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Direct Aqueous Injection LC−ESI/MS/MS Analysis of Water for 11 Chloro- and Thiomethyltriazines and Metolachlor and Its Ethanesulfonic and Oxanilic Acid Degradates

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A multianalyte method is reported for the determination of atrazine, simazine, propazine, and their respective dealkylated chlorotriazine metabolites; ametryn and prometryn and their respective dealkylated thiomethyltriazine metabolites; and S-metolachlor and its ethanesulfonic and oxanilic acid degradates in deionized, ground, surface, and finished drinking water. Water samples are analyzed using direct aqueous injection (DAI) liquid chromatography−electrospray ionization/mass spectrometry/mass spectrometry (LC−ESI/MS/MS). No preanalysis sample manipulation is required other than transfer of a small portion of sample to an injection vial. The lower limit of the method validation is 0.050 μg/L (ppb) for all analytes except 2,4-diamino-6-chloro-*s*-triazine (didealkylatrazine, DDA, or G-28273). For this compound the LLMV is 0.50 μg/L (ppb). The overall mean procedural recoveries (and percent relative standard deviations) for all water types for all analytes ranged from 95 to 101% (4.5−11%). The method validation was conducted under U.S. EPA FIFRA Good Laboratory Practice Guidelines 40 CFR 160.

KEYWORDS: Direct aqueous injection; LC/MS/MS; chlorotriazines; dealkylated; chlorotriazines; thiomethyltriazines; dealkylated thiomethyltriazines; total chlorotriazines; acetanilide; Good Laboratory Practices (GLP)

INTRODUCTION

Atrazine [1,3,5-triazine-2,4-diamine, 6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-], simazine [1,3,5-triazine-2,4-diamine, 6-chloro-*N*,*N*′-diethyl-], and propazine [1,3,5-triazine-2,4-diamine, 6-chloro-*N*,*N*′-bis(1-methylethyl)-] are chlorotriazine herbicides manufactured, formulated, and sold under various trademarks by several agrochemical companies. Atrazine is most often used in corn, sorghum, and sugar cane production for the control of broadleaf and grass weeds, whereas simazine is primarily used for weed control in corn, citrus, grape, and other fruit and vegetable crops. Propazine is used primarily for the control of annual broadleaf weeds and grasses in sorghum, umbelliferous crops, and glasshouse ornamentals. Ametryn [1,3,5-triazine-2,4-diamine, *N*-ethyl-*N*′-(1-methylethyl)-6-(methylthio)-] and prometryn [1,3,5-triazine-2,4-diamine, *N*,*N*′-bis(1-methylethyl)-6-(methylthio)-] are thiomethyltriazines used primarily for the control of annual grasses and broadleaf weeds in citrus fruit, coffee, tea and in cotton, sunflowers, peanuts, respectively. *S*-Metolachlor [acetamide, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]-] is used for the control of numerous winter and annual grasses and broadleaf weeds in a wide variety of vegetables, stone fruits, and tobacco.

The chloro- and thiomethyltriazines metabolize in plants and animals (*1*) and undergo environmental degradation via chemical and microbiological transformation processes to form dealkylated chlorotriazines (or dealkylated thiomethyltriazines) (*2*), conversion to hydroxytriazines (*3, 4*), and eventual mineralization to carbon dioxide and ammonia (*5*). The dealkylated chlorotriazine metabolites consist of deethylatrazine (DEA), deisopropylatrazine (DIA), and didealkylatrazine (DDA). All three of these compounds can result from the degradation/metabolism of atrazine, but only DIA and DDA can result from the degradation/metabolism of simazine, and only DEA and DDA can result from the degradation/metabolism of propazine. Analogous to the chlorotriazines, the dealkylated thiomethyltriazines consist of deethylametryn (DEAM), deisopropylametryn (DIAM), and didealkylametryn (DDAM). All three of these metabolites can arise from the degradation/metabolism of ametryn, but only DEAM and DDAM can be obtained from the degradation/metabolism of prometryn. The acetanilide *S*-metolachlor degrades in the environment to form the ethanesulfonic and oxanilic acid degradates (*6, 7*), which are on EPAs Unregulated Contaminant Monitoring Regulation Second Cycle (*8*).

Chlorotriazine metabolite levels in surface water, when detected solely in the upper percentiles of the most vulnerable water systems, are typically a fraction of the parent and show

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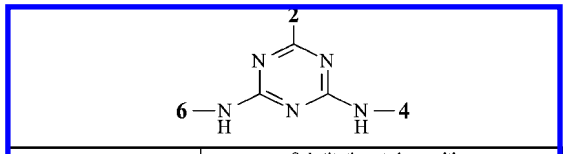
Table 1. Chemical Names and Code Numbers for the Compounds in the Method Validation Study

Syngenta code	common name	CAS chemical name	CAS registry number
G-30027	atrazine	1,3,5-triazine-2,4-diamine, 6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-	1912–24–9
G-27692	simazine	1,3,5-triazine-2,4-diamine, 6-chloro-*N,N*′-diethyl-	122–34–9
G-30028	propazine	1,3,5-triazine-2,4-diamine, 6-chloro-*N,N*′-bis(1-methylethyl)-	
G-30033	deethylatrazine (DEA)	1,3,5-triazine-2,4-diamine, 6-chloro-*N*-(1-methylethyl)-	6190–65–4
G-28279	deisopropylatrazine (DIA)	1,3,5-triazine-2,4-diamine, 6-chloro-*N*-ethyl-	1007–28–9
G-28273	didealkylatrazine (DDA)	1,3,5-triazine-2,4-diamine, 6-chloro-	3397–62–4
G-34162	ametryn	1,3,5-triazine-2,4-diamine, *N*-ethyl-*N*′-(1-methylethyl)-6-(methylthio)-	834–12–8
G-34161	prometryn	1,3,5-triazine-2,4-diamine, *N,N*′-bis-(1-methylethyl)-6-(methylthio)-	7287–19–6
GS-11354	deethylametryn (DEAM)	1,3,5-triazine-2,4-diamine, *N*-(1-methylethyl)-6-(methylthio)-	4147–57–3
GS-11355	deisopropylametryn (DIAM)	1,3,5-triazine-2,4-diamine, *N*-ethyl-6-(methylthio)-	4147–58–4
GS-26831	didealkylametryn (DDAM)	1,3,5-triazine-2,4-diamine, 6-(methylthio)-	5397–01–3
CGA-77102	*S*-metolachlor	Acetamide, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]-	87392–12–9
CGA-354743	metolachlor ESA	ethane sulfonic acid, 2-[(2-ethyl-6-methylphenyl) -(2-methoxy-1-methylethyl)amino]-2-oxo-, Na	171118–09–5
CGA-51202	metolachlor OA	acetic acid, [(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]oxo-, Na	152019–73–3

a clear seasonal pattern, with metabolite to parent ratios lowest in the second quarter of the year and increasing during the rest of the growing season. The names and experimental codes of all the compounds included in this study are listed in **Table 1** and their structures are shown in **Figures 1** and **2**.

The occurrence and fate of atrazine and simazine and their respective dealkylated chlorotriazine degradates in water have been the subjects of numerous publications over the past 2 decades, and as a consequence, more than 1000 methods have been reported using a wide variety of sample preparation procedures and detection schemes (*9*). The analysis of triazine compounds was recently reviewed (*10, 11*). Generally, techniques such as gas chromatography/mass selective detection (GC/MSD) (*12, 13*) have been preferred to support large-scale water monitoring studies, due to its sensitivity and confirmatory ability. However, applications of liquid chromatography/mass spectrometry (LC/MS) continue to increase, particularly for the analysis of aqueous samples, and the technique is directly applicable to the analysis of thermally labile compounds such as the ESA and OA degradates of *S*-metolachlor that are not easily amenable to analysis using GC (*14*).



Compound	Substitution at ring position 2	4	6
Atrazine	-Cl	$-C_2H_5$	$-CH(CH_3)_2$
Simazine	-Cl	$-C_2H_5$	$-C_2H_5$
Propazine	-Cl	$-CH(CH_3)_2$	$-CH(CH_3)_2$
Deethylatrazine (DEA)	-Cl	-H	$-CH(CH_3)_2$
Deisopropylatrazine (DIA)	-Cl	$-C_2H_5$	-H
Didealkylatrazine (DDA)	-Cl	-H	-H
Ametryn	$-SCH_3$	$-C_2H_5$	$-CH(CH_3)_2$
Prometryn	$-SCH_3$	$-CH(CH_3)_2$	$-CH(CH_3)_2$
Deethylametryn (DEAM)	$-SCH_3$	-H	$-CH(CH_3)_2$
Deisopropylametryn (DIAM)	$-SCH_3$	$-C_2H_5$	-H
Didealkylametryn (DDAM)	$-SCH_3$	-H	-H

Figure 1. Structures of the triazine compounds included in this study.

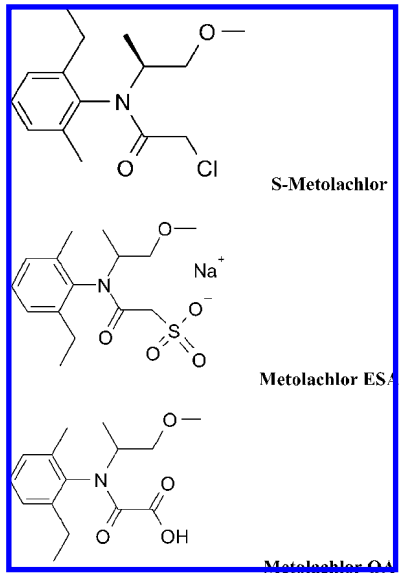


Figure 2. Structures of *S*-metolachlor and its ESA and OA degradates.

Direct injection liquid chromatography−electrospray ionization/mass spectrometry/mass spectrometry (LC−ESI/MS/MS) has been successfully applied to the analysis of a wide range of compounds in various sample matrices, including opioids and cocaine (*15*) and alkyl phosphates (*16*) in urine, propamocarb (*17*) and *N*-methyl carbamate pesticides (*18*) in wine, and organophosphorus pesticides in vegetable extracts (*19*). Several applications to the analysis of pesticides in water have been reported and include the determination of dimethyl tetrachloroterephthalate (*20*), 4-chloro-2-methylphenoxyacetic acid (*21*), various organophosphorus pesticides (*22*), carbamates, thiocarbamates, and phenylureas (*23*), acetanilide degradates (*24*), and chlorotriazines (*25*).

In this work, we describe a direct aqueous injection (DAI) liquid chromatography electrospray ionization mass spectrometry/mass spectrometry method that can be used to quantify 14 compounds: six chloro- and five thiomethyltriazines and *S*-metolachlor and its ESA and OA degradates in water with no sample manipulation prior to injection. Note, this is not a chiral separation, so other isomers of this racemic mixture of metolachlor such as *R*-metolachlor can also be detected and whether or not the source of the detection is from the old or the new product cannot be inferred.

EXPERIMENTAL PROCEDURES

Standards. Analytical grade standards of atrazine (97.9%), simazine (99.7%), propazine (98.5%), DEA (94%), DIA (96%), DDA (97%), ametryn (98.3%), prometryn (99.7%), GS-11354 (97%), GS-11355 (98%), GS-26831 (99%), *S*-metolachlor (97.9%), metolachlor ethanesulfonic acid (95.9%), and metolachlor oxanilic acid (99.7%) were obtained from the Technology and Projects Department and the Chemical Synthesis Group of Syngenta Crop Protection, Inc. (SCP), Greensboro, NC. Individual stock standards were prepared by weighing 10.0 mg of each compound (5.0 mg

Table 2. Characterization Data for the Three Types of Water Used in the Method Validation Study

	finished water	surface water	ground water	deionized water
location	treated well water from Belews Creek, NC	Spring Lake, Kernersville, NC	well water, Summerfield, NC	PicoPure Water System, Syngenta, Greensboro, NC
pH	7.5	6.2	7.1	6.4
calcium (ppm)	24	3.2	11	1.1
magnesium (ppm)	4.9	1.7	3.7	0.2
sodium (ppm)	13	4.4	7.8	<0.1
hardness (mg CaCO/L)	81	15	43	3.0
conductivity (mmhos/cm)	0.23	0.17	0.14	0.01
sodium adsorption ratio (SAR)	0.61	0.50	0.52	0.0
total dissolved solids (ppm)	162	14	108	4.0
turbidity (NTU)	0.58	1.26	0.19	0.18

of DEA, DIA, and DDA), corrected for percent purity, into each of 14 100-mL volumetric flasks (one compound in each flask) followed by dilution to the mark with methanol. The smaller quantities of metabolites weighed and the use of methanol as solvent were due to solubility limitations. A 5.0 μg/mL mixed standard was prepared by transferring 5.0 mL of each stock solution (10 mL of the DEA, DIA, and DDA stock solutions) to a 100-mL volumetric flask followed by dilution to the mark with HPLC grade methanol. Serial dilutions of the mixed standard were prepared in 5/95 (v/v) acetonitrile/water to create mixed working standards in the 0.02−4.0 pg/μL concentration range (equivalent to a range of 1.0−200 pg injected for a 50 μL injection volume). These standards were used for calibration and fortification purposes. All standard solutions were stored in amber-colored glass bottles at refrigerator temperature (4 °C).

Solvents and Reagents. HPLC grade methanol (Fisher Cat. No. A452SK-4), water (Fisher Cat. No. W5SK-4), and acetonitrile (Fisher Cat. No. A998SK-4) were used for preparation of the standards and mobile phases. Deionized water was obtained from the Picopure water purification system in the laboratories of SCP. HPLC grade formic acid (88%, Fisher Cat. No. A118P-500) was used in the preparation of the LC mobile phases.

Preparation of Solutions. Formic acid was added to acetonitrile and water at the 0.10% concentration level to create mobile phase solutions A and B. Methanol was used in the preparation of standards.

Sample Storage. Field water samples to be analyzed should be stored in amber glass bottles in the dark at refrigerator temperature (4 °C) until analyzed. Previous work in this laboratory demonstrated stability for at least 2 years for all 14 analytes when samples were stored under these conditions. Note, all the results reported in this study are for laboratory-fortified ground, surface, and DI water that were analyzed almost immediately after fortification.

Water Sample Sources. The groundwater used in this study was obtained from a well at a private residence in Summerfield, NC; surface water was obtained from Spring Lake in Kernersville, NC; finished drinking water was obtained from a treated community well in Belews Creek, NC; deionized water was obtained from the PicoPure water purification system in the laboratories of SCP in Greensboro, NC. Samples of all four water types were analyzed by Agvise Laboratories, Northwood, ND, and the resultant characterization data are shown in **Table 2**.

Sample Preparation. Small portions of water samples (typically 500−800 μL) are transferred to HPLC injection vials. No sample dilution is necessary prior to injection unless the sample, as demonstrated in previous work, is known to exhibit suppression. Samples visibly containing strong color or particulates may be subjected to centrifugation prior to analysis. Sample injection is typically performed overnight.

Procedural recovery samples can be obtained by judicious choice of mixed standard concentration, its volume, and the volume of sample to be fortified. For example, the addition of 1.0 mL of a 0.010 μg/mL mixed standard to a 100-mL aliquot of water produces a 0.10 ppb fortification. Analyte fortifications at the 0.050, 0.10, 0.50, and 3.0 ppb concentration levels were analyzed during the method validation study. During routine analysis, a minimum of two recovery samples should be included in every analytical set: one at the lower limit of method validation (LLMV) and one at a value believed to be higher than the highest concentration of residue expected in the field samples.

Table 3. LC Gradient Used during These Analyses

Time (min.)	% A	% B	
0.0	95	5	Linear Gradient @ 0.5 mL/min
2.0	5	95	Stay at 95%B @ 0.5 mL/min
3.0	5	95	Step to 5%B @ 1.0 mL/min
4.0	95	5	Stay at 5%B @ 0.5 mL/min
6.0	95	5	

Instrumentation. Analyses were performed using a Perkin-Elmer Series 200 liquid chromatograph (LC) interfaced to an Applied Biosystems, MDS Sciex API-4000 tandem mass spectrometer utilizing electrospray ionization (TurboSpray at 700 °C) in both the positive and negative ion modes. The software was Analyst 1.4.1. A Zorbax SB-AQ (4.6 mm × 50 mm, 3.5 μm particle size, Agilent P/N 835975–914) LC column and a column filter (ColumnSaver, MAC-MOD P/N MMCS210) were used at a flow rate of 0.50 mL/min and maintained at a temperature of 25 °C. Mobile phase A was water and mobile phase B was acetonitrile, each containing 0.10% formic acid. The injection volume was 50 μL and the gradient used is detailed in **Table 3**. The run time per injection was 6 min.

Two injections per sample were required: the first was performed solely in the + ion mode (12 analytes) and the second performed solely in the − ion mode (two analytes), thus increasing the run time per sample to 12 min. This was done because the switching time (700 ms) on the MDS/Sciex API-4000 system between the + and − ion modes of operation was too slow to reproducibly ensure the collection of an adequate number of data points for each of the 14 analytes to provide good peak shape and integration reliability and repeatability. Plus, monitoring in solely one mode provided the sensitivity necessary to quantify the residues at 0.05 μg/L (ppb). The LC gradient used for each of the two injections was identical.

The ionspray voltages employed were 5500 V (+ mode) or 4500 V (− mode). The instrument settings and acquisition parameters were as follows: CUR, 10; GS1, 50; GS2, 50; CAD, 2; EP, +10/-10 (+/− mode); scan type, multiple reaction monitoring (MRM); resolution Q1, unit; resolution Q2, unit. The ions selected for MRM, corresponding operating parameters, and retention times are listed in **Table 4**. Note, that only the most abundant MRM transition was monitored. Although it is good qualitative practice to monitor a second MRM transition for confirmatory purposes, this was not done in this work due to the desire to obtain the lowest LOQ possible. In other words, the more transitions monitored, the less sensitivity for those employed for quantitative purposes due to decreased dwell time for each transition. Thus, analyte detections at some level deemed to be important may require a reanalysis solely for that analyte using one or two additional MRMs to confirm the analyte's identity.

Sample Analysis. Each analytical set consisted of eight analytical standards of various concentrations, reagent blank, control, and controls

Table 4. Various MS/MS Operating Parameters

analyte	MRM transition	ion mode	dwell time (ms)	DP	CE	CXP	retention time (min)
DDA	145.7 > 104.0	+	40	52	27	12	1.91
	145.7 > 79.0[a]						
DDAM	158.2 > 110.0	+	40	60	25	10	1.85
	158.2 > 110.0[a]						
DIA	174.2 > 96.2	+	40	75	24	8	2.16
	174.2 > 146.0[a]						
DIAM	186.1 > 96.0	+	40	80	30	9	2.01
	186.1 > 68.0[a]						
DEA	188.3 > 104.0	+	40	40	35	8	2.29
	188.3 > 146.0[a]						
DEAM	200.3 > 158.0	+	40	70	24	14	2.10
	200.3 > 116.0[a]						
simazine	202.1 > 132.1	+	40	53	27	8	2.48
	202.1 > 104.0[a]						
atrazine	216.1 > 174.2	+	40	70	25	11	2.60
	216.1 > 104.0[a]						
ametryn	228.2 > 186.1[a]	+	40	65	25	20	2.28
	228.2 > 96.0						
prometryn	242.0 > 158.2	+	40	70	32	12	2.38
	242.0 > 200.0[a]						
propazine	230.2 > 145.9	+	40	66	31	12	2.70
	230.2 > 188.1[a]						
S-metolachlor	284.2 > 176.3	+	40	55	40	17	2.87
	284.1 > 252.3[a]						
metolachlor-ESA	328.2 > 121.0	−	100	−114	−32	−9	2.35
	328.2 > 79.0[a]						
metolachlor-OA	278.1 > 206.3	−	100	−62	−16	−9	2.44–2.57
	278.1 > 174.0[a]						

[a] Second MRM transition.

fortified with the analytes at the 0.050−3 μg/L (ppb) concentration level for procedural recovery purposes. Additional standards were dispersed throughout the sequence as a means of checking system stability and column performance. When analyzing true field-collected samples, we also highly recommend analyzing at least one or more in triplicate in order to evaluate repeatability (within-run variance).

RESULTS AND DISCUSSION

LC−MS/MS Analyses. Representative MRM chromatograms of a 1.0 pg injected mixed standard (lowest concentration of standard injected and used to construct the calibration plots and equivalent to 0.020 ppb), controls, and 0.05 ppb procedural recovery samples for DDAM and *S*-metolachlor in surface water are shown in **Figures 3** and **4**, respectively. Figures for surface water are shown because this is generally the most challenging matrix type with regard to suppression or interference issues, and figures for DDAM and *S*-metolachlor were chosen simply because these have the shortest and longest retention times, respectively. The signal-to-noise ratio (*S*/*N*) is ≥5 in all cases for standard injections at the 1.0 pg injected level and the *S*/*N* ratio is ≥10 for the procedural recovery samples fortified at the lower limit of method validation (LLMV). The picograms injected and their respective responses for each analyte were used for construction of the calibration plots, and all were linear (weighted regression) with correlation coefficients >0.99 throughout the study. The responses for peaks detected in the control samples, if any, were subtracted from the responses for the peaks detected in the procedural recovery samples prior to calculation of percent recovery.

A Stable-Bond AQ column was used in this work in order to provide sufficient retention for DDA and DDAM to obtain reproducible peak shape and ensure adequate separation of these polar compounds from the column void volume. DDA and DDAM elute too quickly on C-2, C-8, and C-18 columns and exhibit severe peak asymmetry. Although using the AQ polar column, the mobile phase composition is such that the separation is still in reverse phase mode as demonstrated by increasing retention with decreasing analyte polarity. Interestingly, dissolved components in the four water types used in this validation previously exhibited suppression when analyzing undiluted samples using a cyano LC column (*25*). Thus, dilution was required in order to minimize the effects of suppression. In this work, it appears the sample components responsible for the suppression are either eluted early, possibly in or near the void volume, or after 3 min on the AQ column, since no suppression was observed for any of the analytes. The retention times of all 14 analytes ranged from only 1.8 to 2.9 min. This narrow retention window may be wholly or at least partially responsible for the absence of suppression during the analysis of these samples by isolating or reducing coelution of the analytes of interest from the majority of the suppression causing sample components. The analysis of undiluted samples increases the sensitivity of the measurement and allows lower analyte concentrations to be reliably measured. During the method validation work, the number of analyses that could be realistically performed was limited so only the four water types (well, surface, finished, and DI) were used during the validation. However, in addition to these water types, 20 additional water types (mostly surface water) were tested for potential suppression effects using this method prior to the validation study. Although these 20 water types cannot represent every type of water sample encountered that requires analysis, it does demonstrate a high degree of method ruggedness as far as avoiding matrix suppression effects. Of course, in any analytical work involving the analysis of new water samples, an evaluation for potential matrix suppression effects should be performed before embarking on a massive monitoring study.

It is relatively common practice to add low concentration levels of acetic or formic acid (0.1% or so) to the mobile phase

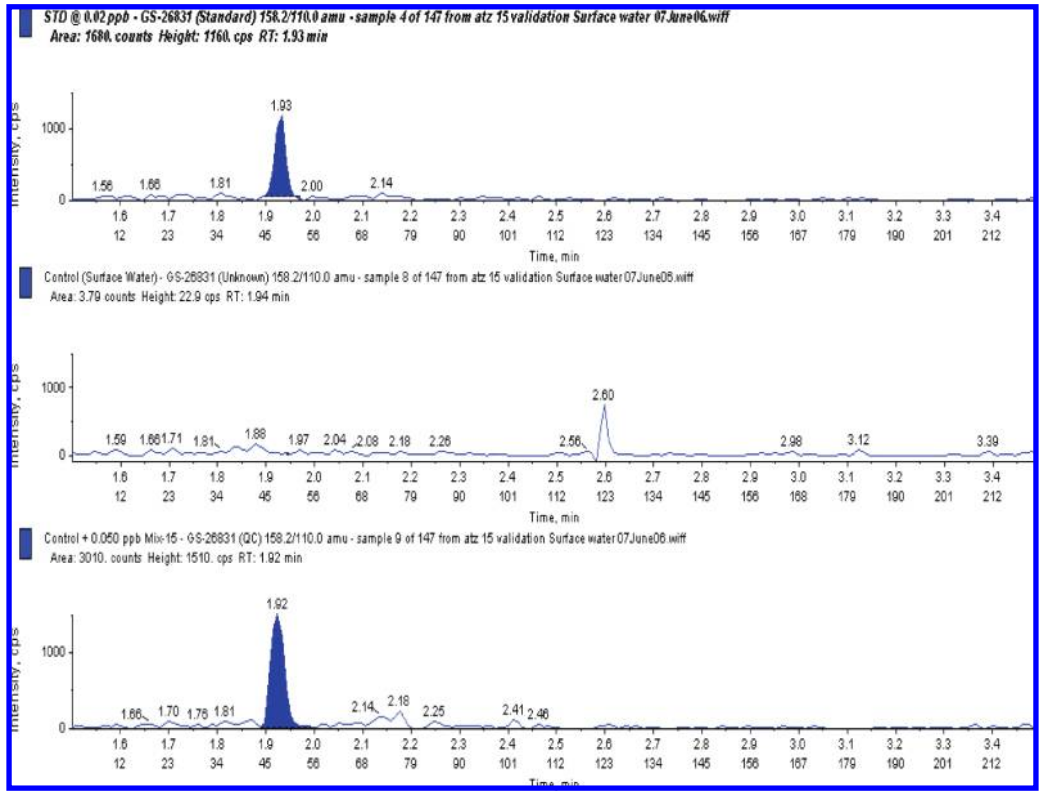


Figure 3. Representative MRM chromatograms from the analysis of surface water for DDAM: (top) 0.02 ppb standard (50 μL injection of a 0.02 pg/μL standard to give a 1.0 pg on-column injection), (middle) control, and (bottom) 0.050 ppb procedural recovery sample.

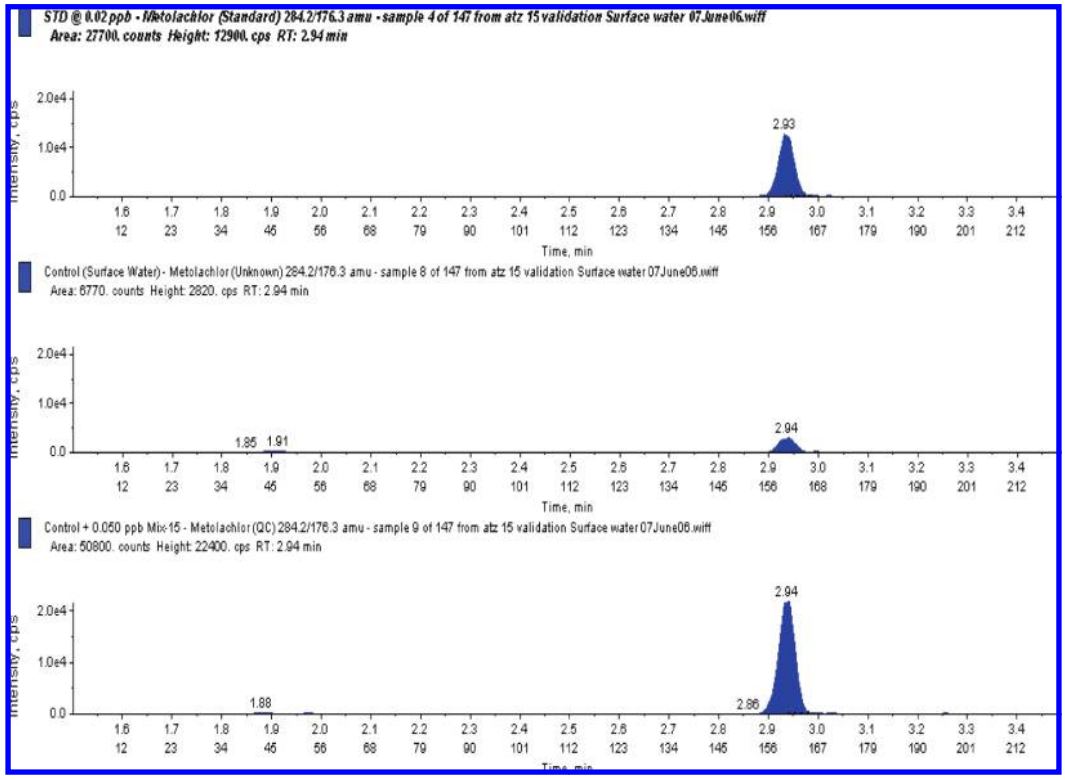


Figure 4. Representative MRM chromatograms from the analysis of surface water for *S*-metolachlor: (top) 0.02 ppb standard (50 μL injection of a 0.02 pg/μL standard to give a 1.0 pg on-column injection), (middle) control, and (bottom) 0.050 ppb procedural recovery sample.

when using electrospray ionization in order to increase the abundance of M + 1 ions. In this work, the addition of formic acid slightly suppressed the observed ESI signal, especially for the parent compounds atrazine and simazine, when using a methanol/water mobile phase system. However, signal enhancement was observed when adding formic acid to the mobile phase when using acetonitrile instead of methanol. In previous work with a cyano column (*25*), methanol provided better overall separation and peak shape for the more polar analytes.

As described earlier, two injections were required to obtain data for all 14 analytes. Although not employed during this method validation study, our laboratory also evaluated the possibility of utilizing one injection followed by splitting the LC effluent into two paths prior to entrance into the ESI source. By splitting at this point in the analysis, each path would contain all 14 analytes. One path would be very short, allowing almost instantaneous entry to the source and mass spectrometer. The instrument would be operated solely in the + ion mode during this time to collect data for the 12 + ion mode analytes. By judicious choice of peek tubing length and diameter, entry of the analytes from the second path into the ESI source would be delayed until after the last analyte from the first path had entered the instrument. The instrument would be operated solely in the − ion mode during this time to collect data for the two ion mode analytes. The immediate advantage is the ability to use one injection per sample, but the disadvantages may be the unequal concentration of all 14 analytes in each path after splitting, and the total run time may be still close to the time required for the two separate injections (12 min). Further evaluation is required to fully determine the advantages and disadvantages of this approach.

Work in our laboratory with a Thermo Electron TSQ Quantum Ultra MS/MS system (mode switching time of 330 ms compared to 700 ms for the API-4000) resulted in the need for only one injection to obtain data for all 14 analytes. However, the frequent mode switching significantly shortened the lifetime of the instrument's ion source high-voltage power supply. Future generation instruments from all manufacturers will likely encompass faster mode switching times and make one injection analysis possible.

As discussed in the instrumentation section, only one MRM transition was monitored for each analyte during these analyses. Pozo et al. (*26*) indicated that there are no specific regulations about confirmation in the environmental field but highly recommended the acquisition of a second transition for each analyte to safely confirm the presence or identity of the detected analyte. While we are in complete agreement with this approach to ensure correct identification of detected analytes, we feel the monitoring of one MRM in this particular application is warranted for the following reasons: (1) our goal is to obtain the lowest possible LOQ, since some of these data could be used for risk assessment purposes (in which case a false positive would be more conservative than using half-LOQ values). (2) Some of these analytes are found at low concentrations in agricultural areas particularly vulnerable to runoff, and these are the areas usually subjected to monitoring studies; thus, true unknown samples are atypical. (3) This method could be considered a screening procedure, and any sample with a positive detection at some predetermined trigger level (e.g., perhaps 3 ppb for atrazine and 4 ppb for simazine) could be reanalyzed using two or more MRM transtions (greatly reducing the number of reanalyses required). (4) Samples with positive detections above a certain action or trigger level in the regulatory arena are typically reanalyzed as a means of obtaining a better estimate of the analyte's concentration via two separate analysis results and reconfirming the analyte's identity for possible enforcement reasons.

On the basis of the intensity ratios of the first MRM to the second MRM transitions for these analytes and taking into consideration the doubling of the number of transitions monitored, the LOQs would increase from 0.10 to the ~1.0 ppb concentration level to monitor two MRM transitions (and to >5 ppb for DDA, which is a small molecule without a satisfactory second MRM transition). Thus, if higher LOQs are adequate for the intended purpose of the analysis, monitoring two MRM transitions could be accomplished for all analytes. If the requirements of a study are still at an LOQ of 0.10 ppb, the monitoring of a second MRM transition for each analyte could be performed easily if data are needed for only about half of the analytes.

Table 5. Compilation of Mean Percent Procedural Recoveries and Percent Relative Standard Deviations for All Analytes in Each Water Type

	mean recoveries/% RSD			
	treated	surface water	ground water	DI water
atrazine	105/4.0	94/5.0	101/4.7	105/3.9
simazine	100/4.4	89/5.1	102/4.2	99/3.9
propazine	97/4.2	93/5.6	98/4.6	97/3.7
DEA	97/6.1	83/5.0	101/3.8	102/5.1
DIA	103/6.9	85/6.9	106/6.0	109/6.3
DDA	95/7.2	94/4.1	109/5.5	99/6.4
ametryn	—[a]	93/3.5	102/3.8	93/3.9
prometryn	—[a]	95/4.2	102/2.8	95/2.1
DEAM	—[a]	83/3.3	101/3.2	100/3.3
DIAM	—[a]	82/5.6	102/3.4	103/5.1
DDAM	—[a]	80/9.9	102/7.3	106/8.0
metolachlor	96/7.3	101/8.5	101/7.2	99/9.6
metolachlor ESA	97/4.5	95/4.4	102/3.4	96/3.8
metolachlor OA	99/4.3	98/6.2	99/3.8	98/4.1

[a] Thiomethyltriazines are not found in chlorine-containing water.

Method Performance. A summary of the procedural recovery data for each water type is shown in **Table 5**. For all analytes, the mean percent recoveries (and percent relative standard deviations, RSD) range from 96 to 105% (4.0–7.3%), 82 to 101% (3.3–9.9%), 98 to 109% (2.8–7.3%), and 93 to 109% (2.1–9.6%) for treated, surface, ground, and deionized water, respectively. The range of mean percent recoveries for the 14 analytes is greatest for surface water, with means in the 80s for six of the analytes. This is not unexpected, since surface water typically contains a larger diversity and wider concentration range of other matrix components than the other three water types. The absence of recovery results for the thiomethyltriazines in treated water is due to the presence of chlorine for disinfectant purposes. The thiomethyltriazine compounds are quickly and easily oxidized in the presence of chlorine to other species and are no longer detectable using the instrumental operating parameters employed using the method described herein.

A summary of data for all analytes in all water sample types is shown in **Table 6**. The mean percent recoveries (% RSDs) range from 95 to 101% (4.5−15%). The overall mean percent recovery and RSD are 98% and 8.3%, respectively. This demonstrates a high degree of accuracy and precision, which is most likely due to the absence of analyte loss mechanisms when performing DAI. No sample extraction or cleanup steps are employed prior to analysis; thus, all the method variability is associated with the water sample matrix type and any effects caused by other dissolved sample components, sample injection variability, and/or fluctuations in sensitivity associated with the instrumentation due to variances in the chromatography, analyte ion formation in the ESI, ion transfer into the MS, etc. The vast majority of the 1929 individual recovery values obtained during this method validation are 70–120% (<1% outside this range), indicating a high degree of method ruggedness and reliability.

The measurement uncertainty associated with these results can be estimated from the validation data (*27*). In this study, the effects of sample inhomogeneity are not an issue, since the

Table 6. Overall Summary of Mean Procedural Recoveries, Relative Standard Deviations, Ranges, and Total Number of Procedural Recovery Samples Analyzed during the Method Validation

	mean %	% RSD	range	*n*
atrazine	101	6.1	73–114	158
simazine	98	6.9	71–111	158
propazine	96	4.9	70–105	158
DEA	96	9.6	75–114	158
DIA	101	11.2	67–123	158
DDA	99	8.4	75–122	80[a]
ametryn	96	5.5	83–109	117[b]
prometryn	98	4.5	85–107	117[b]
DEAM	95	9.4	77–110	117[b]
DIAM	95	11.3	76–114	117[b]
DDAM	96	14.8	64–121	117[b]
metolachlor	99	8.5	66–118	158
metolachlor ESA	98	4.8	80–113	158
metolachlor OA	100	4.8	84–113	158
all	97.8	8.3	67–123	1929

[a] Only data for 0.50 ppb and higher are used, since this is the LLMV for DDA (G-28273). [b] Does not include recovery data from treated water, since these compounds do not survive in the presence of chlorine.

samples are liquids. In addition, the uncertainties associated with analyte loss during sample preparation are almost nonexistent, since the only sample handling step is the transfer of an aliquot portion of water to an injection vial. Thus, the vast majority of the uncertainty comes from the instrumental measurement and this is also usually small when carefully controlling the operating parameters and the appropriate quality control indicators are utilized to warn the analyst of potential problems. The standard uncertainty (bias) of the analytical procedure was calculated for all analytes using the average recovery, relative standard deviation, and number of replicates at each concentration level ($n = 5$) for each of the four water types studied during the method validation (standard uncertainty = standard deviation/$\sqrt{5}$). This value allows calculation of a test statistic t using $t =$ |1 − mean recoveryl/standard uncertainty. If this value is less than or equal to the two-tailed critical value for $n - 1$ degrees of freedom at 95% confidence, the results are not significantly different from 1 and recovery correction is not warranted. In this case, the final result should not be corrected for recovery when using this method to analyze field samples for any of these analytes in these water types, since the test statistic t was much less than the two-tailed critical value.

Since the recovery and precision studies take into account the influence of the calibration of the different volumetric and weighing measurements, only the reference standards and possible nonlinearity in responses need be considered. In this case, the purity of the reference standards is high and accurately known, so the potential uncertainty is expected to be small and negligible. In studies of this type, nonlinearity would contribute to the observed precision, so no additional allowance is required. Therefore, the bias and repeatability are the major contributors to measurement uncertainty in this method. In this study, the repeatability results are almost identical to those used for the calculation of bias, and this allows calculation of the combined uncertainty (0.0005) and the expanded uncertainty ($0.0005 \times 2 = 0.001$). Therefore, the uncertainty associated with a measurement at the LOQ (0.050 ppb) is ±0.001 ppb. Again, this incredible degree of accuracy is attainable only because no sample handling steps are required. However, it is also likely that not all possible sources of measurement uncertainty are accounted for in this assessment. Note that analysts should use measurement uncertainty data with caution when analyzing new water sample types until comparable accuracy (recovery) and precision (standard deviation) data are obtained and the absence of suppression is demonstrated. As expected, the standard deviation consistently decreased as the fortification level increased during this study.

The LLMV is 0.05 μg/L (ppb) for 13 of the analytes and 0.50 ppb for DDA, as these were the lowest procedural recovery concentrations tested. The limit of detection (LOD) is 1.0 pg (10 pg for DDA) and is defined as the lowest concentration of standard injected and used for construction of the calibration plot. These definitions are only slightly more conservative than the 3σ and 10σ (standard deviations) used by the U.S. EPA for LOD and LOQ (*28–30*), respectively, since our *S*/*N* ratios for the lowest concentration of standard injected and lowest procedural recovery tested are about 5 and 10, respectively. Thus, these instrumental figures of merit are likely valid measures of the best attainable LOD and LOQ for the entire procedure, since no sample handling steps are performed.

The results presented herein demonstrate the accuracy and precision of this FIFRA GLP guideline 40 CFR 160 validated analytical method and its applicability to the analysis of atrazine, simazine, and propazine, and their respective dealkylated chlorotriazine metabolites (DEA, DIA, and DDA); ametryn and prometryn and their dealkylated thiomethyltriazine metabolites (DEAM, DIAM, and DDAM); and *S*-metolachlor and its ESA and OA degradates in water. The method is less costly than previously reported methods, since no preinjection sample manipulation is required and therefore meets the objective to be a very cost-efficient alternative to most other methods. Only if signal suppression is encountered is it necessary to dilute samples prior to analysis. Typically, 50 field samples (along with standards, quality-control checks, controls, blanks, procedural recovery samples, etc.) can be injected overnight.

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