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# Determination of Sulfonic Acid Degradates of Chloroacetanilide and Chloroacetamide Herbicides in Groundwater by LC/MS/MS

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**An analytical method is presented which permits qualitative and quantitative analysis of sulfonic acid degradates of three chloroacetanilide herbicides (acetochlor, alachlor, and metolachlor) and one chloroacetamide herbicide (dimethenamid) in groundwater at trace levels with determination by LC/MS/MS. The analytes were isolated from groundwater by solid-phase extraction (SPE). The final samples were analyzed by reversed-phase HPLC with MS/MS detection utilizing a pneumatically assisted, and heat-assisted electrospray interface (TurboIonSpray). Unique precursor/product ion pairs were obtained in the MS/MS mode which permitted conclusive identification of each analyte, even when the analytes coeluted. Quantification was performed by generation of an external calibration curve. Excellent linearity was obtained over a calibration range from 0.25 to 10 ng injected on-column, with all linear correlation coefficients exceeding 0.999. Method performance for this analytical procedure was validated by analyzing groundwater samples fortified at levels of 0.1, 1, and 50 ppb. The average recovery at each fortification level for each analyte exceeded 89%. Excellent method precision was demonstrated with percent relative standard deviations of less than 10% for all analytes at all fortification levels.**

The demands placed on current regulatory and environmental monitoring programs require the development of increasingly sophisticated analytical techniques which are capable of qualitatively and quantitatively determining the presence of analytes of interest at trace levels. Gas chromatography with selected ion monitoring (GC/MSD) has played an important role and has been the technique of choice for monitoring analytes of concern in the environment.[1−4] Unfortunately, many analytes of concern are either too polar or thermally unstable to permit analysis by GC techniques. High-performance liquid chromatography (HPLC) coupled with mass spectrometric detection (LC/MS) provides an ideal system for the analysis of these polar and/or thermally unstable analytes.[4−8] Commercially available LC/MS systems have advanced in reliability and ruggedness to the point where industrial and regulatory laboratories can now use these systems for routine analyses.

The chloroacetanilide and chloroacetamide classes of herbicides are widely used for the control of broadleaf and grass weeds in various crops. Groundwater and surface water systems are commonly monitored for the presence of these herbicides, in addition to other agrochemicals. The parent agrochemical may degrade in soil or water to form other degradates. Recent monitoring studies have demonstrated the presence of the oxoethane sulfonic acid (ESA) degradates of alachlor[9−13] and metolachlor[13] in groundwater. Alachlor−ESA was found more frequently than parent alachlor, and usually at higher levels. Feng[14] determined that acetochlor−ESA was a terminal degradation product of acetochlor in soil, making it highly likely that this degradate would be found in the environment, if monitored. It is highly probable that other chloroacetanilide and chloroacetamide agrochemicals also form sulfonic acid degradates in the environment, on the basis of these findings. The ESA degradates have a great potential to leach into groundwater; thus there is a need for an analytical procedure which permits the identification and accurate quantification of these analytes in water at trace levels.

Initial methodologies for determining chloroacetanilide−ESA degradates in water utilized immunoassay[10] or HPLC with UV detection.[10,12,15] HPLC/UV lacks the specificity required for environmental methods, where the presence of an analyte at unacceptable levels may trigger the need for some type of regulatory action or for restrictions to be placed on the use of that agrochemical. Recently, Thurman et al.[13] published a method

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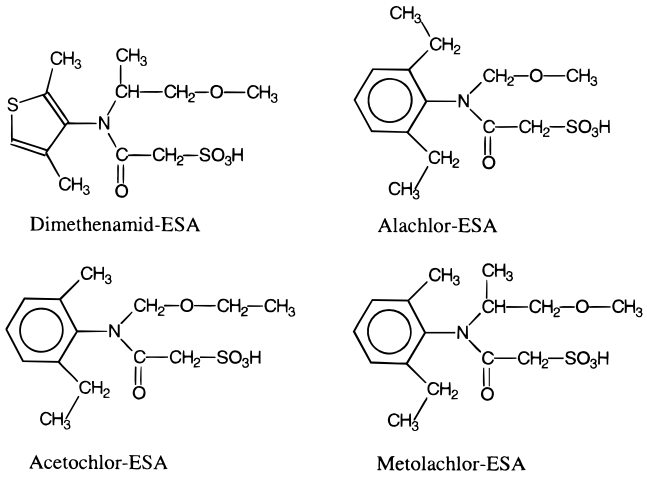


Figure 1. Chemical structures for the ESA analytes.

which utilized LC/MS for the identification of alachlor−ESA, acetochlor−ESA, and metolachlor−ESA in water. While this publication represents the most comprehensive and advanced studies with these degradates to date, it is lacking in several areas of critical importance. The method utilized single-quadrupole LC/MS, which was not able to conclusively distinguish between alachlor−ESA and acetochlor−ESA because they chromatographically coeluted and produced the same molecular ion via electrospray techniques. In addition, it was noted that the procedure was only able to produce semiquantitative analyses, with a coefficient of variation of ±30% observed. Limited data were provided which did not adequately demonstrate that the analytical methodology would work at a wide range of concentrations of the analytes in water.

The analytical method presented in this publication utilizes solid-phase extraction (SPE) to isolate the analytes of interest from water with subsequent analysis by LC/MS/MS. This permits the conclusive identification of the ESA degradates of the chloroacetanilide herbicides of alachlor, acetochlor, and metolachlor and for the ESA degradate of the chloroacetamide herbicide dimethenamid. All of these analytes form unique precursor ion/product ion pairs which are monitored. Accurate quantification is obtained by generation of external calibration curves. Excellent stability and reproducibility of the analysis system is demonstrated. Recovery data is presented for the analytical method for the fortification of water samples at levels of 0.1, 1, and 50 ppb.

## EXPERIMENTAL SECTION

**Chemical Standards.** The sulfonic acid degradates of dimethenamid, acetochlor, alachlor, and metolachlor were synthesized by the Chemical Synthesis Group at Novartis Crop Protection (Greensboro, NC). The structures of the synthesized standards were verified by various spectral and chromatographic techniques. The chemical structures for the four analytes are presented in Figure 1. Standard solutions were prepared by dissolving the analytes in 25% (v/v) methanol/water. Subsequent dilutions for calibration standards and for fortification solutions were prepared in 25% methanol/water. All solutions were stored refrigerated when not in use.

**Reagents and Apparatus.** HPLC-purity solvents were used for the preparation of all mobile phases and solutions. All binary solutions were prepared on a volume/volume basis. The isolation of the analytes from water was achieved using an ENV SPE cartridge, 1 g capacity (Varian Sample Preparation Products, Harbor City, CA). (Note: The ENV SPE cartridge is very similar to a C18 SPE except that the support particle is styrene divinylbenzene-based rather than silica-based.)

**Water Sample.** Groundwater from Guilford County, NC, was used as the water source for all samples presented in this publication. Treated water from Greensboro, NC, has also been used during method development studies with excellent method results, although the results are not presented in this paper.

**Sample Preparation.** A 100-mL water sample was measured, acidified with 200 $\mu$L of concentrated acetic acid, and passed through a preconditioned Varian ENV SPE column. The SPE column was preconditioned with about 5 mL of methanol followed by 5 mL of 0.2% acetic acid. The sample vessel was rinsed with about 5 mL of 0.2% acetic acid, and this rinsate was passed through the SPE column. The SPE column was then rinsed with about 3 mL of purified water. A 50-mL concentration tube, precalibrated to the desired final sample volume, was placed beneath the SPE. The analytes were eluted from the SPE with 5 mL of 70% methanol/water. (Less polar degradates, including the parent herbicides, may be eluted using 10 mL of methanol.) The sample volume was reduced to approximately 50% of the desired final sample volume via rotary evaporation. Methanol was added to the sample so that the final methanol content was 25%. The sample was diluted to the desired calibration mark with water. A final sample volume of 2.0 mL was used for samples fortified at the lowest level of 0.1 ppb.

**Method Validation.** The analytical method was validated by analysis of water samples fortified at 0.1, 1, and 50 ppb, with 10 replicates at each level. The water samples were fortified with 1.0 mL of a mixed fortification solution prior to the acidification step. Two samples were not fortified and served as control samples to ensure that no significant contamination was introduced by the method.

**HPLC.** The analytes were separated by reversed phase HPLC using a YMC ODS-AQ column, 15 cm × 4.6 mm, $d_p = 5\ \mu$m (YMC, Inc., Wilmington, NC). The column was kept in a column oven at 30 °C to ensure reproducible retention times. A sample volume of 100 $\mu$L was injected via an autosampler. A binary mobile phase gradient was used: (A) acetonitrile with 0.1% acetic acid added; (B) water with 0.1% acetic acid added. The initial mobile phase composition was 15% A/B, with a linear ramp to 35% A/B over 5 min, beginning upon injection of the sample. The column was reequilibrated at initial conditions for 7 min prior to the next injection. A mobile phase flow rate of 1.5 mL/min was used.

**LC/MS.** All single-quadrupole analyses were conducted using a PE Sciex API-I system, equipped with an IonSpray interface. The analytes were detected as their deprotonated molecular ions $[(M - H)^-]$ in the negative ion mode. The column effluent was split so that a small wet spot was observed hitting the orifice plate (approximately 50 $\mu$L/min). The system state files were optimized by infusion of the analytes into the system while adjusting the voltages to obtain the optimum signal.

**LC/MS/MS.** All MS/MS data were generated using a PE Sciex API-365 system, equipped with a TurboIonSpray interface. The negative deprotonated molecular ion was passed through Q1,

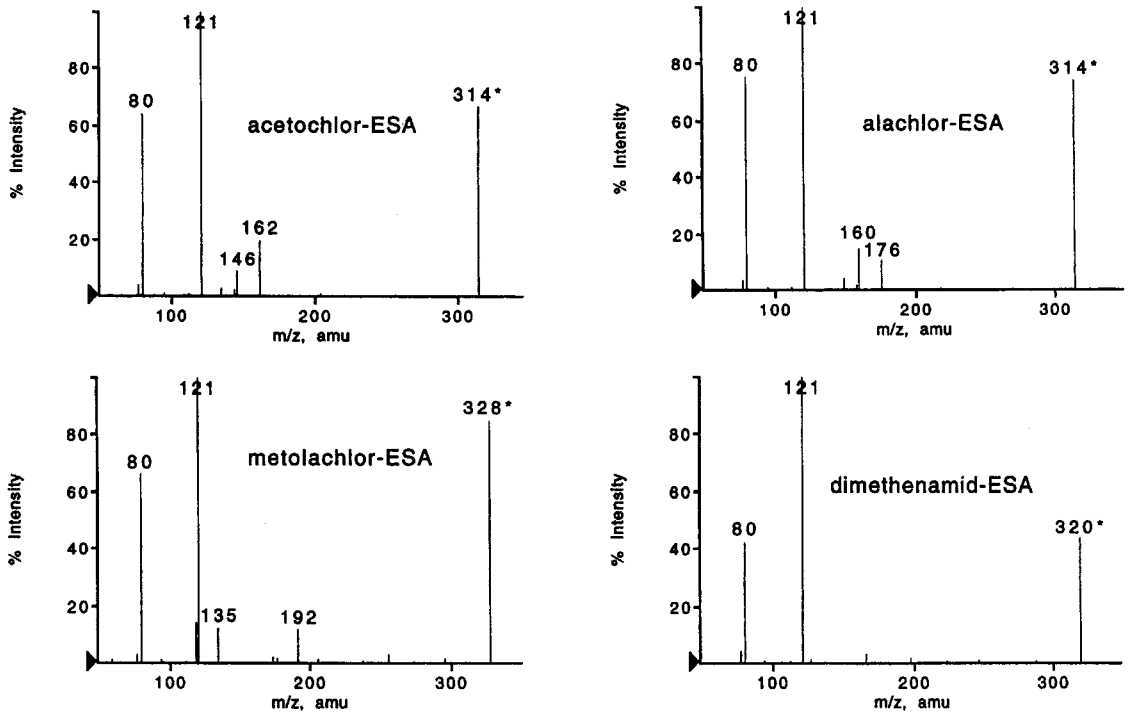


Figure 2. MS/MS fragmentation spectra. The asterisks (*) denote the undissociated deprotonated molecular ions.

fragmentation was induced in the collision cell, and a unique product fragment ion was monitored in Q3. The column effluent to the TurboIonSpray interface was split so that a small wet spot was observed on the side of the interface plate. The system state files were optimized by infusion of the analytes into the system using the IonSpray interface while adjusting the voltages to obtain the optimum signal.

**Quantification.** Quantification was obtained by generation of a linear five-point external calibration curve for each analyte. The calibration standards ranged from 0.0025 to 0.1 ng/$\mu$L (0.25−10 ng injected on-column). Calibration standards were interspersed among samples so that the first and last injections were always calibration standards.

## RESULTS AND DISCUSSION

**Chromatography.** Alachlor−ESA, acetochlor−ESA, and metolachlor−ESA have nearly identical chromatographic retention times when analyzed in a reversed-phase mode. Dimethenamid−ESA elutes earlier and is easily resolved. Peak splitting is observed for dimethenamid−ESA and acetochlor−ESA due to the presence of rotational isomers. Alachlor−ESA also exhibits peak splitting due to rotational isomers, but in the chromatography presented in this work it is suppressed, and not apparent, due to the gradient profile. While rotational isomers exist for metolachlor−ESA, no separation is observed in the chromatogram. The peak splitting becomes more apparent with increased retention of the analytes. Thurman et al.[16] were able to suppress the peak splitting for alachlor−ESA by using a short HPLC column, elevated column temperature, and a neutral pH buffered mobile phase. The chromatographic conditions used by Thurman et al.,[16] along with minor modifications, were tried by the author in an attempt to minimize the peak splitting exhibited by dimethenamid−ESA and acetochlor−ESA, but these efforts were unsuccessful. In addition, the neutral-buffered mobile phase is not compatible with electrospray ionization techniques, and the sensitivity of the analytes is optimal with nonbuffered, acidic mobile phases. Optimum sensitivity for the analytes was obtained by focusing the injected sample onto the column under initial mobile phase conditions where the analytes were highly retained and then running a rapid gradient to elute the analytes as relatively sharp peaks. The rapid gradient permitted samples to be injected every 12 min. Neither temperature, slight variations in the mobile phase pH, or the use of differing brands of reversed-phase HPLC columns were found to have any significant effect on improving the peak splitting.

**Mass Spectrometry.** All of the analytes form intense deprotonated molecular ions [$(M - H)^-$] when monitored as negative ions in the MS mode. Metolachlor−ESA (molecular ion 328.1) and dimethenamid-ESA (molecular ion 320.1) are distinguishable from alachlor−ESA and acetochlor−ESA (molecular ion 314.1) in the MS mode; however, alachlor−ESA and acetochlor−ESA cannot be distinguished from each other on the basis of molecular ion monitoring alone. Analyses can be conducted with a single-quadrupole system if it is not necessary to distinguish between these two analytes. MS/MS induces characteristic fragmentation of the molecular ions which not only greatly diminishes the possibility of an interference from the sample exhibiting the same fragment ion, but also permits alachlor−ESA and acetochlor−ESA to be distinguished from one another. MS/MS fragmentation spectra are presented in Figure 2. All analytes exhibit very similar precursor ion fragmentation spectra with ions at $m/z$ 121 (base ion for all analytes in the MS/MS mode) and $m/z$ 80 being of greatest intensity. Alachlor−ESA exhibits a unique fragment at $m/z$ 176, corresponding to the loss of [CH−O−$CH_3$] from the

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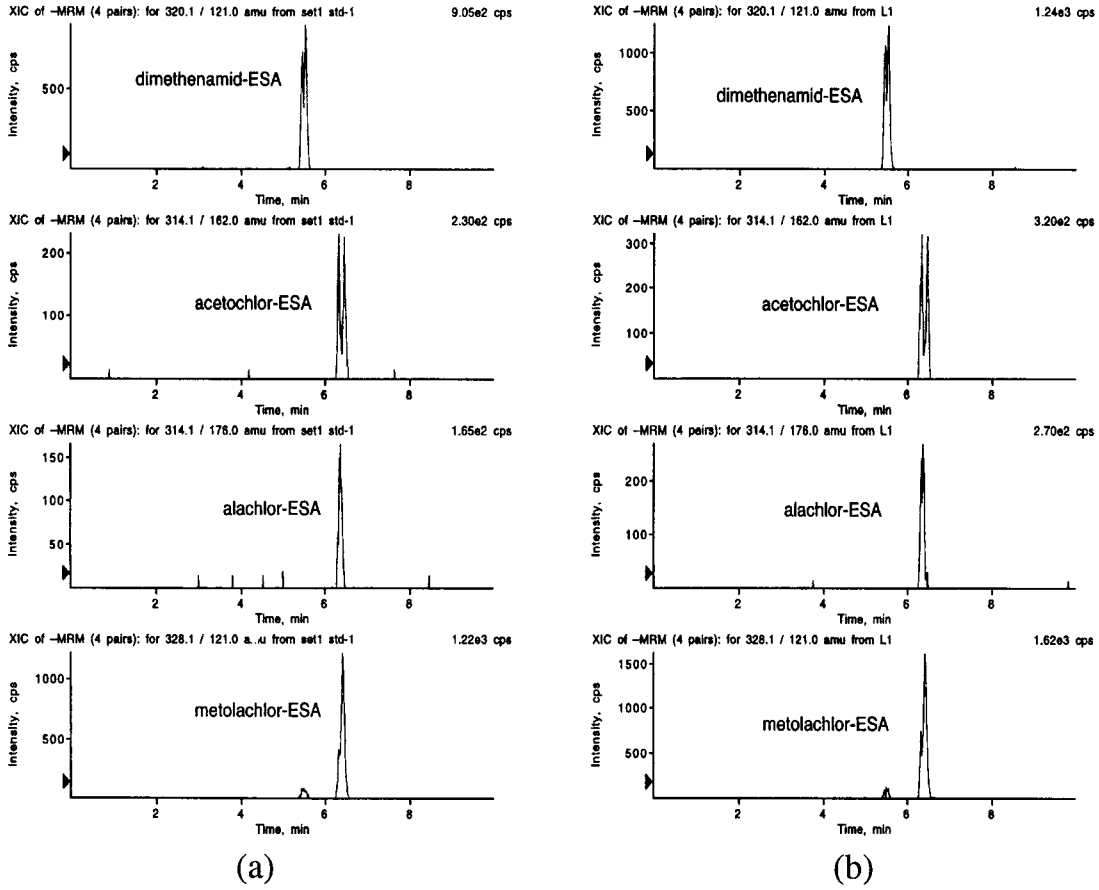


Figure 3. Representative LC/MS/MS chromatograms for (a) the lowest standard injected, 0.25 ng on-column, and (b) water sample fortified at the lowest level (0.1 ppb).

Table 1. Ions Monitored

| analyte | exact MW | MS | MS/MS |
|---|---|---|---|
| dimethenamid−ESA | 321.1 | 320.1 | 320.1/121 |
| alachlor−ESA | 315.1 | 314.1 | 314.1/176 |
| acetochlor−ESA | 315.1 | 314.1 | 314.1/162 |
| metolachlor−ESA | 329.1 | 328.1 | 328.1/121 |

nitrogen. Acetochlor−ESA exhibits a unique fragment at $m/z$ 162, corresponding to the loss of [CH−O−$CH_2$−$CH_3$] from the nitrogen. The base ion fragment at $m/z$ 121 will provide about 5× more sensitivity if there is no need to discriminate between alachlor−ESA and acetochlor−ESA. The ions which are monitored in MS and MS/MS analyses are presented in Table 1.

**LC/MS/MS.** Representative chromatograms for the lowest standard injected (0.25 ng injected on-column) and for a water sample fortified at the lowest fortification level of 0.1 ppb are presented in Figure 3. The lowest standard represents a level of 0.05 ppb in samples. Adequate sensitivity is observed for all analytes at this level. No interferences were observed in the control water samples. Quantification was performed by comparison of the analyte peak area versus an externally generated calibration curve. The external calibration curves used five calibration standards ranging from 0.0025 to 0.1 ng/$\mu$L (100-$\mu$L injection volume). Excellent linearity for all analytes was observed, with correlation coefficients exceeding 0.999 for all of the analyses that were performed while evaluating the analytical method.

**LC/MS.** Excellent results were also obtained by monitoring the analytes' deprotonated molecular ions in the MS mode. The fortified water samples and calibration standards were also analyzed with the single-quadrupole system. Representative chromatograms for the lowest standard injected (0.25 ng injected on-column) and for a water sample fortified at the lowest fortification level of 0.1 ppb are presented in Figure 4. Adequate sensitivity was observed for the lowest calibration standard. Excellent linearity for all analytes was observed with correlation coefficients exceeding 0.999 for all of the analyses. The analytical results for fortified water samples were in excellent agreement with the recovery values obtained using the MS/MS system. No interferences were observed in the control samples. The only apparent shortcoming for this system was the inability to discriminate between alachlor−ESA and acetochlor−ESA, although the peak areas for the two coeluting analytes could be summed and residues accurately quantitated as "alachlor−ESA + acetochlor−ESA" residues.

**Method Performance.** Method performance was evaluated by fortifying water samples at varying levels (0.1, 1, and 50 ppb), subjecting the samples to the analytical method cleanup, and then analyzing the samples by LC/MS/MS. The results of these experiments are summarized in Table 2. Excellent recoveries were observed for all analytes at all fortification levels (average recoveries at each level equaled or exceeded 89%, with a range

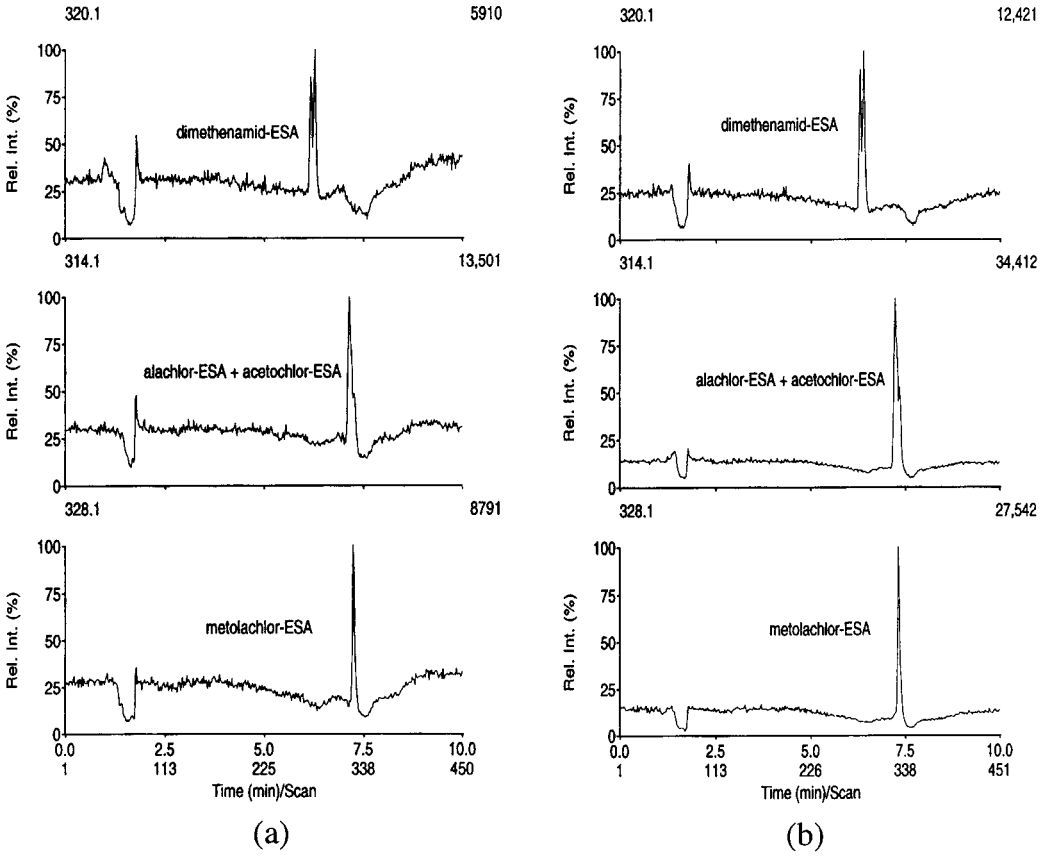


Figure 4. Representative LC/MS chromatograms for (a) the lowest standard injected, 0.25 ng on-column and (b) water sample fortified at the lowest level (0.1 ppb). Note that alachlor−ESA and acetochlor−ESA cannot be distinguished.

Table 2. Method Performance Data (LC/MS/MS Analyses)

| | fortification level | | |
|---|---|---|---|
| analyte | 0.1 ppb | 1 ppb | 50 ppb |
| dimethenamid−ESA | 89 ± 3.6 | 101 ± 9.8 | 94 ± 2.7 |
| alachlor−ESA | 91 ± 6.6 | 100 ± 9.2 | 92 ± 8.0 |
| acetochlor−ESA | 91 ± 6.6 | 101 ± 8.6 | 90 ± 6.3 |
| metolachlor−ESA | 91 ± 5.4 | 102 ± 8.8 | 92 ± 6.9 |

average recovery ± standard deviation
$n = 10$ for all levels

from 89% to 102%). The percent relative standard deviation at each fortification level for each analyte was less than 10%, demonstrating excellent method precision. The method performance easily exceeded the U.S. EPA requirements that environmental methods demonstrate average recoveries of 70−120% and RSD of 20% or less at all fortification levels.[17]

**Ultimate Sensitivity.** The limit of quantification (LOQ) for this method (0.1 ppb) meets EPA's general criteria for the lowest level that an analyte needs to be determined in a water method developed to support the registration, or reregistration, of an agrochemical. Analyses at lower concentrations are considered necessary only when the analyte of interest has special environmental or human health concerns.

The ultimate sensitivity of this method is affected by (1) the volume of water processed, (2) the final volume of the sample prior to injection, (3) the volume of sample injected, (4) the bandwidth of the eluted peaks (i.e., how good is the chromatography?), (5) the sensitivity of the instrument on which the analyses are performed, (6) how well the system is optimized, and (7) the signal/noise ratio the chemist is willing to accept as the lowest quantifiable signal. The conditions used in this analytical method represent reasonable compromises that permit the method to be very rugged and reliable, important traits for methods that will be used on a routine basis involving the analyses of many samples.

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