

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, CCCs, LFBs, LFSMs, FDs and LFSMDs are not counted as samples. The beginning CCC each day must be at or below the MRL in order to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL standards to meet this requirement. Alternatively, the analyte concentrations in the analyte PDS may be customized to meet this criteria. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

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

10.3.2 Determine that the absolute area of the quantitation ion of the internal standard has not changed by more than ± 50% from the average area measured during initial calibration. If the IS area has changed by more than ±50%, remedial action may be necessary (Sect. 10.3.4). If the loss in area cannot be recovered, recalibrate according to Section 10.2 and verify sensitivity by analyzing a CCC at or below the MRL (Sect 10.3.3). Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and surrogate in the CCC. 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 (Sect. 10.3.4) which may require recalibration. Any field or QC samples that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored, with the following exception. **If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular target analyte, and field sample extracts show no detection for that target analyte, non-detects may be reported without reanalysis.**

10.3.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance such a cleaning the atmospheric pressure ionization source, cleaning the mass analyzer, replacing the LC column, etc., require returning to the initial calibration step (Sect. 10.2).

11. PROCEDURE

11.1 This procedure may be performed manually (Sect. 11.4) or in an automated mode (Sect. 11.3) using a robotic or automatic sample preparation device. The data presented in Tables 5-11 demonstrate data collected by both manual and automated extraction. Extraction and/or elution steps may not be changed or omitted in the manual or automated mode.

11.2 SAMPLE PREPARATION

11.2.1 Samples are dechlorinated, collected and stored as presented in Section 8. All field and QC samples must contain the dechlorinating agent listed in Section 8.1.1, 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 11.6 for final volume determination. The LRB and LFB may be prepared by measuring 250 mL of reagent water with a graduated cylinder or filling a 250-mL sample bottle to near the top.

11.2.2 Add an aliquot of the SUR PDS (Sect. 7.2.2.2) to each sample, cap and invert to mix. During method development, a 5-µL aliquot of the 24 µg/mL SUR PDS was added to 250 mL of sample for a final concentration of 0.48 µg/L.

11.2.3 In addition to the SUR and dechlorination agent, if the sample is an LFB, FD, LFSM, or LFSMD, add the necessary amount of analyte PDS (Sect. 7.2.3.2). Cap and invert each sample to mix.

11.2.4 Continue to Section 11.3 if performing automated extractions or 11.4 if performing manual extractions.

11.3 AUTOMATED CARTRIDGE SPE PROCEDURE

11.3.1 The parameters used during method development are shown in the table below. Minor modifications to the automated method are allowed providing the QC criteria in Section 9 are met. Modifications to the solvents used and the volumes of solvents used is not permitted.

Zymark AutoTrace Extraction Workstation Procedure	
Step 1:	Process 6 samples using the following procedure:
Step 2:	Condition cartridge with 10 mL of 10 mM ammonium acetate/MeOH
Step 3:	Condition cartridge with 10 mL of 10 mM ammonium acetate/MeOH
Step 4:	Condition cartridge with 10 mL of reagent water
Step 5:	Condition cartridge with 10 mL of reagent water
Step 6:	Condition cartridge with 10 mL of reagent water
Step 7:	Load 275 mL of sample onto cartridge**
Step 8:	Rinse cartridge with 5 mL of reagent water
Step 9:	Dry cartridge with nitrogen gas for 3 minutes
Step 10:	Soak and collect 5-mL fraction using 10 mM ammonium acetate/MeOH
Step 11:	Collect 10-mL fraction using 10 mM ammonium acetate/MeOH
Step 12:	Clean each sample path with 50 mL of reagent water
Step 13:	End

Flow Rates		SPE Parameters	
Condition:	10 mL/min	Push Delay:	5 sec
Load:	10 mL/min	Air Factor:	0.0
Rinse:	5 mL/min	Autowash Vol.:	2.00 mL
Elute:	5 mL/min		
Cond Air Push:	10 mL/min		
Rinse Air Push:	10 mL/min		
Elute Air Push:	5 mL/min		

**A sample volume larger than the actual sample size is entered to ensure that all of the sample is used.

11.3.2 After automated collection of the eluant into the collection tubes, transfer the collection tubes to the nitrogen evaporator and proceed to Section 11.5.

11.4 MANUAL CARTRIDGE SPE PROCEDURE

11.4.1 CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 20 mL of 10 mM ammonium acetate/methanol (Sect. 7.1.4). Next, rinse each cartridge with 30 mL of reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Add approximately 3 mL of reagent water to the cartridge, attach the transfer tube, turn on the vacuum, and begin adding sample to the cartridge.

11.4.2 SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is 10-15 mL/min. Do not allow the cartridge to go dry before all the sample has passed through. After all of the sample has passed through the cartridge, remove the transfer tubing from the top of the cartridge and rinse the cartridge with 5 mL of reagent water. Draw air or nitrogen through the cartridge for 3 min at high vacuum (10-15 in. Hg).

11.4.3 CARTRIDGE ELUTION – Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridge. Elute the analytes from the cartridge with 15 mL of 10 mM ammonium acetate/methanol at a low vacuum (5 mL/min used to collect method data), such that the solvent exits the cartridge in a dropwise fashion.

11.5 EXTRACT CONCENTRATION – Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (60-70 °C) to remove all the ammonium acetate/methanol. Add the appropriate amount of 5 mM ammonium acetate/reagent water (Sect. 7.1.5) and the IS PDS (Sect. 7.2.1.2) to the collection vial to bring the volume to 1 mL (10 µL of the 8 µg/mL IS PDS for an extract concentration of 80 µg/L used for method development). Transfer to autosampler vial.

11.6 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). Assume a sample density of 1.0 g/mL. In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 12.2).

11.7 EXTRACT ANALYSIS

11.7.1 Establish operating conditions equivalent to those summarized in Tables 1-4 of Section 17. Instrument conditions and columns should be optimized prior to the initiation of the IDC. Confirm that resolution of alachlor ESA and acetochlor ESA peaks meets the requirements of Section 9.10.

11.7.2 Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the LC over the course of time. Plus or minus three times the standard deviation of the retention time obtained 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.

11.7.3 Calibrate the system by either the analysis of a calibration curve (Sect. 10.2) or by confirming the initial calibration is still valid by analyzing a continuing calibration check as described in Section 10.3. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2. Confirm that the system meets the resolution check criteria as described in Section 9.10 once for every 24-hour analytical batch.

11.7.4 Begin analyzing field and QC samples at their appropriate frequency by injecting the same size aliquots (100 µL was used in method development), under the same conditions used to analyze the initial calibration.

11.7.5 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding analyte peak in a reference standard. Comparison of the mass spectra is not particularly useful given the limited ±1-2 dalton mass range around a single product ion for each target analyte.

CAUTION: Alachlor ESA and acetochlor ESA are not baseline resolved under the conditions in this method and they have the same quantitation ion. Each chromatogram must be manually checked by the analyst to verify that the data system has correctly identified the peaks in the alachlor ESA/acetochlor ESA retention time window.

11.7.6 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 5 mM ammonium acetate/reagent water (Sect. 7.1.5) with the appropriate amount of internal standard added to match the original concentration. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable surrogate performance (Sect. 9.7) should be determined from the undiluted sample extract. The resulting data should be documented as a dilution, with an increased MRL.

12. DATA ANALYSIS AND CALCULATION

12.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS with the exception of alachlor ESA and acetochlor ESA (Sect. 9.10). In validating this method, concentrations were calculated by measuring the product ions listed in Tables 3 and 4. Other ions may be selected at the discretion of the analyst.

12.2 Calculate analyte and surrogate concentrations, using the multipoint calibration established in Section 10. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.6.

12.3 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification (especially in the case of the closely resolved acetochlor ESA and alachlor ESA) or poor integration.

12.4 Calculations must utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

Note: Some data in Section 17 of this method are reported with more than two significant figures. This is done to better illustrate the method performance.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, DLs AND LCMRLs – Demonstration data are presented using two commercially available tandem mass spectrometers in two separate laboratories. DLs and LCMRLs are presented in Section 17, Table 5. Precision and accuracy are presented for four water matrices: reagent water (Table 6 and 7); chlorinated (finished) surface water (Table 8); high TOC chlorinated surface water (Table 9); and day zero of the holding time in chlorinated ground water (Table 10).

13.2 EVALUATION OF ADDITIONAL ACETANILIDE DEGRADATES – Propachlor sulfinyl acetic acid (SAA) and alachlor SAA were evaluated for inclusion in this

method. The two compounds were not included due to low and inconsistent recoveries on the carbon cartridges.

13.3 ANALYTE STABILITY STUDIES

13.3.1 AQUEOUS SAMPLES – Chlorinated ground water samples, fortified with target analytes at 0.10-0.80 µg/L, were preserved and stored as required in Section 8. The average of seven replicate analyses, conducted on days 0, 7, and 14 are presented in Section 17, Table 10. These data document the 14-day sample holding time.

13.3.2 EXTRACTS – Extracts from the first day of the holding time study described above were stored at 4 °C, and analyzed in replicate (N=7) on days 0, 7, 14, 21, and 28. The results of these analyses are presented in Table 11 and document the 28-day extract holding time.

13.4 PROBLEM COMPOUNDS

13.4.1 MATRIX ENHANCEMENT – Propachlor OA and flufenacet OA were prone to ESI matrix enhancement in chlorinated surface waters containing approximately 2 mg/L or higher TOC. ESI matrix enhancement of propachlor OA (132%) and flufenacet OA (158%) was observed in typical chlorinated surface water using the ion trap instrument (Table 8) due to co-eluting TOC. This matrix enhancement was not observed using the triple quadrupole instrument due to the different LC conditions (Table 1) used. The TOC eluted earlier in the chromatogram when using the triple quadrupole LC conditions instead of the ion trap LC conditions, thus less matrix enhancement was observed using the triple quadruple LC conditions. Because ion trap instruments have much longer MS/MS scan cycles than triple quadrupole instruments, different LC conditions were necessary on the ion trap instrument in order to broaden the chromatographic peaks to obtain a minimum of 20 scans across the peak. Additional information on LC/MS matrix enhancement can be found in the literature.[6,7]

This matrix enhancement is the primary reason for not allowing modifications to the LC mobile phase constituents. Where the TOC elutes in the chromatogram versus the target analytes will determine the amount of matrix enhancement as well as which analytes are affected. Matrix enhancement was well characterized during method development, using the LC conditions listed in Table 1, to ensure minimal inferences due to TOC.

13.4.2 SPE BREAKTHROUGH – Without the presence of matrix enhancement to obscure SPE recovery losses, the data using a triple quadrupole instrument in Table 9 illustrates the loss in recovery for propachlor OA (33%) and

flufenacet OA (42%) due to excessive TOC (8.6 mg/L) affecting retention on the graphitized carbon solid phase sorbent. The recoveries of propachlor OA (75%) and flufenacet OA (96%) on the ion trap instrument do not appear to be low, but similar to the values in Table 8, the propachlor OA and flufenacet OA recoveries in Table 9 are really matrix enhanced. Thus, the actual SPE sorbent recoveries for propachlor OA and flufenacet OA are probably more similar to those observed for the triple quadrupole data in Table 9.

13.5 SECOND LABORATORY DEMONSTRATION – The performance of this method was demonstrated on the triple quadrupole instrument by a second laboratory and included in Section 17, Tables 5-9. The detection limits and LCMRLs observed on the triple quadrupole instrument are higher than the ion trap instrument. Different LC/MS/MS systems and ESI interfaces respond very differently to the various method parameters (e.g., LC mobile phase and modifiers) and the target analytes. For example, a second triple quadrupole LC/MS/MS system investigated during the method development was more similar in sensitivity to the ion trap instrument. The authors wish to acknowledge the work of Dr. Glynda Smith of Shaw Environmental Incorporated for her participation in the second laboratory demonstration.

14. POLLUTION PREVENTION

14.1 This method utilizes SPE technology to remove the analytes from water. It requires the use of small volumes of solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment when compared with 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, on-line at http://membership.acs.org/c/ccs/pub_9.htm.

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. 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.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND DEMONSTRATION DATA*

TABLE 1. LC METHOD CONDITIONS

Triple Quadrupole Conditions			Ion Trap Conditions		
Time (min)	**% 5mM ammonium acetate**	**% Methanol**	**Time (min)**	**% 5mM ammonium acetate**	**% Methanol**
initial	90.0	10.0	initial	95.0	5.0
7.0	80.0	20.0	5.0	72.0	28.0
10.0	75.0	25.0	10.0	72.0	28.0
18.0	75.0	25.0	35.0	20.0	80.0
20.0	20.0	80.0	35.1	95.0	5.0
25.0	20.0	80.0	57.0	95.0	5.0
25.1	90.0	10.0			
40.0	90.0	10.0			
Agilent Hypersil 2.1 x 100 mm packed with 5.0 µm C_{18} stationary phase Flow rate of 0.25 mL/min Column Temperature of 65°C 100 µL injection			Agilent Hypersil 2.1 x 200 mm packed with 5.0 µm C_{18} stationary phase Flow rate of 0.4 mL/min Column Temperature of 65°C 100 µL injection		

TABLE 2. ESI-MS METHOD CONDITIONS

Triple Quadrupole ESI Conditions		Ion Trap ESI Conditions	
Polarity	Negative ion	Polarity	Negative ion
Capillary needle voltage	-3 kV	Capillary needle voltage	-3 kV
Cone gas flow	50 L/hr	Nitrogen sheath gas	80 (unitless)
Nitrogen desolvation gas	500 L/hr	Nitrogen auxillary gas	20 (unitless)
Desolvation gas temp.	210°C	Heated capillary	275°C
Cone voltage	40 V		

*Instrumentation, when specified, does not constitute endorsement. Brand names are included for illustration only.

TABLE 3. TRIPLE QUADRUPOLE MS/MS METHOD CONDITIONS [a]

Segment #[b]	Analyte	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)[c,d]	Collision Energy (ev)
1	Propachlor OA	7.33	206	134	8
1	Flufenacet OA	8.67	224	152	10
2	Propachlor ESA	10.01	256	80	25
2	Flufenacet ESA	10.81	274	80	25
2	Dimethenamid OA	13.25	270	198	10
3	Dimethenamid ESA	14.87 & 15.11 [e]	320	80	25
3	Alachlor OA	15.86	264	160[f]	10
3	Acetochlor OA	16.34	264	146[f]	10
4	Alachlor ESA	18.46	314	80	25
4	Metolachlor OA	18.60	278	206	8
4	Acetochlor ESA	19.12	314	80	30
4	Metolachlor ESA	20.95	328	80	25
2	Dimethachlor ESA (SUR)	12.18 [e]	300	80	25
5	Butachlor ESA (IS)	36.95	356	80	25

[a] Argon used as collision gas at 2 mtorr.
[b] Segments are time durations in which single or multiple scan events occur.
[c] Ions used for quantitation purposes.
[d] Scan width was ±0.5 daltons.
[e] Analyte has two partially resolved chromatographic peaks due to stereoisomers. Both peaks summed for quantitation purposes.
[f] Scan range was 145-161 daltons.

TABLE 4. ION TRAP MS/MS METHOD CONDITIONS [a]

Segment # [b]	Analyte	Retention Time (min)	Precursor Ion (*m/z*) [c]	Product Ion (*m/z*) [d,e]	Normalized Collision Energy (%)
1	Propachlor OA	11.20	206	134	28
2	Flufenacet OA	11.95	224	152	28
3	Propachlor ESA	12.58	256	121	36
4	Flufenacet ESA	13.01	274	121	36
5	Dimethenamid OA	13.43	270	198	28
6	Dimethenamid ESA [f]	14.34	320	121	36
7	Alachlor OA	15.27	264	160[g]	28
7	Acetochlor OA	16.46	264	146[g]	28
8	Alachlor ESA	16.97	314	121	36
8	Metolachlor OA	18.99	278	206	28
8	Acetochlor ESA	19.24	314	121	36
9	Metolachlor ESA	19.39	328	121	36
4	Dimethachlor ESA (SUR) [f]	13.38	300	121	36
10	Butachlor ESA (IS)	29.26	356	121	36

[a] An ion trap LC/MS/MS chromatogram of the analytes is shown in Figure 1.
[b] Segments are time durations in which single or multiple scan events occur.
[c] Isolation width was ±3 daltons
[d] Ions used for quantitation purposes.
[e] Scan width was ±2 daltons.
[f] Analyte has two partially resolved chromatographic peaks due to stereoisomers. Both peaks summed for quantitation purposes.
[g] Scan range was 145-161 daltons.

TABLE 5. DETECTION LIMITS AND LOWEST CONCENTRATION MRL IN REAGENT WATER

	Triple Quadrupole MS [a]			Ion Trap MS [b]		
Analyte	**DL Fortification Level (µg/L)**	**DL [c] (µg/L)**	**LCMRL [d] (µg/L)**	**DL Fortification Level (µg/L)**	**DL [c] (µg/L)**	**LCMRL [d] (µg/L)**
Propachlor OA	0.20	0.093	0.55	0.080	0.036	0.10
Flufenacet OA	0.10	0.057	0.22	0.010	0.012	0.016
Propachlor ESA	0.23	0.11	0.40	0.020	0.010	0.035
Flufenacet ESA	0.21	0.099	0.35	0.020	0.0060	0.034
Dimethenamid OA	0.18	0.063	0.41	0.020	0.013	0.031
Dimethenamid ESA	0.24	0.093	0.45	0.040	0.029	0.048
Alachlor OA	0.20	0.12	0.52	0.040	0.032	0.076
Acetochlor OA	0.25	0.11	0.48	0.040	0.019	0.074
Alachlor ESA	0.20	0.082	0.32	0.020	0.0080	0.032
Metolachlor OA	0.20	0.090	0.44	0.010	0.010	0.017
Acetochlor ESA	0.18	0.095	0.41	0.020	0.012	0.030
Metolachlor ESA	0.23	0.11	0.40	0.080	0.043	0.11

[a] Samples extracted manually and analyzed using conditions in Table 3.
[b] Samples extracted by automation and analyzed using conditions in Table 4.
[c] Detection Limits were determined by analyzing 7 replicates over 3 days using the conditions outlined in Tables 1-4.
[d] LCMRLs were calculated according to the procedure in reference 1.

TABLE 6. PRECISION AND ACCURACY OF LOW LEVEL FORTIFIED REAGENT WATER

	Triple Quadrupole MS [a]			Ion Trap MS [b]		
Analyte	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**
Propachlor OA	0.50	89	7.5	0.16	98	3.9
Flufenacet OA	0.26	88	6.3	0.020	103	15
Propachlor ESA	0.59	89	11	0.040	103	14
Flufenacet ESA	0.54	88	9.3	0.040	108	14
Dimethenamid OA	0.45	74	12	0.040	98	16
Dimethenamid ESA	0.59	78	15	0.080	98	7.7
Alachlor OA	0.50	76	3.9	0.080	93	17
Acetochlor OA	0.62	82	4.7	0.080	99	15
Alachlor ESA	0.51	83	7.2	0.040	108	13
Metolachlor OA	0.49	77	6.0	0.020	91	16
Acetochlor ESA	0.44	84	5.8	0.040	103	13
Metolachlor ESA	0.58	87	7.0	0.16	101	10
Dimethachlor (SUR)	1.8	94	7.8	0.48	108	7.6

[a] Samples (N=7) extracted manually and analyzed using conditions in Table 3.
[b] Samples (N=7) extracted by automation and analyzed using conditions in Table 4.

TABLE 7. PRECISION AND ACCURACY OF HIGH LEVEL FORTIFIED REAGENT WATER

	Triple Quadrupole MS [a]			Ion Trap MS [b]		
Analyte	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**
Propachlor OA	2.0	89	5.8	0.80	102	3.6
Flufenacet OA	1.0	90	4.2	0.10	106	3.2
Propachlor ESA	2.3	100	1.8	0.20	106	3.9
Flufenacet ESA	2.1	98	1.4	0.20	104	3.5
Dimethenamid OA	1.8	85	3.3	0.20	96	7.1
Dimethenamid ESA	2.4	94	3.1	0.40	108	6.0
Alachlor OA	2.0	80	6.2	0.40	80	9.9
Acetochlor OA	2.5	82	4.6	0.40	88	9.9
Alachlor ESA	2.0	92	2.4	0.20	100	5.3
Metolachlor OA	2.0	85	6.5	0.10	91	12
Acetochlor ESA	1.7	96	3.3	0.20	108	7.7
Metolachlor ESA	2.3	94	2.8	0.80	104	9.4
Dimethachlor (SUR)	1.8	95	1.6	0.48	100	5.6

[a] Samples (N=7) extracted manually and analyzed using conditions in Table 3.
[b] Samples (N=6) extracted by automation and analyzed using conditions in Table 4.

TABLE 8. PRECISION AND ACCURACY IN CHLORINATED SURFACE WATER [a]

	Triple Quadrupole MS [b]			Ion Trap MS [c]		
Analyte	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**
Propachlor OA	1.5	63	1.4	0.80	132	12
Flufenacet OA	0.78	68	2.0	0.10	158	14
Propachlor ESA	1.8	81	2.4	0.20	93	6.0
Flufenacet ESA	1.6	85	1.7	0.20	103	1.8
Dimethenamid OA	1.4	73	1.4	0.20	99	8.8
Dimethenamid ESA	1.8	82	2.4	0.40	106	3.3
Alachlor OA	1.5	84	1.8	0.40	97	11
Acetochlor OA	1.9	83	1.8	0.40	103	10
Alachlor ESA	1.5	80	2.0	0.20	119	4.3
Metolachlor OA	1.5	69	2.7	0.10	89	4.7
Acetochlor ESA	1.3	80	1.9	0.20	117	2.9
Metolachlor ESA	1.7	72	3.8	0.80	106	2.0
Dimethachlor (SUR)	1.8	81	1.2	0.48	100	5.6

[a] TOC = 1.6 mg/L and hardness = 120 mg/L.
[b] Samples (N=6) extracted manually and analyzed using conditions in Table 3.
[c] Samples (N=4) extracted by automation and analyzed using conditions in Table 4.

TABLE 9. PRECISION AND ACCURACY IN HIGH TOC CHLORINATED SURFACE WATER [a]

	Triple Quadrupole MS [b]			Ion Trap MS [c]		
Analyte	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**	**Fortification Level (µg/L)**	**Mean % Recovery**	**RSD (%)**
Propachlor OA	1.5	33	4.0	0.80	75	11.6
Flufenacet OA	0.78	42	4.5	0.10	96	10.1
Propachlor ESA	1.8	87	2.4	0.20	71	6.5
Flufenacet ESA	1.6	91	2.7	0.20	84	8.8
Dimethenamid OA	1.4	60	2.4	0.20	83	6.1
Dimethenamid ESA	1.8	82	3.0	0.40	73	4.2
Alachlor OA	1.5	63	3.0	0.40	88	4.5
Acetochlor OA	1.9	64	3.1	0.40	73	8.6
Alachlor ESA	1.5	87	2.9	0.20	74	9.4
Metolachlor OA	1.5	67	3.8	0.10	122	2.5
Acetochlor ESA	1.3	89	2.8	0.20	84	6.6
Metolachlor ESA	1.7	77	3.0	0.80	109	5.6
Dimethachlor (SUR)	1.8	83	2.4	0.48	86	4.1

[a] TOC = 8.6 mg/L.
[b] Samples (N=7) extracted manually and analyzed using conditions in Table 3.
[c] Samples (N=4) extracted by automation and analyzed using conditions in Table 4.

TABLE 10. SAMPLE HOLDING TIME DATA (N=7) FOR CHLORINATED GROUND WATER SAMPLES [a,b]

Analyte	Fortification Level (µg/L)	Day 0		Day 7		Day 14	
		Mean % Recovery	RSD (%)	Mean % Recovery	%RSD	Mean % Recovery	RSD (%)
Propachlor OA	0.80	95	3.7	112	6.3	107	10
Flufenacet OA	0.10	116	2.4	128	4.5	128	8.2
Propachlor ESA	0.20	106	3.6	112	5.0	102	9.4
Flufenacet ESA	0.20	101	4.9	112	6.2	107	7.9
Dimethenamid OA	0.20	105	1.8	107	6.0	108	4.9
Dimethenamid ESA	0.40	105	1.3	108	4.3	100	5.8
Alachlor OA	0.40	94	5.3	102	5.9	100	10
Acetochlor OA	0.40	100	4.1	103	4.8	96	8.3
Alachlor ESA	0.20	89	4.9	105	1.9	93	3.3
Metolachlor OA	0.10	91	4.1	96	5.6	90	4.5
Acetochlor ESA	0.20	91	5.0	104	6.9	82	5.9
Metolachlor ESA	0.80	97	4.9	104	4.5	97	4.7

[a] TOC = 1.6 mg/L and hardness =360 mg/L.
[b] Samples dechlorinated, extracted by automation and analyzed using ion trap conditions in Table 4.

TABLE 11. EXTRACT HOLDING TIME DATA (N=7) FOR CHLORINATED GROUND WATER SAMPLES [a,b]

Analyte	Fortification Level (µg/L)	Day 0		Day 7		Day 14		Day 21		Day 28	
		Mean %Rec	RSD (%)	Mean %Rec	RSD (%)	Mean %Rec	RSD (%)	Mean %Rec	RSD (%)	Mean %Rec	RSD (%)
Propachlor OA	0.80	95	3.7	98	4.7	99	5.5	100	5.0	95	6.1
Flufenacet OA	0.10	116	2.4	112	4.6	121	3.9	114	3.0	112	4.4
Propachlor ESA	0.20	106	3.6	93	4.5	95[c]	4.3	92[c]	7.7	100	6.0
Flufenacet ESA	0.20	101	4.9	102	6.6	107	4.2	97	7.4	100	5.5
Dimethenamid OA	0.20	105	1.8	98	3.7	111	1.8	103	4.6	106	6.6
Dimethenamid ESA	0.40	105	1.3	102	6.3	102	3.4	97	7.6	95	4.4
Alachlor OA	0.40	94	5.3	86	7.3	101	7.2	100	9.8	104	11.4
Acetochlor OA	0.40	100	4.1	86	11.1	112	5.7	97	8.9	102	8.8
Alachlor ESA	0.20	89	4.9	100	6.4	105	5.1	94	6.4	106	4.3
Metolachlor OA	0.10	91	4.1	81	5.7	103	2.7	92	4.0	101	7.6
Acetochlor ESA	0.20	91	5.0	92	6.1	97	5.5	93	3.9	88	4.7
Metolachlor ESA	0.80	97	4.9	102	4.9	107	4.6	98	3.1	98	5.3
Dimethachlor (SUR)	0.48	102	3.9	94	5.8	103	6.7	101	6.3	103	6.1

[a] TOC = 1.6 mg/L and hardness =360 mg/L.
[b] Samples dechlorinated, extracted by automation and analyzed using ion trap conditions in Table 4.
[c] N=6

TABLE 12. 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, or anytime a new lot of SPE materials or reagents are used.	Demonstrate that all target analytes are ≤1/3 the MRL and that possible interferences from reagents, extraction media and glassware do not prevent the identification and quantitation of target analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs/CCCs fortified at midrange concentration	%RSD must be ≤ 20%.
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP	Mean recovery ±30% of true value.
Sect. 9.2.4	Minimum Reporting Level (MRL) Confirmation	Fortify, extract and analyze 7 replicate LFBs at the proposed MRL. Use the equation provided to verify the MRL. Repeat after major instrument or operational changes.	See Section 9.2.4 for confirmation criteria.
Sect. 9.2.5	Detection Limit (DL) Determination (optional)	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the DL. Analyze the replicates through all steps of the analysis. Calculate the DL using the equation in Sect. 9.2.5.	Data from DL replicates are not required to meet method precision and accuracy criteria. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

TABLE 13. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect 8.4	Sample Collection, Preservation and Holding Time	14 days, protected from light, with addition of ammonium chloride.	Iced or refrigerated at 10 ^{0}C or less for up to 48 hours to allow time for shipping; refrigerated at 6 ^{0}C or less after arrival at the laboratory.
Sect. 8.4	Extract Holding	28 days	Stored at 4 ^{0}C or less in amber vials.
Sect. 9.3	Laboratory Reagent Blank (LRB)	Include a LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free from interferences.	Demonstrate that all target analytes are below 1/3 the MRL, and that possible interference from reagents and glassware do not prevent the identification and quantitation of target analytes.
Sect. 9.4 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) 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 within 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be within 50-150% of the true value.
Sect. 9.5	Laboratory Fortified Blank (LFB)	Analyze at least one LFB daily or one for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each analyte and surrogate for all fortified concentrations greater than the lowest CAL point. Results of LFBs corresponding to the lowest CAL point must be 50-150% of the true value.
Sect. 9.6	Internal Standard (IS)	Butachlor ESA is added to all standards and extracts.	Peak area counts of the IS in LFBs, LRBs and sample extracts must be within 50-150% of the average peak area in the initial calibration.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.7	Surrogate Standards (SUR)	The surrogate, dimethachlor ESA, is added to all calibration standards, samples, LFBs, LFSMs, LFSMDs, FDs, and LRBs.	Surrogate recovery must be within 70-130% of the true value. Samples that fail criteria, must be reported as suspect due to surrogate recovery or potential matrix effect.
Sect. 9.8	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 field samples or less) fortified with method analytes at a concentration ≥ the native concentration.	Recoveries not within 60-140% (50-150% at the MRL) of the fortified amount may indicate a matrix effect.
Sect. 9.9	Field Duplicates (FD) or Laboratory Fortified Sample Matrix Duplicates (LFSMD)	Analyze 1 FD for each 20 samples, or 1 per extraction batch, whichever is greater. See the referenced section for a discussion of when LFSMDs should be analyzed.	RPDs should be within 0-30%.
Sect. 9.10	Resolution Check	Monitor once for every 24 hour analysis period.	Alachlor ESA and acetochlor ESA that are not baseline resolved must have a resolution of 1.0 or greater using the equation in Sect. 9.10.
Sect. 9.11	Quality Control Sample (if available)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	If analyzed as a calibration sample, CCC criteria apply. If analyzed as an LFB, those criteria apply.
Sect. 10.2	Initial Calibration	Use internal standard calibration technique to generate an average RF, or first or second order calibration curve for each analyte. Use a minimum of 5 CAL standards.	When each calibration standard is calculated using the calibration curve, the results should be 70-130% of the true value for all but the lowest standard. The lowest standard should be 50-150% of the true value.

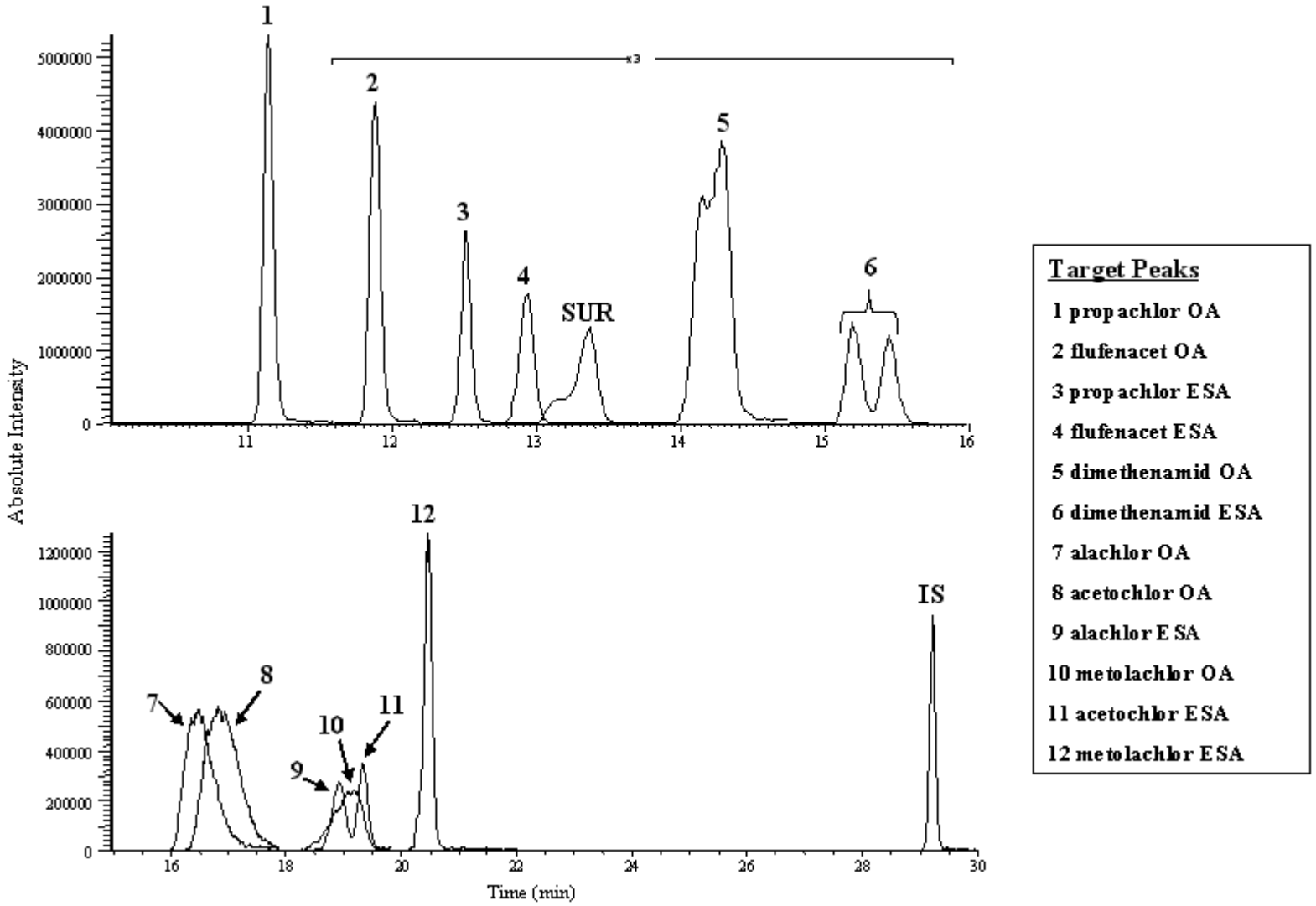

Figure 1. Ion trap LC/MS/MS chromatogram of a standard containing the target analytes at the concentrations listed in Table 10. Note there some of the analytes (SUR, 5, and 6) have two partially resolved peaks due to steroisomers.

METHOD 535 APPENDIX A

A.1 SCOPE AND APPLICATION

This appendix is intended to be used to synthesize butachlor ESA, which is the suggested internal standard (IS) used in Method 535. During method development, the following directions, adapted from Feng,[a] were used to synthesis the IS; however, these directions are not intended to be restrictive. The following steps may be modified provided the MS/MS mass spectrum matches that provided in Figure A1 and that no contaminant peaks are present which could interfere in the quantitation of the target analytes.

A.2 SAFETY

A.2.1 The toxicity or carcinogenicity of each reagent used in this appendix has not been precisely defined; each chemical reagent should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this appendix. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis.

A.2.2 Pure standard materials and stock standards should be handled with suitable protection to skin and eyes. Care should be taken not to breathe the vapors or ingest the materials.

A.2.3 At a minimum, the refluxing, acidification and separatory funnel extractions should be performed in a chemical hood.

A.3 EQUIPMENT AND SUPPLIES (Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

A.3.1 GLASSWARE – 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.

A.3.2 GRADUATED CYLINDERS – Various sizes.

A.3.3 BALANCE – Analytical, capable of accurately weighing to 0.0001 g.

A.3.4 DISPOSABLE PIPETTES – Various sizes, used to transfer solutions.

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[a] Feng, P.C.C. (1991) *Pestic. Biochem. Physiol.* **40**, 136-142.

A.3.5 DRYING OF THE PRODUCT

A.3.5.1 VACUUM DESICCATOR – To dry the synthesized product (Fisher Cat.# 08-632 or equivalent).

A.3.5.2 FREEZE DRYER (OPTIONAL) – To dry the synthesized product (Labconco, Freeze Dry System/Freezone 4.5, Cat. # 77510-00N or equivalent).

A.3.6 ROUND BOTTOM OR FLAT BOTTOM FLASK – 50-mL pyrex flask (or equivalent) to contain and reflux the reactants.

A.3.7 FRIEDRICH CONDENSER – 250 mL, used to prevent evaporation losses during refluxing (Kimble-Kontes Cat.# 456250-0022 or equivalent).

A.3.8 SEPARATORY FUNNEL – 60 mL, used in the liquid-liquid extraction of the synthesized IS after refluxing.

A.3.9 HEATING MANTLE – Sized to fit and heat a 50-mL flask to 85-95 °C.

A.3.10 EXTRACT CONCENTRATION SYSTEM – Synthesized product is concentrated by blowdown with nitrogen using a water bath set at 60-65 °C (Meyer N-EVAP, model 111, Organomation Associates, Inc. or equivalent).

A.4 REAGENTS AND GASES

A.4.1 BUTACHLOR ($C_{17}H_{26}ClNO_2$, CAS#: 23184-66-9) – Reactant in IS synthesis (Supelco, Cat. #PS-348 or equivalent).

A.4.2 ETHANOL (C_2H_6O, CAS#: 64-17-5) – $\geq$190 proof (Sigma Cat.# E7148 or equivalent).

A.4.3 BOILING CHIPS (CAS#: 1344-28-1) – Used in refluxing step (Fisher Cat.# B365, or equivalent). A stir bar and stir plate can be used in place of the boiling chips, if preferred.

A.4.4 SODIUM SULFITE (Na_2SO_3, CAS#: 7757-83-7) – Reactant in IS synthesis (Fisher Cat.# S-447 or equivalent).

A.4.5 SULFURIC ACID (H_2SO_4, CAS#:7664-93-9) – Used to acidify synthesized IS before extracting (Fisher Cat.# A300-500 or equivalent).

A.4.6 METHYLENE CHLORIDE (CH_2Cl_2, CAS#: 75-09-2) – Extraction solvent (Fisher Cat.# D151 or equivalent).

A.4.7 NITROGEN – Used to evaporate the methylene chloride (Ultra High Purity or equivalent).

A.4.8 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes.

A.5 SYNTHESIS OF BUTACHLOR ESA

A.5.1 Add 13.5 mL of reagent water to a 50-mL round-bottom flask with a few boiling chips.

A.5.2 Add 1.5 mL of ethanol to the flask.

A.5.3 Add 0.5g of butachlor and 3g of sodium sulfite to the flask containing the ethanol and water. Not all the butachlor and sodium sulfite will be soluble and the solution may look cloudy.

A.5.4 Gently heat and reflux the solution overnight (~20 hours). The solution will become clear during the refluxing.

A.5.5 After refluxing, allow the solution to cool. Add 2 mL of concentrated sulfuric acid to the round-bottom flask containing the refluxed and cooled solution. At this point the solution will become milky white in appearance.

A.5.6 Transfer the reaction solution to a 60-mL separatory funnel with a disposable pipette. Wash the round-bottom flask with several milliliters of reagent water and transfer the washes to the separatory funnel.

A.5.7 Add 20 mL of methylene chloride to the separatory funnel. Shake the funnel for approximately 2 minutes with frequent venting. A milky white emulsion will form. Allow the phases to separate for approximately 24 hours. The emulsion should separate into 2 phases: a methylene chloride phase and a water phase. If the emulsion has not cleared, allow the phases to stand longer until separation is complete.

A.5.8 Obtain the dry weight of the collection apparatus (beaker, tube, etc.) and collect the bottom layer (methylene chloride layer) of the separatory funnel. Discard the water fraction.

A.5.9 Evaporate the methylene chloride to dryness in a nitrogen evaporator at room temperature. After evaporation the residue will appear viscous. Weigh the residue.

A.5.10 Place the residue in a vacuum desiccator. Dry the product until a constant weight is achieved. This may take several weeks. Other methods of evaporation (such as freeze drying) are also acceptable.

A.6 VERIFICATION OF BUTACHLOR ESA

A.6.1 Follow the procedure in Sect. 7.2.1 of Method 535 to prepare an IS PDS solution of the IS near a concentration of 10 µg/mL in methanol.

A.6.2 Using the IS PDS, prepare a "calibration standard" containing only the synthesized IS at an appropriate concentration. During method development, the IS concentration in standards and extracts was 80 µg/L.

A.6.3 Tune the LC/MS using the procedures outline in Section 10.2.1.1 and 10.2.1.2 of Method 535.

A.6.4 Initially, perform a full scan LC/MS run to locate the IS peak and verify the molecular weight (butachlor ESA MW=357). Set the MS to scan at least 200-400 daltons in the negative ion ESI mode.

A.6.5 Inject an aliquot of the "calibration standard" into the LC/MS using the suggested LC parameters outlined in Section 17, Table 1 of Method 535. Other LC conditions may be used at the discretion of the analyst. If other LC conditions are used, more emphasis will have to be placed on the interpretation of the mass spectra since the IS retention time will not match the retention time demonstrated in Method 535.

A.6.6 Locate the chromatographic peak containing the $[M-H]^-$ ion at *m/z* 356. Note the retention time. In addition, carefully observe the whole chromatogram and verify that no other peaks (contaminants) are present which will interfere in the analysis of the other target analytes. Since the final analysis is MS/MS, the main concern at this point should be whether any potential contaminants are present in large enough quantities to cause suppression in the electrospray source. A suggested guideline is: if the contaminant is present at 10x the IS level, it may cause suppression of a co-eluting target analyte. If such a contaminant is present, further purification of the synthesized IS may be necessary. Figure A1-A and A1-B demonstrate the relative purity of the synthesized standard. A full scan mass spectra of synthesized butachlor ESA is demonstrated in Figure A1-C.

A.6.7 Optimize the LC/MS/MS using the procedures outlined in Section 10.2.1.3 of Method 535 Confirm the identity of the synthesized IS with LC/MS/MS by scanning all products ions produced by fragmentation of *m/z* 356. Suggested MS/MS parameters are found in Tables 3 or 4 of Method 535. Obtain a full scan MS/MS by scanning the product ions over a mass range of 75-357 daltons, if possible. (Ion trap instruments may not be able to scan this large of a mass range.

In that case, use the lowest starting mass the ion trap will allow.) A product ion mass spectra of synthesized butachlor ESA is demonstrated in Figure A2 using an ion trap instrument. MS/MS fragmentation and relative abundances can vary significantly between instruments and depends heavily on various instrument settings. At a minimum, *m/z* 121 must be present in the synthesized IS and if a triple quadrupole instrument is used, *m/z* 80 must be present. Other ions present in Figure A2 should be used, at the discretion of the analyst, to further verify the synthesis of the IS.

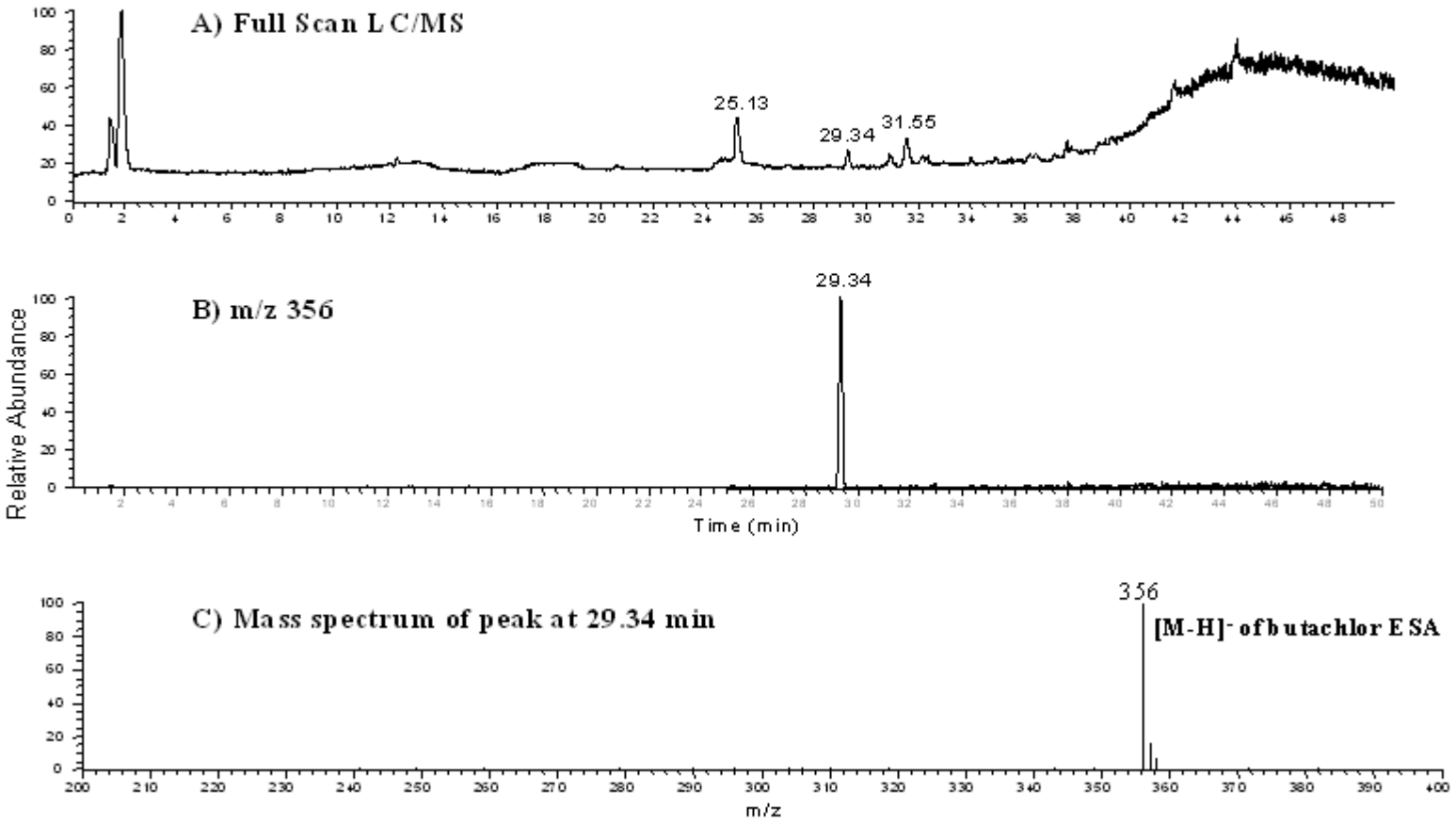

Figure A1. LC/MS analysis of synthesized butachlor ESA demonstrating relative purity and confirmation of the molecular weight using an ion trap mass spectrometer.

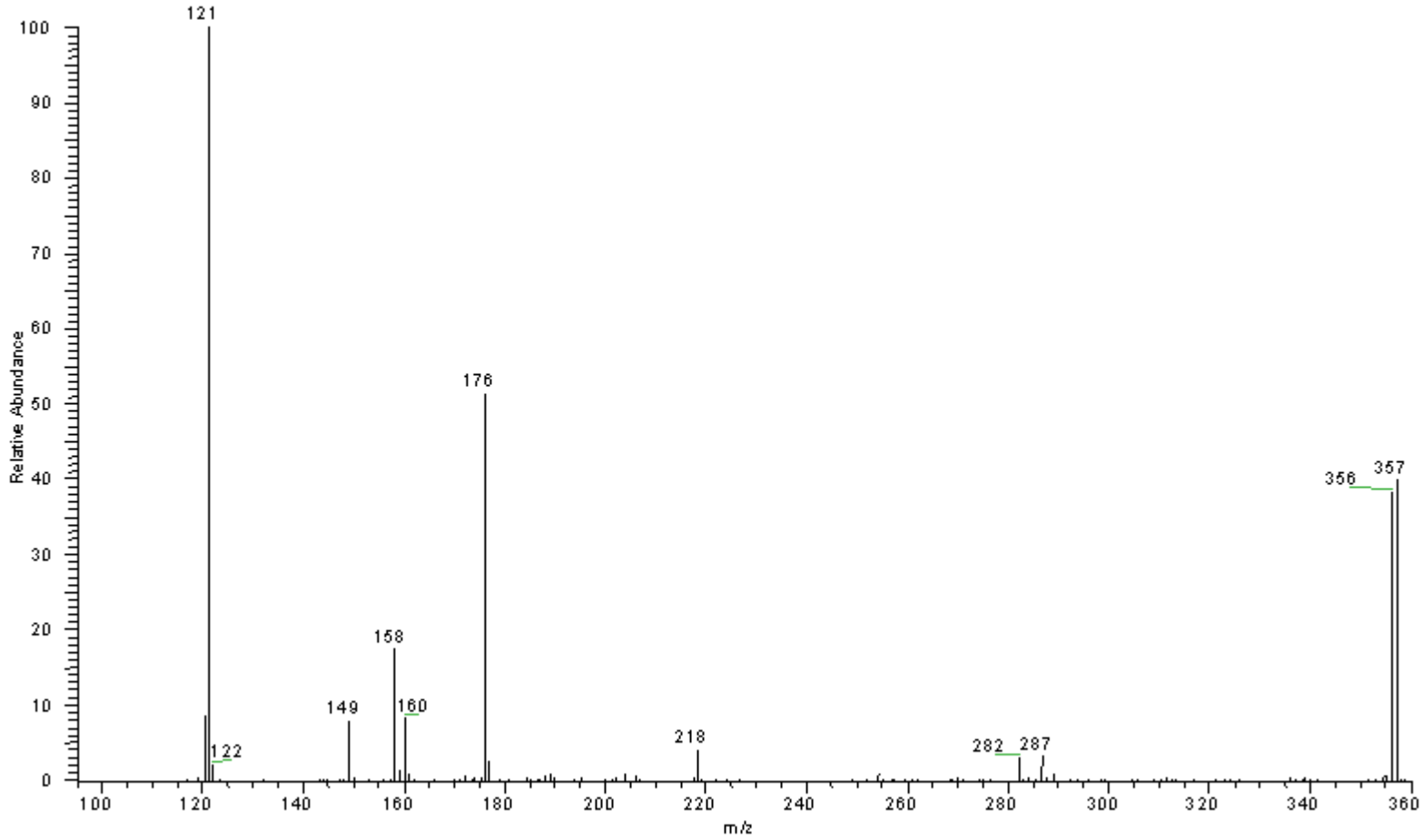

Figure A2. Product ion mass spectrum of *m/z* 356 used for confirmation of synthesized butachlor ESA using an ion trap mass spectrometer.

Method 535 APPENDIX B

B.1 SCOPE AND APPLICATION

This appendix is intended to be used to synthesize dimethachlor ESA which is the suggested surrogate (SUR) standard used in Method 535. During method development, the following directions, adapted from Feng,[a] were used to synthesis the SUR; however, these directions are not intended to be restrictive. The following steps may be modified provided the MS/MS mass spectrum matches that provided below and that no contaminant peaks are present which could interfere in the quantitation of the target analytes.

B.2 SAFETY

B.2.1 The toxicity or carcinogenicity of each reagent used in this appendix has not been precisely defined; each chemical reagent should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this appendix. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis.

B.2.2 Pure standard materials and stock standards of these compounds should be handled with suitable protection to skin and eyes. Care should be taken not to breathe the vapors or ingest the materials.

B.2.3 At a minimum, the refluxing, acidification and separatory funnel extractions should be performed in a chemical hood.

B.3 EQUIPMENT AND SUPPLIES (Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

B.3.1 GLASSWARE – 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.

B.3.2 GRADUATED CYLINDERS – Various sizes.

B.3.3 BALANCE – Analytical, capable of accurately weighing to 0.0001 g.

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[a] Feng, P.C.C. (1991) *Pestic. Biochem. Physiol.* **40**, 136-142.

B.3.4 DISPOSABLE PIPETTES – Various sizes, used to transfer solutions.

B.3.5 CONICAL COLLECTION TUBES –15 mL and 50 mL, or other glassware suitable for collection of the eluent from the solid phase cartridge after extraction of the synthesized SUR.

B.3.6 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

B.3.6.1 SPE CARTRIDGES – 5.0 g, 20 mL SPE cartridges containing a nonporous graphitized carbon sorbent phase (Supelclean ENVI-Carb SPE cartridges, Supelco Catalog No. 57129 or equivalent).

B.3.6.2 VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold (Supelco Cat. No. 57030 and 57275 or equivalent) for cartridge extractions.

B.3.6.3 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 25 cm (10 in) of mercury.

B.3.7 DRYING OF THE PRODUCT

B.3.7.1 VACUUM DESICCATOR – To dry the synthesized product (Fisher Cat.# 08-632 or equivalent).

B.3.8 ROUND BOTTOM OR FLAT BOTTOM FLASK – 50-mL pyrex flask (or equivalent) to contain and reflux the reactants.

B.3.9 FRIEDRICH CONDENSER – 250 mL, used to prevent evaporation losses during refluxing (Kimble-Kontes Cat.# 456250-0022 or equivalent).

B.3.10 HEATING MANTLE – Sized to fit and heat a 50-mL flask to 85-95 °C.

B.3.11 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by blowdown with nitrogen using a water bath set at 60-65 °C (Meyer N-EVAP, model 111, Organomation Associates, Inc. or equivalent).

B.4 REAGENTS AND GASES

B.4.1 DIMETHACHLOR ($C_{13}H_{18}ClNO_2$, CAS#: 50563-36-5) – Reactant in SUR synthesis (Sigma-Aldrich, Cat. #45447 or equivalent).

B.4.2 ETHANOL (C_2H_6O, CAS#: 64-17-5) – $\geq$190 proof (Sigma Cat.#E7148 or equivalent).

B.4.3 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of target analytes and interferences (Fisher Optima grade or equivalent).

B.4.4 AMMONIUM ACETATE ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade or equivalent).

B.4.5 10 mM AMMONIUM ACETATE/METHANOL – To prepare 1L, add 0.7708 g ammonium acetate to 1L of methanol. During method development, this solution was shown to be stable for at least 18 days.

B.4.6 BOILING CHIPS (CAS#: 1344-28-1) – Used in refluxing step (Fisher Cat.# B365, or equivalent). A stir bar and stir plate can be used in place of the boiling chips, if preferred.

B.4.7 SODIUM SULFITE (Na_2SO_3, CAS#: 7757-83-7) – Reactant in SUR synthesis (Fisher Cat.# S-447 or equivalent).

B.4.8 NITROGEN – Used to evaporate the methylene chloride (Ultra High Purity or equivalent).

B.4.9 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes.

B.5 SYNTHESIS OF DIMETHACHLOR ESA

B.5.1 Add 13.5 mL of reagent water to a 50-mL round-bottom flask with a few boiling chips.

B.5.2 Add 1.5 mL of ethanol to the flask.

B.5.3 Add 0.25 g of dimethachlor and 1.5 g of sodium sulfite to the flask containing the ethanol and water.

B.5.4 Gently heat and reflux the solution overnight (~20 hours).

B.5.5 Allow the refluxed solution to cool. Divide the 15-mL refluxed solution into two approximately equal portions. Prepare two SPE cartridges according to the procedure in Section B.5.6.1 and extract each portion on the cartridges according to the procedure in Section B5.6.2.

B.5.6 MANUAL CARTRIDGE SPE PROCEDURE

B.5.6.1 CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 60 mL of 10 mM ammonium acetate/methanol (Sect. B.4.5). Next, rinse each cartridge with 120 mL of reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Leave a small layer of reagent water on the top of the cartridge. Begin adding the synthesized SUR solution to the cartridge.

B.5.6.2 SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is 5 mL/min. Do not allow the cartridge to go dry before all the solution has passed through. After all of the solution has passed through the cartridge, rinse the cartridge with 50 mL of reagent water and draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

Note: If the reagent water rinse is not performed, substantial residual sodium sulfite remaining in the cartridge will be eluted with the SUR. Sodium sulfite is not soluble in methanol thus a white precipitate will be observed. In this case, the extract will have to be taken to dryness, reconstituted in reagent water and re-extracted according to Section B.5.6.

B.5.6.3 CARTRIDGE ELUTION – Lift the extraction manifold top and insert a rack with two 50-mL collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridge. Elute the analytes from the cartridge with 50 mL of 10 mM ammonium acetate/methanol at a low vacuum (~5 mL/min), such that the solvent exits the cartridge in a dropwise fashion.

B.5.7 EXTRACT CONCENTRATION – Concentrate the extracts under a gentle stream of nitrogen in heated water bath (60-65 °C) to 3-5 mL each. Weigh a 15-mL collection tube. Transfer the two extracts to the weighed 15-mL collection tube rinsing with 2-3 mL of 10 mM ammonium acetate/methanol. Evaporate the extract to dryness. Due to the amount of water (from the cartridges) in the extracts, the evaporation process will take in excess of 24 hours. After evaporation the clear residue will appear viscous. Weigh the residue.

B.5.8 Place the residue in a vacuum desiccator. Periodically weigh the residue. Continue drying until constant weight is achieved. This may take several weeks.

B.6 VERIFICATION OF DIMETHACHLOR ESA

B.6.1 Follow the procedure in Section 7.2.2 of the method to prepare a SUR PDS solution of the SUR near a concentration of 24 µg/mL in methanol.

B.6.2 Using the SUR PDS, prepare a "calibration standard" containing only the synthesized SUR at an appropriate concentration. During method development, the SUR concentration in standards and extracts was 120 µg/L.

B.6.3 Tune the LC/MS using the procedures outline in Section 10.2.1.1 and 10.2.1.2 of Method 535.

B.6.4 Initially, perform a full scan LC/MS run to locate the SUR peak and verify the molecular weight (dimethachlor ESA MW=301). Set the MS to scan at least 200-400 daltons in the negative ion ESI mode.

B.6.5 Inject an aliquot of the "calibration standard" into the LC/MS using the suggested LC parameters outlined in Section 17, Table 1 of the Method 535. Other LC conditions may be used at the discretion of the analyst. If other LC conditions are used, more emphasis will have be placed on the interpretation of the mass spectra since the SUR retention time will not match the retention time demonstrated in Method 535.

B.6.6 Locate a chromatographic peak containing an $[M-H]^-$ at *m/z* 300. Note the retention time. In addition, carefully observe the whole chromatogram and verify that no other peaks (contaminants) are present which will interfere in the analysis of the other target analytes. Since the final analysis is MS/MS, the main concern at this point should be whether any potential contaminants are present in large enough quantities to cause suppression in the electrospray source. A suggested guideline is, if the contaminant is present at 10x the SUR level, it may cause suppression of a co-eluting target analyte. If such a contaminant is present, further purification of the synthesized SUR may be necessary. Figure B1-A and B1-B demonstrate the relative purity of the synthesized standard. A full scan mass spectra of synthesized dimethachlor ESA is demonstrated in Figure B1-C.

B.6.7 Optimize the LC/MS/MS using the procedures outlined in Section 10.2.1.3 of Method 535. Confirm the identity of the synthesized SUR with LC/MS/MS by scanning all products ions produced by fragmentation of *m/z* 300. Suggested MS/MS parameters are found in Tables 3 or 4 of the Method 535. Obtain a full scan MS/MS by scanning the product ions over a mass range of 75-305 daltons, if possible. (Ion trap instruments may not be able to scan this large of a mass range. In that case, use the lowest mass the ion trap will allow.) A product ion mass spectra of synthesized dimethachlor ESA is demonstrated in Figure B2 using a triple quadrupole instrument. MS/MS fragmentation and relative abundances can vary significantly between instruments and depends heavily on various instrument settings. At a minimum, *m/z* 121 must be present in the synthesized SUR and if a triple quadrupole instrument is used, *m/z* 80 must be present. Other ions present in Figure B2 should be used, at the discretion of the analyst, to further verify the synthesis of the SUR. Note: *m/z* 212 was not observed in the ion trap product ion mass spectrum of dimethachlor ESA.

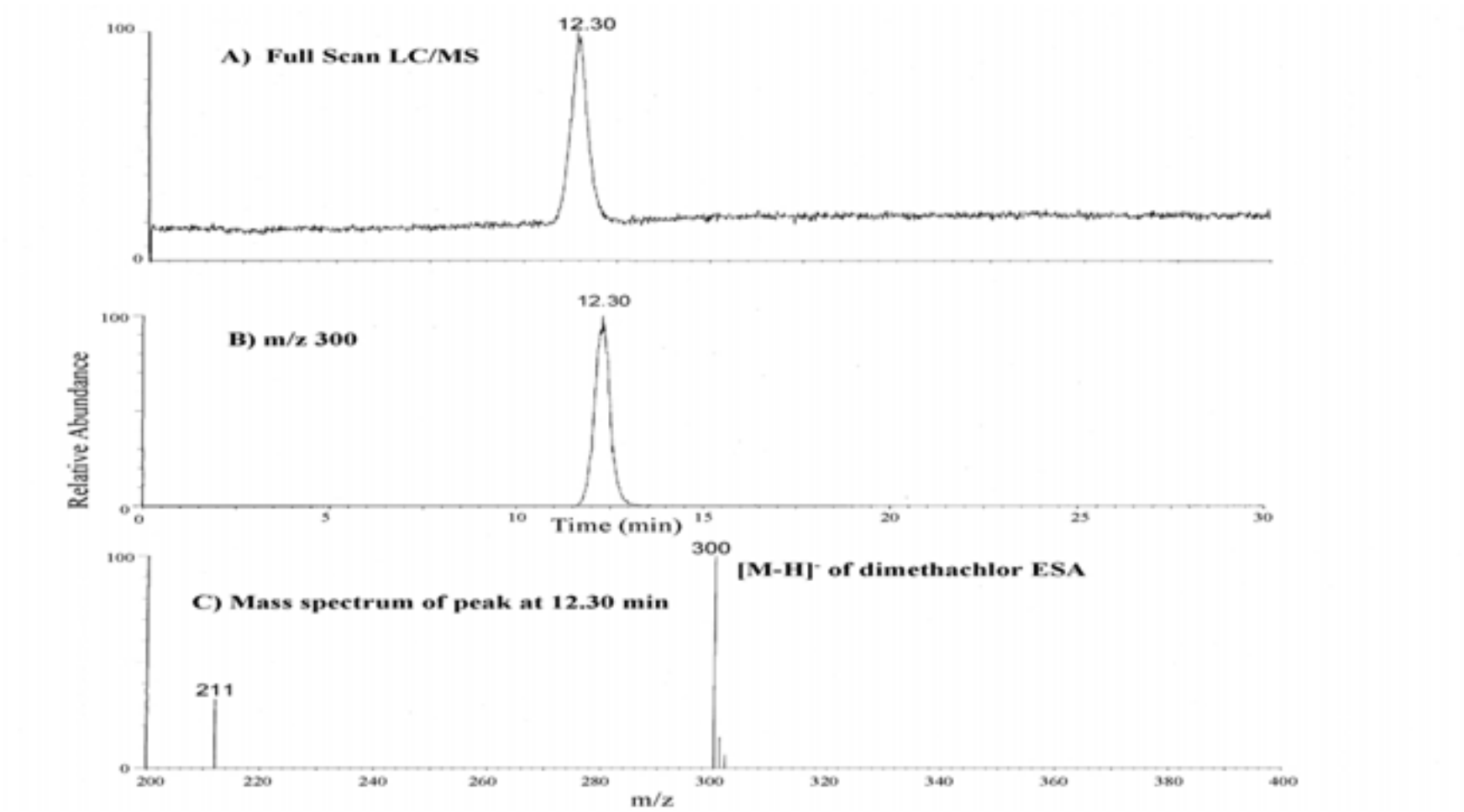

Figure B1. LC/MS ananlysis of synthesized dimethachlor ESA demonstrating purity and confirmation of the molecular weight using a triple quadrupole mass spectrometer.

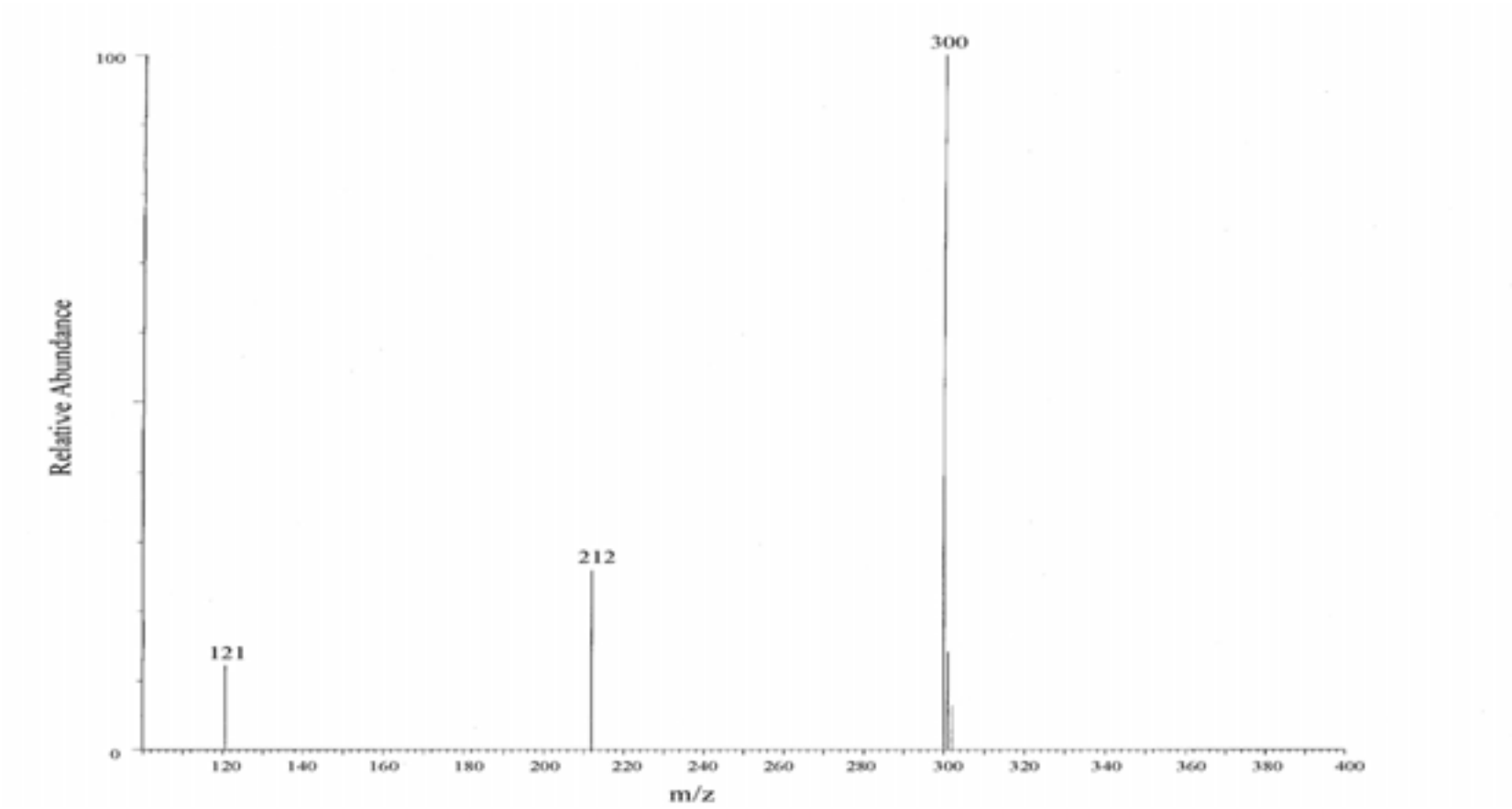

Figure B2. Product ion mass spectrum of *m/z* 300 used for confirmation of synthesized dimethachlor ESA using a triple quadrupole mass spectrometer.