EPA Method 535 -Analytical Method Development for Chloroacetanilide and Other Acetamide Degradation Products in Drinking Water - Research Summary

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OUTLINE

Research Summary

1 Introduction

1.1 Purpose. The purpose of this documentation is to provide supplemental information not found in Method 535 which will aid in understanding the decisions made during the development of the method. Data included in Method 535 are not repeated in this document.

1.2 Instrumentation. Method 535 was developed to detect and quantitate 6 ethane sulfonic acid (ESA) and 6 oxanilic acid (OA) degradation products of acetanilide herbicides in drinking water for the next Unregulated Contaminant Monitoring Regulation (UCMR). Since methodology developed in our laboratory will be used to determine the occurrence of acetanilide degradates in drinking water, positive confirmation by mass spectrometry (MS) was desired. Due to the ionic nature of these compounds, electrospray (ESI)-LC/MS, operated in the negative ion mode, was believed to be the best choice of analytical methodology.

1.3 Data Quality Objectives. The goal was to find a solid phase extraction (SPE) procedure that, combined with LC/MS (or LC/MS/MS) analysis, produced a method that met our data quality objectives of 70-130% mean recovery (% of true value) and <30% relative standard deviation (RSD). The toxicological effect of these ESA and OA acetanilide degradates has not been extensively studied, thus no health effect level has been established. The lowest concentration minimum reporting level (LCMRL) goal was to strive for the lowest possible LCMRL using a cost-effective, but selective analytical methodology.

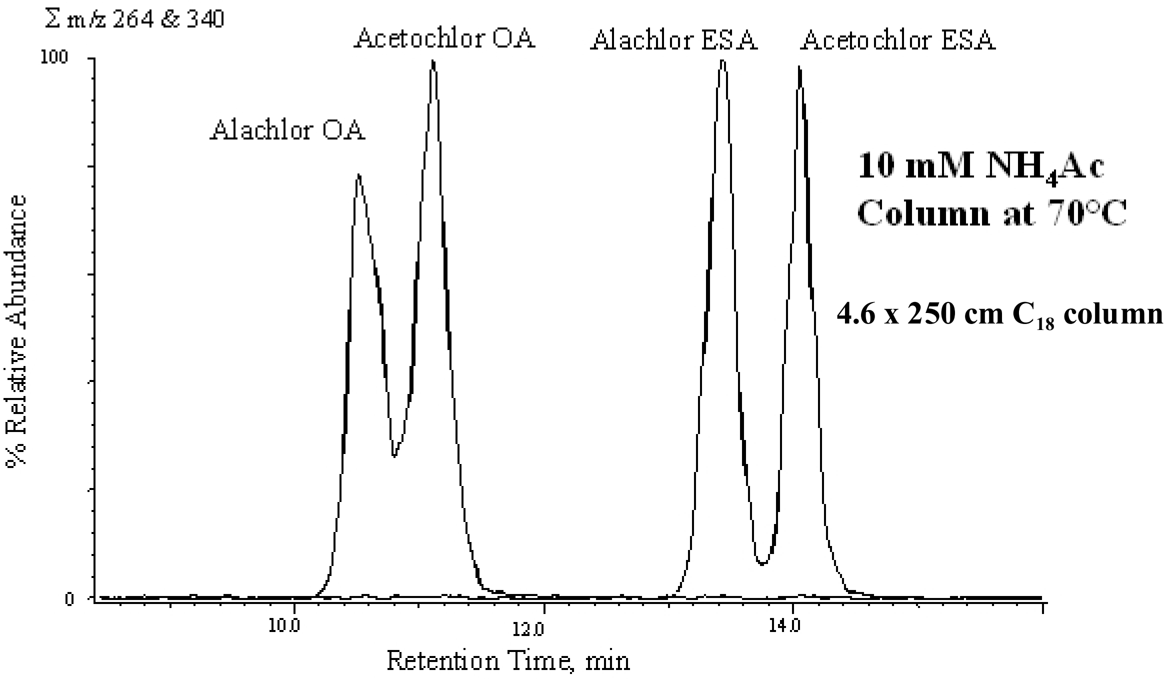
2 LC Optimization

2.1 Analytical Column Choice. There are two structural isomer pairs (alachlor ESA/acetochlor ESA and alachlor OA/acetochlor OA) in the Method 535 target analyte list which have the same molecular weight and are known to co-elute on C_{18} columns under conventional conditions. Thus, various LC analytical columns and mobile phase conditions were evaluated in an effort to separate these structural isomer pairs. The structural isomers were not successfully resolved on any of the LC columns evaluated. To further complicate the analyses, many of these ESA and OA target analytes have rotational stereoisomers which are resolved on many of these columns. As a result, some of the target analytes had multiple peaks with several minutes between each stereoisomer peak elution.

Brand Name	Stationary Phase
Agilent Hypersil C_{18}	octadecyl bonded silica gel
Thermo Hypercarb	porous spherical carbon
Hamilton PRP-1	polystyrene divinylbenzene
Supelco ABZ+plus	alkyl amide bonded silica gel
Zirchrom Diamond bond C_{18}	octadecylphenyl modified carbon bonded on zirconia

The stereoisomer peaks also tended to be broad peaks. Since the standards are only available as an unknown mixture of the stereoisomers, it is preferable to detect all of a target's stereoisomers as one peak.

2.2 LC Separation. The literature indicated some success with combining the stereoisomers of the acetanilide ESAs and OAs into single sharp peaks by heating the analytical column.[1,2] It was determined during method development that heating a C_{18} column to 65-70 °C did indeed prevent the stereoisomer separation of the target analytes, although it did not provide sufficient separation of the



alachlor ESA/acetochlor ESA and alachlor OA/acetochlor OA structural isomer pairs. It was discovered that heating the C_{18} column (70 °C), in combination with a 10 mM ammonium acetate/methanol mobile phase gradient, provided near baseline resolution of the ESA pair and reasonable resolution of the OA pair in LC/MS analysis.

2.3 Optimization of Column Temperature. During the course of method development, several observations were made regarding the optimum column temperature. The optimum column temperature, needed to separate the structural isomers, was dependent on the type of C_{18} column used and on the manufacturer and model of LC system used. The heat transfer and/or the location of the temperature sensor may not be equivalent between all models and manufacturers of LC systems. Thus, when different LC systems were tried, the column temperature required was typically 65 ± 5 °C to get the needed resolution. Resolution between alachlor OA and acetochlor OA required a column temperature of 70 °C (maximum temperature rating of a C_{18} column) on all models and columns tested. If resolution between alachlor OA and acetochlor OA is not necessary (Sect. 4.2), it was possible to use lower column temperatures (60 to 65 °C) to lengthen the column lifetime, while maintaining resolution between alachlor ESA and acetochlor ESA. The Hypersil C_{18} columns used in the development of Method 535 were used for over 10 months with no measurable degradation due to the elevated column temperatures (60 to 70 °C).

2.4 Acetic Acid vs. Ammonium Acetate. Many of the reports[1,3] on the analysis of the acetanilide ESA and OA degradates cite acetic acid as the mobile phase modifier. Many researchers preferred using acetic acid over ammonium acetate due to the decreased ESI sensitivity when using ammonium acetate. Therefore, acetic acid was evaluated for inclusion in Method 535. While sensitivity for the ESAs is 3 to 5 times higher when using acetic acid (depending on the concentration of acetic acid used), than when using ammonium acetate, the acetic acid had the disadvantage of lowering the mobile phase pH, thus causing the OAs to be neutral. As a result, sensitivity for the OAs was approximately 5-10 times lower when using acetic acid than when using 10 mM ammonium acetate in the mobile phase. In addition, acetic acid did not provide the necessary resolution between the alachlor and acetochlor ESA and OA structural isomers, even when the LC column was heated to 70 °C. Analysis by LC/MS requires LC separation of the alachlor and acetochlor ESA and OA degradates. If analyzed by LC/MS/MS, alachlor OA and acetochlor OA have prominent dissimilar products ions which eliminate the need for chromatographic resolution between this pair. However, alachlor ESA and acetochlor ESA have nearly identical product ions in LC/MS/MS. There are dissimilar products ions at *m/z* 176 (alachlor ESA) and *m/z* 162 (acetochlor ESA), however their relative intensity is less than 20% of the base peak. Sensitivity would be severely compromised if these product ions were chosen as would be required with the use of the acetic acid mobile phase. Thus, ammonium acetate remained the mobile phase modifier of choice for the rest of method development.

It was also decided not to allow other mobile phases in Method 535 for the UCMR because of the humic/fulvic background problem discussed in Section 4.1. Many of the literature reports demonstrate retention times under 10 minutes for all the target analytes using acetic acid as the mobile phase modifier. The humic/fulvic material also elutes in this retention window. While this may be acceptable for ground water samples, surface waters are higher in humic/fulvic material and will cause suppression of the target analytes eluting early in the chromatogram.

3 Solid Phase Extraction Optimization

3.1 SPE Sorbent Choice. Various SPE sorbents were evaluated for their ability to extract all the target analytes in 100 mL of fortified deionized water. Literature reports indicated that C_{18} quantitatively retains the ESAs and OAs of alachlor,

Target Analyte	%Mean Recovery [a]				
	Varian 0.5 g C_{18} Cartridges [b]	Varian 0.5 g C_{18}OH Cartridges [c]	Varian 0.5 g PPL Cartridges [b]	Waters 60 mg Oasis HLB Cartridges [b]	Supelco 0.5 g Carbon Cartridges [c,d]
propachlor OA	5 (17)	11 (7.4)	52 (12)	17 (69)	104 (3.0)
propachlor ESA	4.6 (69)	1.4 (30)	127 (4.9)	55 (44)	103 (4.2)
dimethenamid OA	25 (15)	29 (5.7)	109 (2.3)	58 (33)	97 (5.7)
dimethenamid ESA	32 (30)	32 (24)	106 (7.1)	92 (40)	91 (3.2)
alachlor OA	31 (24)	30 (17)	129 (2.8)	107 (14)	92 (7.0)
acetochlor OA	39 (25)	28 (7.5)	119 (1.4)	86 (18)	94 (4.7)
alachlor ESA	65 (28)	37 (6.9)	119 (4.3)	112 (7.7)	100 (6.2)
metolachlor OA	75 (33)	47 (7.6)	114 (12)	97 (16)	98 (3.4)
acetochlor ESA	62 (23)	46 (13)	119 (14)	112 (3.8)	105 (3.2)
metolachlor ESA	100 (31)	70 (18)	125 (7.2)	122 (14)	99 (4.8)

[a] 100 mL reagent water sample fortified at 5 µg/L with the analytes. Elution solvent was methanol.
[b] N=3
[c] N=4
[d] To elute the analytes,10 mM ammonium acetate was added to the elution solvent (methanol).

metolachlor and acetachlor.[2,4] However, the recoveries of the propachlor and dimethenamid degradates were unacceptably low on the C_{18} (Varian) sorbent. (Flufenacet OA and ESA standards had not been obtained at this point in the method development.) Low recoveries were also observed for the $C_{18}OH$ (Varian), PPL (Varian) and Oasis HLB (Waters) cartridges. Initially, the carbon cartridges retained all the analytes well, but they were difficult to elute from the carbon. Adding 10 mM ammonium acetate to the methanol eluent resolved this issue and allowed all the target analytes to be quantitatively retained and eluted from the carbon cartridges. Therefore, carbon SPE cartridges (Supelco, 6 mL, 0.5 g) were determined to be the best choice for extraction of the target analytes.

3.2 **Optimization of Sample Volume.** To determine the optimum sample volume using the carbon SPE cartridges, recoveries of the acetanilide ESA and OA degradates were evaluated using 100 mL, 200 mL and 500 mL of reagent water spiked at 2.5, 1.25, and 0.5 µg/L, respectively. Recoveries met the 70-130% recovery goal for all sample volumes studied and precision was ≤12% relative percent deviation (%RSD). This indicated that breakthrough was not an issue up to 500 mL in reagent water. However, due to the matrix effects described in Section 4.1, the sample volume was eventually limited to 250 mL to minimize the breakthrough of a few target analytes in water samples containing ≥5 mg/L total organic carbon (TOC), as well as to limit the amount of TOC in the extract which could cause suppression in the ESI source.

	100 mL sample volume		200 mL sample volume		500 mL sample volume	
Target Analyte	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
propachlor OA	104	3.0	99	8.8	95	9.8
propachlor ESA	103	4.2	99	3.0	91	6.5
dimethenamid OA	97	5.7	95	4.5	82	3.8
dimethenamid ESA	91	3.2	91	7.0	83	3.0
alachlor OA	92	7.0	86	12	82	4.5
acetochlor OA	94	4.7	89	6.6	87	4.0
alachlor ESA	100	6.2	93	4.6	90	4.9
metolachlor OA	98	3.4	96	6.7	87	1.1
acetochlor ESA	105	3.2	96	6.0	97	11
metolachlor ESA	99	4.8	89	6.4	86	8.2

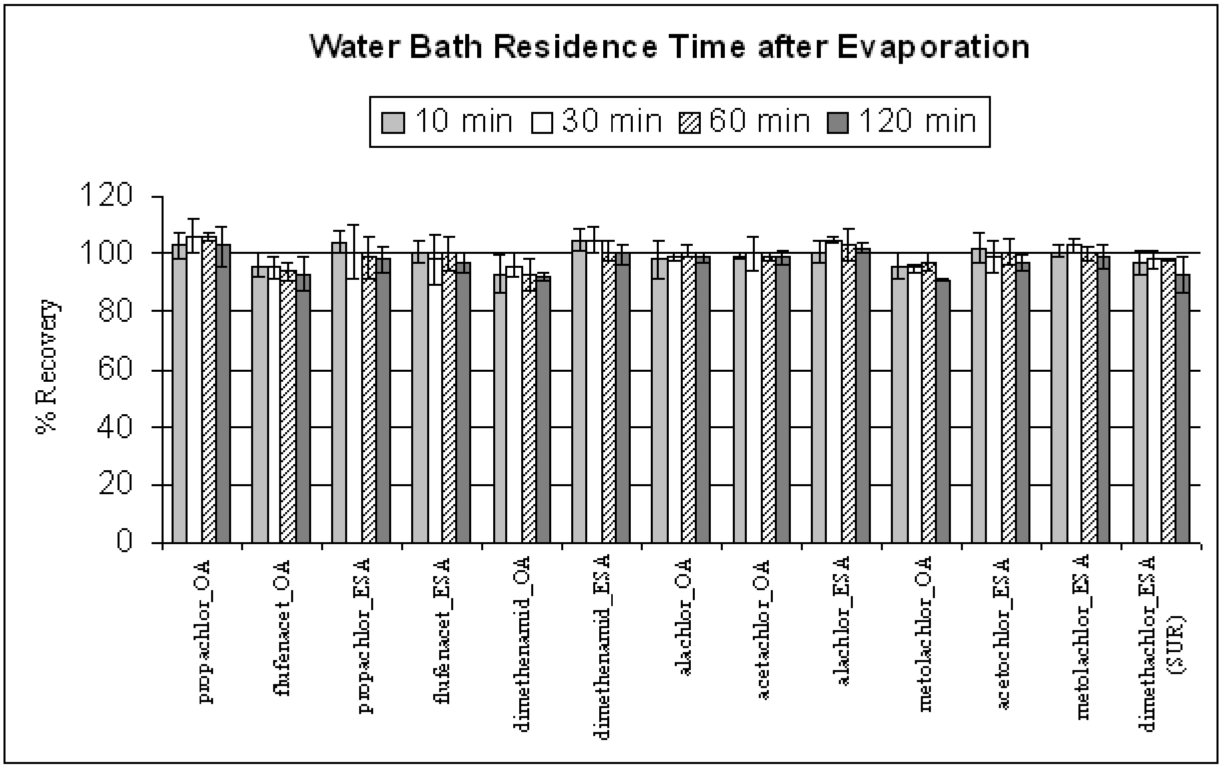
3.3 **SPE Flowrates.** The data collected in Method 535 was collected using a sample loading flowrate of 10 mL/min and an elution flowrate of 5 mL/min. To ensure that faster flowrates did not adversely affect the recovery of the target analytes, faster flowrates were evaluated. Sample loading flowrates up to 25 mL/min and elution flowrates up to 15 mL/min yielded no significant losses in the target analyte recoveries. The extreme flowrates demonstrated in this study aid in determining method ruggedness; however, the more reasonable sample loading flowrate of 10 mL/min and elution flowrate of 5 mL/min are recommended in Method 535.

Target Analyte	Spike Level, µg/L	25 mL/min sample loading flowrate [a]		15 mL/min elution flowrate [b]	
		%Recovery	%RSD	%Recovery	%RSD
propachlor OA	0.80	100	2.3	108	1.3
flufenacet OA	0.10	98	1.5	98	2.1
propachlor ESA	0.20	107	8.0	93	2.7
flufenacet ESA	0.20	105	3.7	99	6.3
dimethenamid OA	0.20	92	2.9	86	3.3
dimethenamid ESA	0.40	101	1.5	96	3.6
alachlor OA	0.40	88	1.4	82	4.3
acetochlor OA	0.40	91	1.1	87	3.4
alachlor ESA	0.20	109	7.4	97	1.7
metolachlor OA	0.10	92	2.9	89	1.7
acetochlor ESA	0.20	106	4.8	96	8.0
metolachlor ESA	0.80	106	0.5	95	3.0
dimethachlor ESA (SUR)	0.48	90	8.3	99	5.0

[a] Elution flowrate was 5 mL/min.

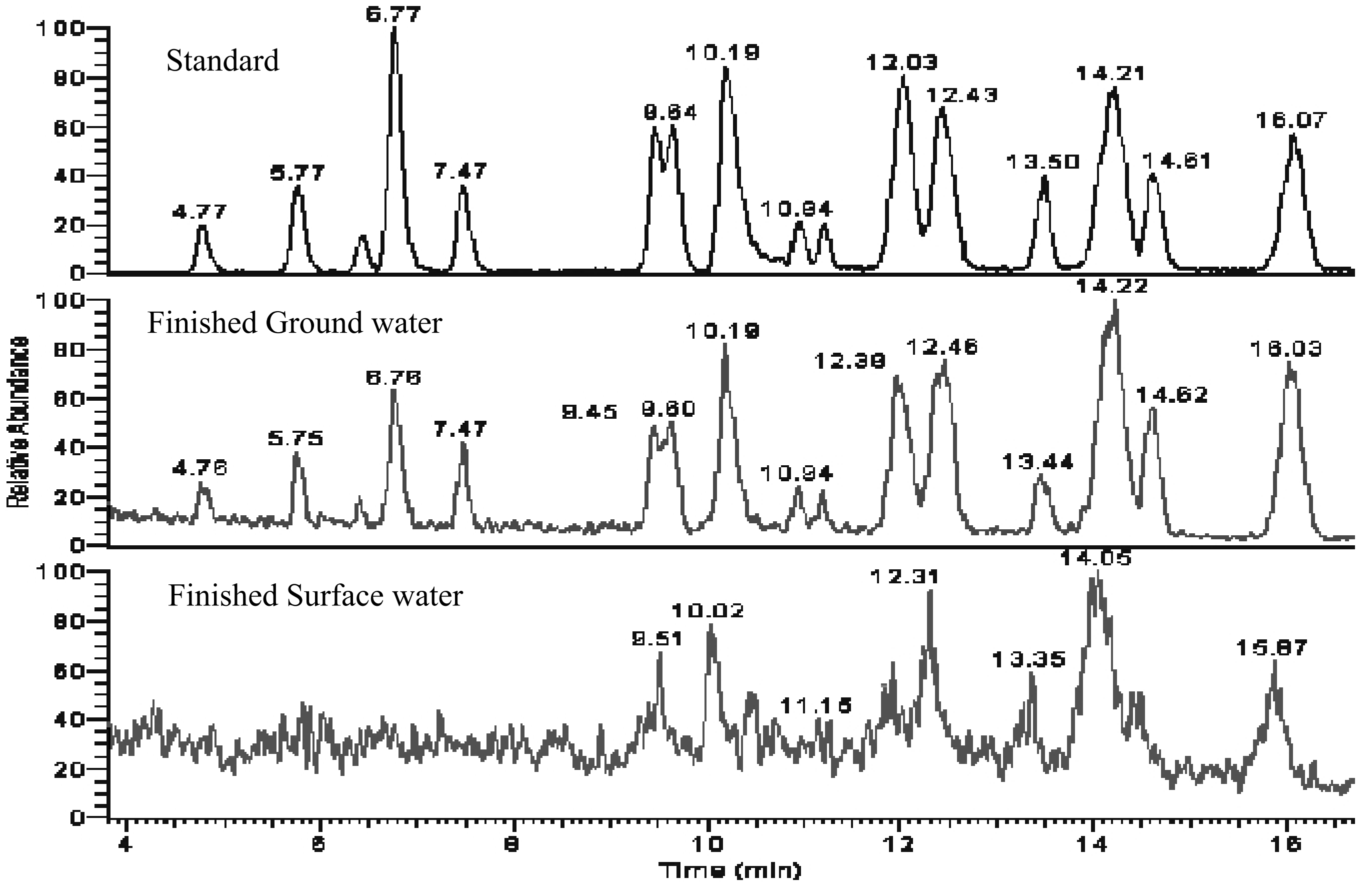
[b] Sample loading flowrate was 10 mL/min.

3.4 **Stability of Analytes during Evaporation.** After elution from the SPE cartridge, Method 535 instructs the user to evaporate the extract to dryness in a heated water bath (60-65 °C). To test the ruggedness of the evaporation step, 15 mL of elution solvent was spiked with the target analytes and left in the heated water bath at 65 °C for 10, 30, 60 and 120 minutes (N=3 for each time point) after the solvent had completely evaporated. Recoveries at all time intervals were well within the QC requirements of the method and indicated that no degradation or losses of the analytes occurred even if the extract was left in the heated water bath up to120 minutes after dryness.



4 Selection and Discussion of LC/MS/MS

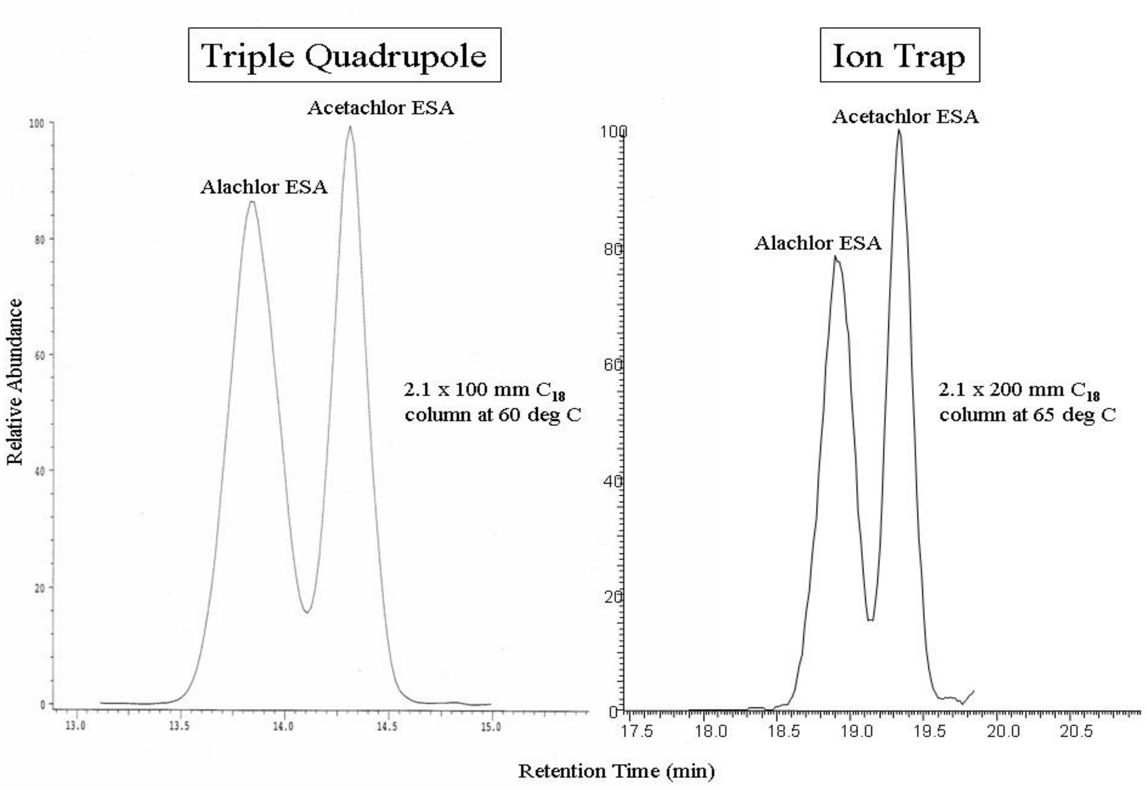
4.1 **Effect of Humic/Fulvic Material.** Porous graphitic carbon retains many organic compounds. While treated drinking water is relatively pure, it can contain high levels (mg/L quantities) of naturally occurring humic and/or fulvic material. Thus, studies were conducted in various drinking water matrices to determine if co-extracted materials would interfere in the SPE or the analysis steps of the method. Not surprisingly, humic and/or fulvic material was co-extracted using the carbon cartridges. Unfortunately, this co-extracted humic and/or fulvic material was observed as an elevated background in the LC/MS analysis spanning the first 10-12 minutes of the chromatogram. In negative ESI, the humics/fulvics produce an ion at every mass between 200-700 daltons. The chromatograms shown in this section (summation of the quantitation ions displayed) of a



calibration standard, a fortified ground water extract, and a fortified surface water extract demonstrate the effect of increasing humic/fulvic material on the LC/MS background. Many of the early analyte peaks are below the baseline in the finished surface water extract. For this method, total organic carbon (TOC) is a good indicator of humic content of the sample. This particular finished surface water was measured at 2 mg/L TOC which is not extremely high and the finished ground water was <1 mg/L. Various clean-up steps were performed (e.g., solvent washes, ion exchange cartridges, molecular weight cut-off filters, etc.) in an effort to remove the interfering humics/fulvics from the carbon sorbent, but none were effective. Other types of SPE sorbents (e.g., C_{18}, polystyrene divinylbenzene, Varian PPL) were tried, but they all retained the humics/fulvics. The interfering material appeared to be anionic, similar to the target analytes, so anything that removed the humics/fulvics from the SPE sorbents in a wash step also removed the target analytes. At this point, it was decided that LC/MS/MS would have to be investigated to determine if the target analytes could be accurately quantitated by LC/MS/MS in the presence of humics and/or fulvics. This elevated MS background due to high TOC levels can cause enhancement[5,6] in the electrospray ionization source and/or low recoveries on the carbon SPE.

4.2 **Chromatographic Resolution for LC/MS/MS**. LC/MS analyses were performed using 10 mM ammonium acetate in the aqueous mobile phase to achieve chromatographic separation of the alachlor OA/acetochlor OA and alachlor ESA/acetochlor ESA structural isomers. Once MS/MS became necessary, chromatographic separation between alachlor OA and acetochlor OA was no longer needed to distinguish these compounds. However, chromatographic separation was still necessary for alachlor ESA and acetochlor ESA because they have no abundant dissimilar product ions (Sect. 2.4). In an effort to boost sensitivity, attempts were made to decrease the concentration of the ammonium acetate in the LC mobile phase. Decreasing the ammonium acetate concentration resulted in less resolution between the structural isomers. Studies indicated that a 5 mM concentration increased the analyte signals (less ESI suppression) by 2-3 times, while retaining near baseline resolution of alachlor ESA and acetochlor ESA. In addition, analyte retention times were not stable in ammonium acetate concentrations lower than 5 mM. Thus, 5 mM ammonium acetate was chosen as the final mobile phase modifier concentration for the method.

4.3 **Ion Trap vs. Triple Quadrupole.** During the course of method development, several LC/MS/MS instruments (triple quadrupole and quadrupole ion trap) were utilized which led to different optimum conditions. This highlighted differences between the instrumentation. The main difference between these two LC/MS/MS instruments that affected Method 535 performance was the scan duty cycle. The triple quadrupole instrument can scan much faster than the ion trap in the selected reaction monitoring mode. This meant that more resolution between chromatographic peaks was needed in



order to get enough scans across the peak on the ion trap instrument. Typically, more than two scan functions per time segment resulted in higher %RSDs for the ion trap instrument. Thus, more separation between the analyte peaks (defined as ≤ 2 scans functions/segment time) had to be achieved for good quantitation on the ion trap. The LC conditions were modified from those of the triple quadrupole: 1) longer C_{18} column, 2) modified mobile phase gradient, and 3) higher column temperature. Similar resolution for alachlor ESA and acetochlor ESA was achieved on the ion trap versus the triple quadrupole using these modified conditions. Calibration on the ion trap instrument was not nearly as stable (1-2 days) as for the triple quadrupole instrument (2 to 3 weeks). Since a solution to the calibration stability could not be found, daily calibration was performed when using the ion trap. Finally, the LCMRLs obtained on the two instruments were significantly different and compound dependent. For most compounds, the ion trap was more sensitive, but quantitation was more difficult due to the duty cycle issues and the calibration instability. Provided the QC criteria in Method 535 is met, any tandem mass spectrometer can be used for analyses of the target analytes.

5 Selection of Preservatives

EPA drinking water regulatory methods typically use preservatives to prevent microbial degradation (e.g., acid, copper(II) sulfate, diazolidinyl urea (DZU), tris(hydroxymethyl)-nitromethane) and to dechlorinate (e.g., sodium sulfite [Na_2SO_3], trizma, ammonium chloride) the sample. Microbial degradation of the target analytes cannot be predicted in all types of matrices containing various types of microbiological contaminants, thus an anti-microbial agent is desirable. While chlorine may not adversely affect the stability of acetanilide degradates, it can interfere in the solid phase extraction; thus, the residual chlorine should be removed. A number of preservative combinations were investigated based on research conducted by Winslow and colleagues:[7] copper(II) sulfate/trizma, DZU/trizma, tris(hydroxymethyl)-nitromethane/trizma, hydrochloric acid (pH=2)/sodium sulfite and ammonium chloride. Only the hydrochloric acid (pH=2)/sodium sulfite combination and the ammonium chloride met the method recovery (%REC) and precision (%RSD) DQOs with the exception of the surrogate (benzoylbenzoic acid, BBA). Extractions performed using only trizma (no anti-microbial) resulted in acceptable recoveries for all analytes indicating that trizma was not the problem. In the case of copper(II) sulfate, the sulfate anion may be interfering with the adsorption of the target analytes onto the carbon SPE sorbent. In the case of DZU, retention of the highly concentrated DZU on the carbon cartridges was probably exceeding the capacity of the cartridge, thereby preventing retention of the target analytes. The extracts produced using the tris(hydroxymethyl)nitro-methane/trizma combination were yellow, indicating retention of the tris(hydroxymethyl)nitro-methane on the carbon sorbent, similar to DZU. Analysis of the tris(hydroxymethyl)nitro-methane/trizma extracts overwhelmed the electrospray and yielded poor target analyte recoveries (not shown), thus studies with tris(hydroxymethyl)nitro-methane/trizma were discontinued.

	$CuSO_4$ (0.5 g/L)[a] trizma (7.78 g/L)		DZU (1.0 g/L)[a] trizma (7.78 g/L)		HCl (pH=2)[b] Na_2SO_3 (50 mg/L)		NH_4Cl[c] (100 mg/L)	
Target Analyte	%REC	%RSD	%REC	%RSD	%REC	%RSD	%REC	%RSD
propachlor OA	75	9.3	0	0	90	2.1	88	6.0
flufenacet OA	79	5.8	0	0	87	1.3	89	9.0
propachlor ESA	89	9.1	34	18	97	4.5	97	8.3
flufenacet ESA	82	10	32	15	95	4.1	90	7.5
dimethenamid OA	77	6.0	29	12	88	6.3	96	3.1
dimethenamid ESA	80	6.0	47	21	91	10	103	4.2
alachlor OA	63	15	38	15	71	3.7	84	6.8
acetochlor OA	64	12	45	18	84	7.1	95	6.6
alachlor ESA	70	13	63	23	86	3.7	95	3.2
metolachlor OA	67	7.6	42	16	84	4.2	97	4.3
acetochlor OA	77	4.3	65	20	88	2.5	94	6.5
metolachlor ESA	78	8.2	46	21	94	4.0	104	6.1
BBA - SUR	44	16	79	29	66	8.7	65	2.8

[a] target analytes spiked at 0.25 µg/L in 200 mL of reagent water.
[b] target analytes spiked at 0.50 µg/L in 200 mL of reagent water.
[c] target analytes spiked at 0.05-0.50 µg/L in 200 mL of reagent water.

The sodium sulfite/HCl preservative combination was initially considered, but literature reports[8-10] indicated that the ESAs of the acetanilide herbicides can be synthesized by reaction of the parent herbicide with sodium sulfite. This raised some concern regarding the use of sodium sulfite as a dechlorinating agent. To determine if sodium sulfite could be used, a worst-case-scenario was studied. To 250 mL of reagent water, 50 µg/L of alachlor and metolachlor, and

Compound	48 days[a]		19 hours[a]		1 hour[a]	
	Calc. Amount µg/L	% created	Calc. Amount µg/L	% created	Calc. Amount µg/L	% created
alachlor ESA	4.5	9.0	0.42	0.84	0.023	0.046
metolachlor ESA	0.86	1.7	0.089	0.18	0.010	0.020

[a] Fifty mg Na_2SO_3 added to 250 mL of chlorinated surface water fortified with 50 µg/L each of metolachlor and alachlor.

50 mg of sodium sulfite were added. These samples were stored at room temperature and extracted and analyzed at various time intervals (48 days, 19 hours, 1hour). The data in the table show that if the parent compound was present, up to 10% conversion to the ESA occurred. Although the percent conversion was small under these conditions, any measurable conversion of the parent herbicide into the ESA by the dechlorinating agent is unacceptable. Thus, sodium sulfite was eliminated as a potential dechlorination agent.

Ammonium chloride became the only choice for a dechlorination agent for Method 535. Since no antimicrobial could be found that did not adversely affect the recoveries of the target analytes, the decision was made to not add an antimicrobial and set the maximum aqueous holding time to 14 days (Sect. 8).

6 Selection of IS and SUR

Many compounds were evaluated as potential internal standards (IS) and surrogates (SUR) for Method 535. Deuterated analogs of the targets would be optimum, but they did not exist for these target analytes. Also, it was difficult to find compounds similar in structure to the target analytes. Thus, the search for suitable IS and SUR began with commercially available carboxylic and sulfonic acids. Specifically, acids were sought which respond well in negative ion ESI and elute near or within the LC retention window of the target analytes. Many of the compounds that were tried were eliminated because of elution in the LC void volume or because of poor chromatographic peak shapes in the gradient system employed for the target analytes. Phenylbenzoic acid, dibromosalicylic acid, diphenylacetic acid, and dichlorophenyl-acetic acid were both used for a while as internal standards, but demonstrated poor day-to-day reproducibility which did not always accurately track the target analytes. Benzoylbenzoic acid showed promise as a surrogate but its recovery tended to be low (60-80%) and erratic in the presence of the preservatives studied.

Compound	Use	Results
2,4-dichlorophenylacetic acid	IS/SUR	poor day-to-day reproducibility/poor recoveries
4-phenoxylbenzoic acid	IS	poor day-to-day reproducibility/poor recoveries
2-benzoylbenzoic acid	SUR	low recoveries in preserved samples
4'-bromoacetoacetanilide	IS	peak tailed significantly under mobile phase conditions
2,2-diphenylpropionic acid	IS	poor sensitivity
1,2-naphthoquinone-4-sulfonic acid	IS	elution in the void volume
3,5-dibromosalicylic acid	IS	poor reproducibility
diphenylacetic acid	IS	poor reproducibility
butachlor ESA	IS	chosen IS for Method 535
dimethachlor ESA	SUR	chosen SUR for Method 535

In the end, butachlor ESA and dimethachlor ESA were chosen as the IS and SUR, respectively. These compounds were synthesized by Snow et al.[11] for use as an IS and SUR in their laboratory. Although dimethachlor and butachlor are herbicides, they are not currently approved for use in the U.S. Thus, their ESA degradates should not be found in the U.S. environment. Unfortunately, these compounds are not commercially available, so they must be synthesized by the Method 535 user. Directions for the synthesis of the IS and SUR are included in Method 535 Appendices A and B. For the purposes of the UCMR, dimethachlor ESA and butachlor ESA will be synthesized in our laboratory, ampulized and distributed to the UCMR participating laboratories. Butachlor ESA proved to be difficult to elute from the carbon cartridges, thus was chosen as the IS. Although more stable than some of the other internal standards tried, area counts for butachlor ESA occasionally changed significantly (30-50%) from one day to the next, then stayed constant for weeks. In the case of butachlor ESA, the target analytes tracked this increase or decrease in area counts, unlike the other failed internal standards. This apparent ESI imprecision was accounted for in Method 535 by allowing a 50% drift of the IS area counts in the continuing calibration check (CCC) compared to the initial calibration. The typical CCC requirement used to verify that the IS area counts are within 30% of the most recent CCC (used in other EPA methods) was removed from Method 535 to allow for more daily drift in the CCC as long as the target analytes still meet the 70-130% accuracy requirement.

Dimethachlor ESA was chosen as the SUR because it mimicked the target analytes which are more likely to breakthrough the carbon cartridges due to matrix effects. Compounds that elute early in the LC chromatogram were those most easily eluted from the carbon SPE cartridge. Dimethachlor ESA eluted early in the chromatogram, and the earlier LC eluters (propachlor OA and flufenacet OA) tended to breakthrough the carbon SPE in high TOC matrices. Thus, the use of dimethachlor ESA as a SUR will aid in monitoring for extraction problems.

7 Evaluation of Carbon SPE disks

In addition to cartridges, carbon is available as disks. Thus, carbon disks were evaluated for inclusion into Method 535. Using SPE conditions similar to those used for cartridges, low recoveries were initially obtained for the majority of the analytes using 47 mm carbon disks. Assuming the analytes had not exhibited breakthrough on the disks, the elution volume was increased to 30 mL and the ammonium acetate concentration in the methanol was increased to 50 mM. However, only slight increases in the recoveries were observed. Recoveries greater than 70% were finally obtained by flipping the carbon disk over before eluting with 30 mL of 50 mM ammonium acetate in methanol. Several compounds were still marginally recovered (74-79%) in reagent water. Given the demonstrated difficulty in removing the target analytes from the carbon disks, the carbon disk work was discontinued and carbon cartridges were chosen as the only option for SPE.

	0.4 µg/L fortification of Reagent water						0.4 µg/L fortification of high TOC surface water	
	12 mL elution of 10 mM NH_4OAc		30 mL elution of 50 mM NH_4OAc		30 mL elution of 50 mM NH_4OAc flipped disk		30 mL elution of 50 mM NH_4OAc flipped disk	
Target Analyte	%REC	%RSD	%REC	%RSD	%REC	%RSD	%REC	%RSD
propachlor OA	88	6.4	86	5.9	98	9.9	89	2.3
flufenacet OA	85	3.1	85	8.8	101	12	86	6
propachlor ESA	45	19	67	2.4	97	3.6	95	5.1
flufenacet ESA	42	22	61	11	93	4.4	86	18
dimethenamid OA	8.7	20	14	14	108	2.2	91	9.8
dimethenamid ESA	N.D.[a]	N.D.	N.D.	N.D.	79	11	81	17
alachlor OA	5.0	5.7	9.4	6.0	116	2.1	84	7.0
acetochlor OA	N.D.	N.D.	16	4.4	115	7.1	80	1.4
alachlor ESA	9.8	3.6	9.8	3.6	74	3.1	66	11
metolachlor OA	N.D.	N.D.	14	4.1	95	0.40	79	5.7
acetochlor ESA	N.D.	N.D.	N.D.	N.D.	76	5.5	72	27
metolachlor ESA	N.D.	N.D.	N.D.	N.D.	84	5.3	65	9.7
BBA-SUR	N.D.	N.D.	9.6	0.74	66	11	80	9

[a] Not detected.

8 Aqueous Storage and Holding Time Study

An aqueous holding time study was performed to evaluate the chemical stability of the target analytes during shipping and during the 14 day holding time. Replicate samples of a chlorinated ground water were collected, dechlorinated, fortified with target analytes, and stored as described in Method 535, Sect. 8. The samples were stored at 10 °C for 48 hours, the maximum allowed by the method, before being moved to 6 °C storage for the remainder of the storage period. A randomly selected set of seven samples was extracted and analyzed on the day of preparation (day 0) and at days 7, 14, and 21. The data from these analyses are provided in Method 535, Section 17, and support the established 14 day aqueous holding time.

9 Extract Storage and Holding Time Study

Extracts prepared on day 0 of the aqueous holding time study (N=7) were stored at 4 °C, and reanalyzed on days 7, 14, 21, 28 and 35. Data from these analyses are provided in Method 535, Section 17, and support the established 28 day extract holding time.

10 Disclaimer

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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