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Analytical Method for the Determination of Atrazine and Its Dealkylated Chlorotriazine Metabolites in Water Using SPE Sample Preparation and GC-MSD Analysis

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A method is reported for the determination of atrazine and its dealkylated chlorotriazine metabolites in ground, surface, and deionized water. Water samples are adjusted to pH 3−4 prior to loading onto two SPE cartridges in series: C-18 and C-18/cation exchange mixed-mode polymeric phases. The analytes are eluted from each of the two cartridges separately, and the pooled and concentrated fraction is analyzed using gas chromatography−mass selective detection in the selected ion monitoring mode. The lower limit of method validation is 0.10 μg/L (ppb) for 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 2-amino-4-chloro-6-(isopropylamino)-*s*-triazine (G-30033, deethylatrazine), 2-amino-4-chloro-6-(ethylamino)-*s*-triazine (G-28279, deisopropylatrazine), and 2,4-diamino-6-chloro-*s*-triazine (G-28273, didealkyatrazine). The overall mean procedural recoveries (and standard deviations) are 96 (6.9), 96 (5.5), 95 (6.8), and 100% (10%) for atrazine, G-30033, G-28279, and G-28273, respectively ($n = 49$). The method validation study was conducted under U.S. EPA FIFRA Good Laboratory Practice Guidelines 40 CFR 160. The reported procedure accounts for residues of G-28273 in water.

KEYWORDS: Atrazine; dealkylated chlorotriazine metabolites; diaminochlorotriazine (DACT); deethyldeisopropylatrazine (DEDIA); didealkylatrazine (DDA); G-28273; ground water; surface water; Good Laboratory Practices (GLP)

INTRODUCTION

Atrazine is a restricted-use herbicide manufactured, formulated, and sold under various trademarks by several agrochemical companies. It is most often used in corn, sorghum, and sugar cane production for the control of annual broadleaf and grass weeds. It metabolizes in plants and animals (*1*) and undergoes environmental degradation via physical, chemical, and microbiological transformation processes to form dealkylated chlorotriazines (*2*), further conversion to hydroxytriazines (*3*, *4*) (no apparent toxicological significance), and eventually mineralization (*5*) to carbon dioxide and ammonia. As the parent chlorotriazine molecule metabolizes or degrades, the subsequent and succeeding products increase in polarity, thus increasing their potential mobility. Other chlorotriazine compounds may also metabolize and/or degrade to form dealkylated chlorotriazines [e.g., simazine can transform to 2-amino-4-chloro-6-(ethylamino)-*s*-triazine (G-28279, deisopropylatrazine) and 2,4-diamino-6-chloro-*s*-triazine (G-28273, didealkyatrazine) but not 2-amino-4-chloro-6-(isopropylamino)-*s*-triazine (G-30033, deethylatrazine)]; thus, the presence of these compounds in the environment is not unique to atrazine. The U.S. EPA established a maximum concentration level (MCL) in drinking water of 3 μg/L (ppb) for atrazine (*6*). The structures and chemical names for these compounds are shown in **Figure 1**. The common names for the degradates (metabolites) will be used throughout the remainder of this report: deethylatrazine or DEA (G-30033); deisopropylatrazine or DIA (G-28279); didealkylatrazine or DDA (G-28273).

The occurrence and fate of atrazine and its dealkylated chlorotriazine degradates in ground and surface waters have been the subjects of many publications over the past two decades and, as a consequence, numerous water sample preparation procedures using various instrumental methods of analysis have been described. However, most reported methods were specifically developed for the analysis of parent moieties from several chemical classes of compounds (e.g., triazines, carbamates, phenylureas, and acetanilides) in water for generalized multiresidue screening or monitoring purposes and, thus, were not applicable to the analysis of the degradates of atrazine. Some methods were developed to analyze for atrazine, DEA, and DIA but not DDA. This was primarily due to the difficulty of quantitatively extracting the polar DDA from water.

There are few papers in which the analysis of DDA in water is addressed. A procedure was reported solely for the analysis of DDA using graphitized carbon black solid-phase extraction (SPE) and final analysis using gas chromatography (GC) high-resolution mass spectrometry (HRMS) (*7*). Recoveries of 75 ± 8% for DDA in deionized water at the 5 ng/L (parts per trillion, ppt) and 91 ± 12% in ground water at the 0.5 μg/L (parts per

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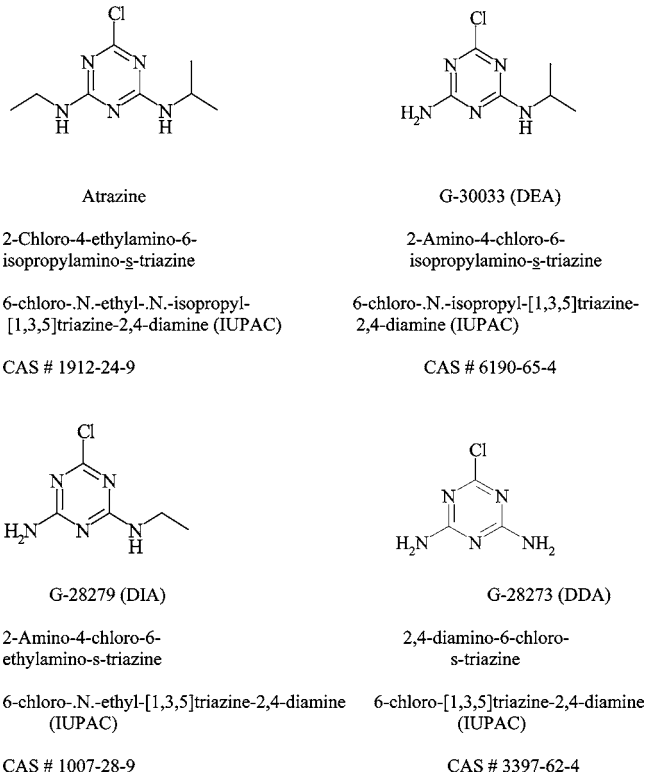


Figure 1. Structures and chemical names of atrazine and its dealkylated chlorotriazine degradates.

billion, ppb) concentration levels were reported. In another study, recoveries of 92−101% were obtained for atrazine, DEA, DIA, and DDA (and three hydroxyatrazine degradates) in drinking water at the 3 ng/L (ppt) concentration level using graphitized carbon black SPE and final analysis using liquid chromatography−electrospray ionization mass spectrometry (LC-ESI/MS) (*8*). Recoveries in the 92−98% range were obtained for these four analytes in ground water at the 0.2 μg/L (ppb) concentration level. The sample size required for analysis was 4 L for drinking and ground water samples. Recoveries were also acceptable (80−101%) for 1 L surface water samples at the 10 and 200 ng/L (ppt) concentration levels. The relative standard deviations for all of the recovery data ranged from 2 to 8% ($n = 6$). Recoveries of 93−107% at the 0.8 and 8 μg/L (ppb) concentration levels were reported in reagent water and soil pore water for all four analytes using graphitized carbon black SPE and final analysis using GC-MS after derivatization of the analytes (*9*). Envi-carb (another carbon-based SPE) was used for sample preparation followed by analysis using GC-MS (*10*). The recoveries ranged from 96 to 109% in distilled water at the 0.05 μg/L (ppb) concentration level for atrazine and DIA and at the 0.10 and 0.20 μg/L (ppb) concentration levels for DEA and DDA, respectively. Recovery data for ground water were not reported. The use of mixed-mode SPE for water and soil sample preparation was reported for the analysis of atrazine, DEA, and DIA (*11*, *12*). The authors compared the retention efficiencies of atrazine, DEA, DIA, etc. on copolymerized (more than one functionality bonded onto a common frame) and blended mixed-mode resins and found that the closeness of the reverse-phase and ion exchange moieties was an important factor affecting the retention of these basic compounds. On the copolymerized resin, the closeness of the functionalities resulted in higher retention of both polar and nonpolar *s*-triazine molecules than on blended resin. Retention on resin containing solely C-18 functionality was dependent on the length of the alkyl side chain; thus, breakthrough volumes for the dealkylated chlorotriazine metabolites were smaller than those for the parent molecules. A mixed-mode (reverse phase/cation exchange) sample preparation procedure was reported for atrazine, DEA, DIA, and DDA at a limit of quantitation (LOQ) of 0.20 μg/L (ppb) in drinking water (*13*). The average recovery for DDA was 75% at the method LOQ, but no data were reported for ground or surface water. The principles and practice of SPE were very well described in the text of Thurman and Mills (*14*) for the interested reader.

Immunoassay techniques were developed for both atrazine and DDA, but the technique can still suffer from cross-reactivity issues and interferences, the number of analytes that can be simultaneously monitored is limited (*15*), and positive detections are still dependent on mass spectrometric confirmation. Methods for the analysis of triazine herbicides were reviewed (*16*−*18*).

We previously reported a Good Laboratory Practices (GLP) validated analytical method that was applicable to the analysis of atrazine, all three of its dealkylated chlorotriazine degradates, and several other herbicides and degradates in ground and surface water (*19*). However, this method relies on classical liquid/liquid partitioning, which is not only labor intensive but requires the use of large volumes of organic solvents (and their subsequent disposal). Furthermore, the coextracted, organic components of the water sample (especially surface water) greatly decreased the useful lifetime of the capillary column used for analysis [gas chromatography−mass selective detection (GC-MSD)]. The coextractives irreversibly adsorb to the bonded stationary phase in the column and adversely affect the peak shape and retention time of DDA after only two to three sets of sample injections. This required daily GC-MSD injection port maintenance and frequent column changing to consistently obtain reliable data for DDA. The cost per analyte per sample using this method was acceptable only as long as the monitoring included DDA (if needed) and a large number of analytes. To facilitate studies wherein data are needed for atrazine and all three of its dealkylated chlorotriazine degradates in ground or surface water samples, it would be desirable to use a faster and less costly alternative to liquid/liquid partitioning for water sample extraction.

In this study, mixed-mode SPE, tandem reverse phase, and copolymerized cation exchange/reverse phase were employed for ground (well), surface (lake), and laboratory (deionized) water sample preparation followed by analysis using GC-MSD in the selected ion monitoring (SIM) mode. The method was validated under GLP guidelines.

EXPERIMENTAL PROCEDURES

Standards. Analytical standards of atrazine (97.9%), DEA (94%), DIA (96%), and DDA (97%) were obtained from the Technology and Projects Department of Syngenta Crop Protection, Inc. (SCP), Greensboro, NC. Individual stock standards were prepared by weighing 10.0 mg of atrazine and 5.0 mg of DEA, DIA, and DDA (corrected for percent purity) into each of four 100-mL volumetric flasks, 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 4.0 μg/mL mixed standard was prepared by transferring 10 mL of the atrazine stock standard and 20 mL of each of the DEA, DIA, and DDA stock standards to a 250-mL volumetric flask, followed by dilution to the mark in acetone. Serial dilutions of the mixed standard were prepared in acetone to create working standards at the 1.0, 0.80, 0.40, 0.20, 0.10, 0.08, 0.04, and 0.02 μg/mL (ng/μL) concentration levels. These standards were used for analytical and fortification purposes.

Solvents and Reagents. HPLC grade methanol (EM-MX0475-1), acetone (EM-AX0115P-1), ACS grade 37% HCl (EM-B10125-74), and

25−30% ammonium hydroxide (EM-AX1303-75) were all obtained from VWR International. Deionized (DI) water was obtained from the Picopure water purification system in the laboratories of SCP.

Preparation of Solutions. The 2% NH_4OH/methanol solution was prepared by adding 2 mL of NH_4OH to 98 mL of methanol. The 0.2% NH_4OH/acetone solution was prepared by adding 200 μL of NH_4OH to 100 mL of acetone.

SPE Cartridges. Waters Oasis MCX cartridges, 500 mg/6 mL (catalog no. 186000776), and a Varian Bond Elut C-18, 500 mg/6 mL (catalog no. 12102052), were used.

Sample Storage. Water samples to be analyzed for residues of atrazine and its dealkylated chlorotriazine metabolites 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 2 years for all four analytes when samples were stored under these conditions (*20*). Note: all of the results reported in this study are laboratory-fortified ground, surface, and DI water samples that were immediately subjected to sample preparation after fortification.

Water Sample Sources. For this study, ground water was obtained from a private residence utilizing well water containing ~120 mg/L (ppm) hardness as $CaCO_3$, and the surface water was obtained from High Point Lake, both in Guilford County, North Carolina. The laboratory deionized water was obtained from a Picopure water purification system located in the laboratories of SCP, Greensboro, NC.

Sample Preparation Procedure. The MCX and C-18 SPE cartridges were conditioned separately under gravity using 5 mL of methanol followed with 5 mL of DI water. To avoid dryness, another 3 mL of DI water was added, and the valve to the vacuum manifold was closed. A 60-mL solvent reservoir was attached to top of the C-18 cartridge, and the C-18 was attached to the top of the MCX cartridge (the MCX was connected to the manifold) to complete the configuration.

The pH of a 200-mL water sample was adjusted by first adding 250 μL of 1.0 M HCl followed by incremental additions of 25 μL of acid until the pH was between 3 and 4. In this work, EM colorpHast pH strips (range 0−6) were used to measure the pH of the sample. It was important to ensure the pH was not inadvertently adjusted to <3 because base cannot be added to the sample to increase the pH (the addition of base "swamps" the sample with cations that compete with the cationic analytes for available ion exchange sites in the MCX cartridge).

The acidified water sample was transferred to the reservoir and allowed to load onto the C-18/MCX SPE cartridges under gravity at a flow rate of ≤2 mL/min. Adjustment of the manifold valves was sometimes required to obtain uniform flow through each configuration due to particle size variances between SPE cartridges. The analyst must remain attentive and add additional water sample as needed to avoid inadvertent drying of the cartridges during sample loading. The load step typically required 2−2.5 h.

Upon completion of the sample load, the still-connected SPE cartridges were washed with 5 mL of DI water. The two cartridges were separated, and the MCX cartridge was washed with an additional 5 mL of DI water. Both cartridges were connected to the vacuum manifold and subjected to vacuum for 20−30 min to remove as much water as possible (we recommend emptying the vacuum manifold of the load water prior to the initiation of the drying step).

After drying, the MCX cartridge was eluted under gravity with four 2-mL portions of 2% NH_4OH/methanol and collected in a 12-mL test tube. A pipet bulb was used to force the last remaining drops of elution solvent from the cartridge and into the test tube. The sides of the test tube were rinsed with <1 mL of acetone, and the solution was concentrated to <1 mL using a gentle stream of N_2 and a water bath temperature of 30−35 °C. The C-18 cartridge was eluted under gravity with four 2-mL portions of 0.2% NH_4OH/acetone and collected in the same test tube containing the MCX cartridge fraction. This combined fraction was quantitatively transferred to a 10-mL disposable syringe fitted with a PTFE syringe filter (0.20 μm) and filtered into a clean 12-mL test tube. This pooled and filtered fraction was reduced to dryness under the same N_2 gas and water bath conditions described previously. The residue was reconstituted in an appropriate volume of acetone for GC-MSD analysis. A final fraction volume of 0.5 mL was required to establish the lower limit of method validation (LLMV).

Table 1. Target and Qualifier Ions Used during the GC-MSD Analyses

analyte	t_R[a] (min)	target	Q1	Q2	Q3
atrazine	11.1	200	215	173	158
G-30033	11.9	172	187	174	145
G-28279	12.3	158	173	175	145
G-28273	13.8	145	110	147	*b*

[a] This will vary according to column length and other operating parameters. [b] A suitable Q3 ion is not available.

Instrumentation. Analyses were performed using an Agilent 6890 gas chromatograph (GC) interfaced (capillary direct) to a 5973 mass selective detector (MSD) and operated in the SIM mode. The MSD transfer line was maintained at 235 °C (to better accommodate the DB-Wax column upper temperature limit), and tuning was performed on a daily basis with perfluorotributhylamine (PFTBA) to ensure accurate mass calibration. The GC was equipped for splitless injection, and electronic pressure programming (EPP) was utilized in conjunction with the following operating parameters: inlet *T*, 225 °C; inlet *P*, 13.6 psi; pulse *P*, 40 psi; pulse *t*, 1 min; purge flow, 50 mL/min; purge *t*, 1 min; carrier gas, He; flow rate, 1 mL/min; initial *T*, 110 °C; initial *t*, 2 min; ramp 1, 10 °C/min to 180 °C; ramp 2, 25 °C/min to 250 °C; final *t*, 6 min; total run *t*, 17.8 min. An Agilent DB-WAX, 15 × 0.25 mm i.d., 0.25-μm film thickness capillary column was employed (catalog no. 122-7012) for the analysis.

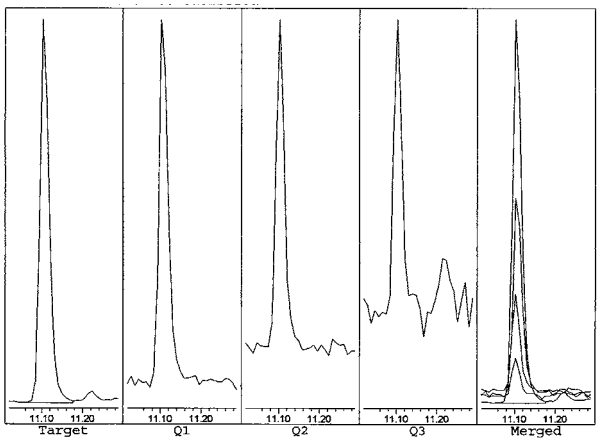
The ions monitored, shown in **Table 1**, were selected after inspection of the full-scan mass spectra for each analyte obtained via electron ionization at 70 eV. In general, the most abundant ion was chosen for quantification purposes (target ion) to maximize sensitivity. The qualifier ions selected were generally the ions of next highest abundance. However, any of the qualifier ions can be switched to the target ion for quantification purposes if interferences are encountered in field samples.

Sample Analysis. Each analytical method validation set consisted of eight analytical standards of various concentrations, reagent blank (on alternate sets only), a control, and seven controls fortified with the analytes at the 0.10−10 μg/L (ppb) concentration level for procedural recovery purposes. Additional standards were dispersed throughout the sequence as a means of checking the stability of the system for variances in MSD sensitivity and/or column performance.

RESULTS AND DISCUSSION

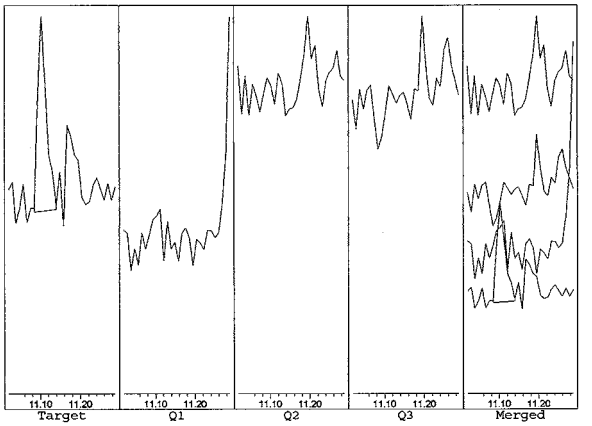
GC-MSD Analyses. Representative SIM chromatograms of a 0.04-ng injected standard (lowest concentration of standard injected), control, and 0.10 ppb procedural recovery sample for atrazine, DEA, DIA, and DDA in ground water are shown in **Figures 2**, **3**, **4**, and **5**, respectively. The signal/noise ratio is ≥10 in all cases, and there is unambiguous identification of each compound based on the acquisition of acceptable qualifier/target ion ratios (±30%). The nanograms injected and respective responses of the target ions for each analyte were used for construction of the calibration plots. All plots were linear, and correlation coefficients were >0.994 throughout the method validation. There was a small, negative intercept for each analyte.

The responses for target ion peaks detected in the control samples were subtracted from the responses for the target ion peaks detected in the procedural recovery samples prior to calculation of the percent recovery. This was done even when the qualifier ions were absent. Peaks with the same *m*/*z* ratios of the target ions and the same retention times (t_R) were occasionally detected in the ground and DI water control samples, but the qualifier ions were absent and the concentrations, if residues, would have been ≪0.10 μg/L (ppb). The surface water control samples appeared to have authentic residues of atrazine but the concentrations, if real, were ≪0.10 μg/L (ppb). The lake water used in this study was collected



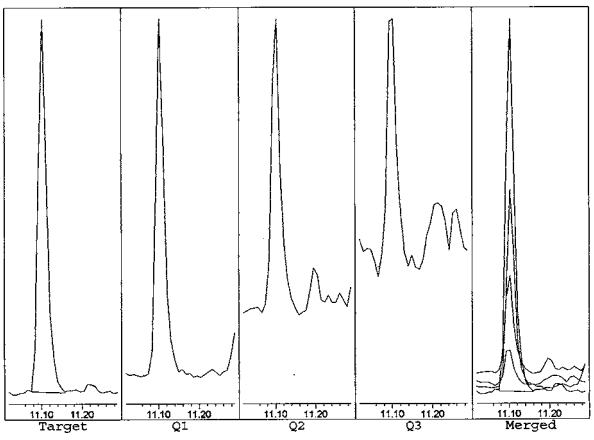
	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	200.000	100.0%			11.10	10.60	14296	sys def
Q1	215.000	54.7	42.2-	63.2	11.10	to	7825	sys def
Q2	173.000	25.5	19.0-	28.6	11.10	11.60	3649	sys def
Q3	158.000	12.5	7.7-	11.5	11.10		1782	sys def

(A)



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	200.000	100.0%			11.10	10.60	794	sys def
Q1	215.000	81.4	42.2-	63.2	11.11	to	646	sys def
Q2	173.000	21.4	19.0-	28.6	11.12	11.60	170	sys def
Q3	158.000	54.7	7.7-	11.5	11.11		434	sys def

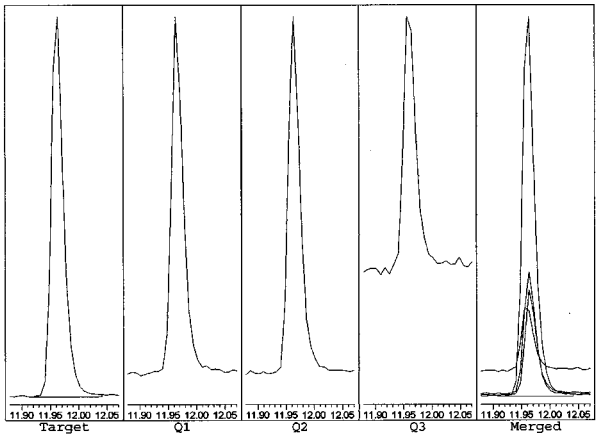
(B)



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	200.000	100.0%			11.10	10.60	27349	sys def
Q1	215.000	53.9	42.2-	63.2	11.10	to	14736	sys def
Q2	173.000	28.6	19.0-	28.6	11.10	11.60	7828	sys def
Q3	158.000	12.0	7.7-	11.5	11.10		3279	sys def

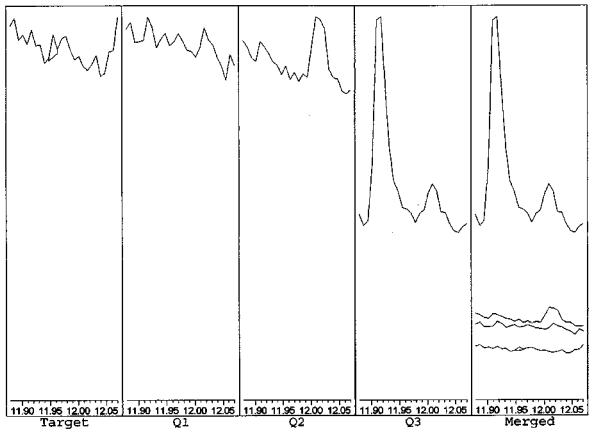
(C)

Figure 2. Representative SIM chromatograms for atrazine: (**A**) 0.04 ng injected standard; (**B**) ground water control; (**C**) 0.10 ppb procedural recovery sample.



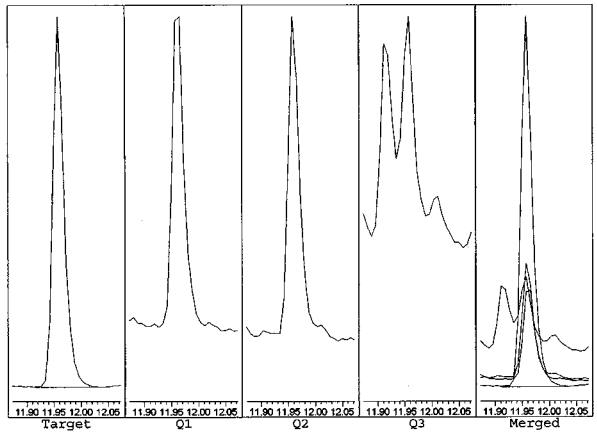
	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	172.000	100.0%			11.96	11.46	21016	sys def
Q1	187.000	27.6	21.7-	32.5	11.96	to	5801	sys def
Q2	174.000	32.0	25.6-	38.4	11.96	12.46	6720	sys def
Q3	145.000	18.7	14.2-	21.4	11.96		3938	sys def

(A)



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	172.000	100.0%			11.96	11.46	44	sys def
Q1	187.000	277.3	21.7-	32.5	11.92	to	122	sys def
Q2	174.000	943.2	25.6-	38.4	11.91	12.46	415	sys def
Q3	145.000	17720.5	14.2-	21.4	11.92		7797	sys def

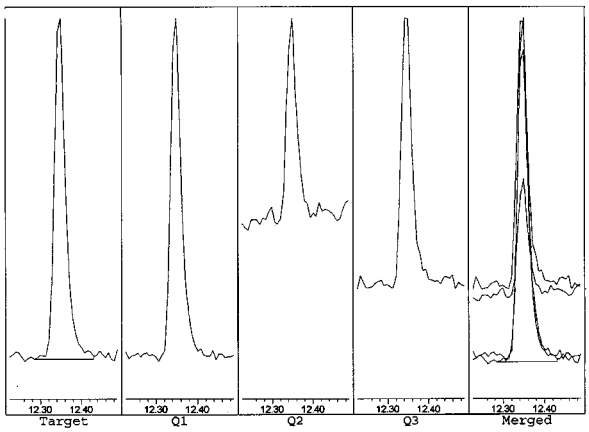
(B)



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	172.000	100.0%			11.96	11.46	39370	sys def
Q1	187.000	27.5	21.7-	32.5	11.96	to	10837	sys def
Q2	174.000	34.2	25.6-	38.4	11.96	12.46	13456	sys def
Q3	145.000	21.3	14.2-	21.4	11.96		8370	sys def

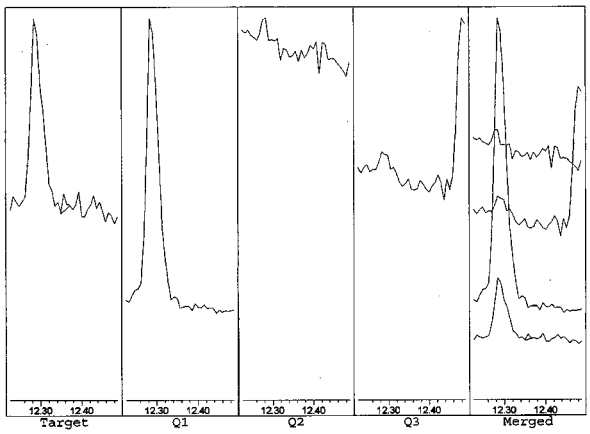
(C)

Figure 3. Representative SIM chromatograms for DEA: (**A**) 0.04 ng injected standard; (**B**) ground water control; (**C**) 0.10 ppb procedural recovery sample.



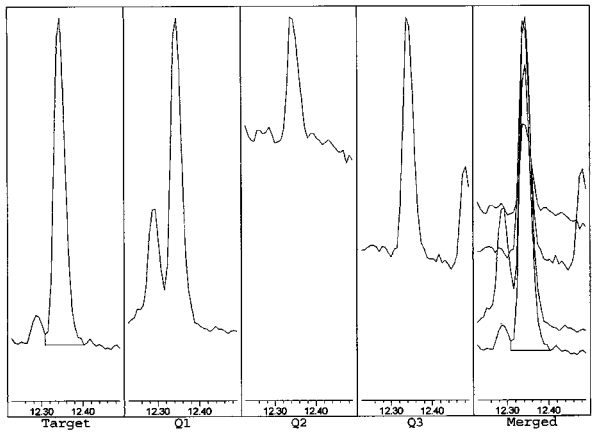
	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	158.000	100.0%		12.35	11.84	7321	sys def
Q1	173.000	106.9	84.7- 127.1	12.35	to	7826	sys def
Q2	175.000	36.4	28.5- 42.7	12.35	12.84	2663	sys def
Q3	145.000	86.3	62.9- 94.3	12.34		6315	sys def

(A)



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	158.000	100.0%		12.36	11.84	67	sys def
Q1	173.000	0.0	84.7- 127.1	0.00	to	0	sys def
Q2	175.000	0.0	28.5- 42.7	0.00	12.84	0	sys def
Q3	145.000	0.0	62.9- 94.3	0.00		0	sys def

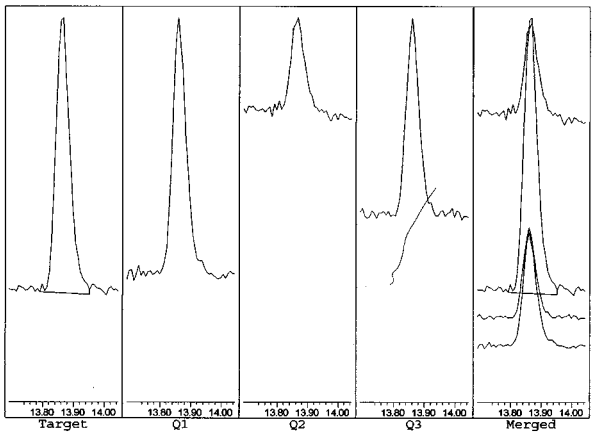
(B)



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	158.000	100.0%		12.34	11.84	12997	sys def
Q1	173.000	112.1	84.7- 127.1	12.34	to	14571	sys def
Q2	175.000	34.2	28.5- 42.7	12.34	12.84	4445	sys def
Q3	145.000	83.1	62.9- 94.3	12.34		10804	sys def

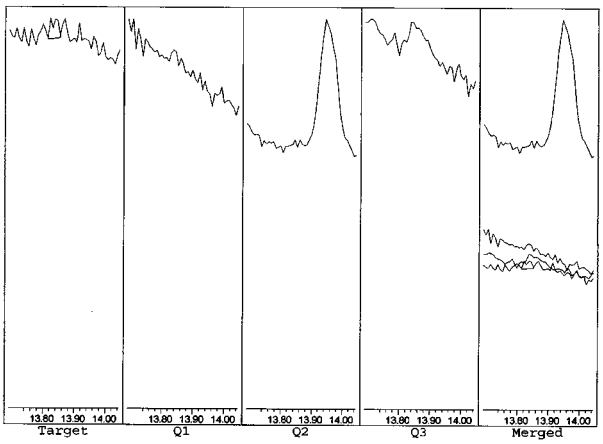
(C)

Figure 4. Representative SIM chromatograms for DIA: (**A**) 0.04 ng injected standard; (**B**) ground water control; (**C**) 0.10 ppb procedural recovery sample.



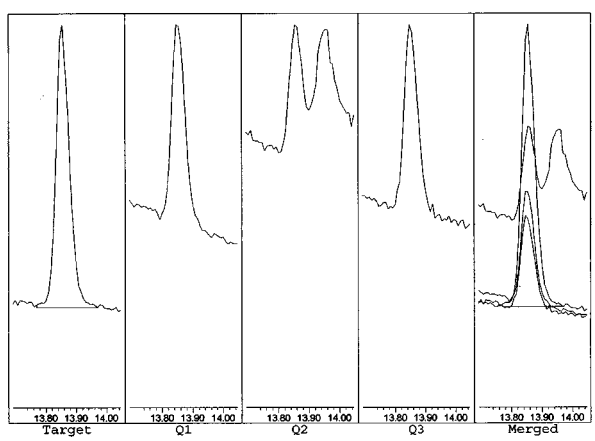
	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	145.000	100.0%		13.87	13.36	12352	sys def
Q1	110.000	43.1	32.3- 48.5	13.86	to	5323	sys def
Q2	147.000	46.0	26.4- 39.6	13.87	14.36	5681	sys def
Q3	68.000	30.2	25.6- 38.4	13.86		3731	sys def

(A)



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	145.000	100.0%		13.82	13.36	213	sys def
Q1	110.000	0.0	32.3- 48.5	0.00	to	0	sys def
Q2	147.000	168.1	26.4- 39.6	13.84	14.36	358	sys def
Q3	68.000	861.5	25.6- 38.4	13.84		1835	sys def

(B)



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	145.000	100.0%		13.85	13.36	20302	sys def
Q1	110.000	47.1	32.3- 48.5	13.85	to	9569	sys def
Q2	147.000	33.2	26.4- 39.6	13.85	14.36	6739	sys def
Q3	68.000	37.9	25.6- 38.4	13.85		7686	sys def

(C)

Figure 5. Representative SIM chromatograms for DDA: (**A**) 0.04 ng injected standard; (**B**) ground water control; (**C**) 0.10 ppb procedural recovery sample.

Table 2. Summary of Procedural Recovery Data Obtained for Fortified Deionized, Ground, and Surface Water Samples

	mean (standard deviation)			
	atrazine	DEA	DIA	DDA
DI water				
0.10 ($n = 9$)	92 (5.5)	94 (5.8)	92 (6.6)	96 (6.2)
1.0 ($n = 6$)	93 (6.8)	97 (5.8)	95 (5.0)	107 (6.6)
10 ($n = 6$)	98 (6.4)	93 (7.4)	93 (9.5)	100 (10)
mean ($n = 21$)	94	95	93	101
SD	6.4	6.1	7.0	8.6
ground water				
0.10 ($n = 6$)	94 (5.8)	96 (1.7)	93 (9.3)	94 (13)
1.0 ($n = 4$)	95 (5.6)	100 (2.4)	101 (2.6)	108 (5.1)
10 ($n = 4$)	94 (2.1)	93 (2.4)	93 (4.6)	103 (14)
mean ($n = 14$)	94	96	95	101
SD	4.6	3.3	7.3	12
surface water				
0.10 ($n = 6$)	103 (7.2)	100 (6.4)	97 (5.8)	88 (6.6)
1.0 ($n = 4$)	105 (5.0)	102 (4.1)	102 (4.6)	108 (6.4)
10 ($n = 4$)	98 (2.9)	95 (2.4)	95 (3.3)	105 (6.0)
mean ($n = 14$)	102	99	98	99
SD	5.9	5.3	5.4	11
overall				
mean ($n = 49$)	96	96	95	100
SD	6.9	5.5	6.8	10
range	84–111	78–108	74–108	79–118

immediately after a large, multiday rainfall event, and the water contained a lot of suspended, reddish, clay soil sediment.

Method Performance. A summary of the procedural recovery data is shown in **Table 2**. Ground water (well) and surface water (lake) were used in the validation because these represent the sample types most often included in water monitoring or survey studies. DI water was used in the validation because it is sometimes used for control and procedural recovery purposes when field samples are analyzed and it can serve as a benchmark to help isolate potential problems that may occur with ground and surface water samples that contain other dissolved substances, almost always at concentrations much higher than the analytes of interest discussed herein. All of the individual recoveries obtained during the validation ranged from 74 to 118%. The mean procedural recoveries (standard deviations) for atrazine, DEA, DIA, and DDA were 96 (6.9), 96 (5.5), 95 (6.8), and 100% (10%), respectively. Overall, there does not appear to be a relationship between procedural recovery and the type of water sample or between procedural recovery and fortification level. The only apparent trend is the slightly higher standard deviation for DDA compared to the other analytes, and this appears to be due primarily to the results for the ground water samples.

The LLMV for all four analytes is 0.10 μg/L (ppb) because this was the lowest procedural recovery concentration tested through the sample preparation procedure. The estimated GC-MSD instrument detection limit (IDL) and quantification limit (IQL) are 0.02 ng injected and 0.066 ng (0.08 μg/L based on an injection of 0.8 g of equivalent water matrix), respectively. These figures of merit were obtained using the root mean square error (RMSE) method (*21*−*23*) and represent only an initial estimate of limit of detection (LOD) and limit of quantification (LOQ) based on 3 and 10 times the standard deviation (RMSE), respectively. The more rigorous estimation of LOQ obtained by analyzing 7 clean control samples fortified at the newly estimated LOQ was not performed in this work. However, standards of increasingly lower concentration were injected (0.02, 0.01, 0.005, and 0.001 ng injected) using the same GC-MSD operating parameters and state of sensitivity as reported herein for the validation experiments. It became increasingly difficult to acquire acceptable qualifier/target ion ratios below the 0.02 ng injected concentration level for these standards primarily due to the generally lower abundances of the qualifier ions compared to the target ions (used for quantification). Thus, the lowest practical method LOQ possible when confirmatory ability is maintained is 0.025 μg/L (ppb) based on the method sample size (200 mL), final fraction volume (0.5 mL), and volume (2 μL) of final fraction injected (0.02 ng/0.8 g water matrix = 0.025 ng/g). For DDA, confirmatory qualifier/target ion ratios were inconsistent below 0.04 ng injected; thus, 0.050 μg/L is the lowest practical LOQ for this analyte. Of course, method LOQs of 0.025 or 0.050 μg/L could be justified only if it was demonstrated that (1) this amount of each analyte could be quantitatively retained and eluted from the SPE cartridges and (2) the injection of water matrix did not adversely affect the t_R, peak shape, or the qualifier/target ion ratios needed for confirmation purposes at these concentration levels.

We reported previously (*19*) that the analysis of surface water samples required more frequent instrumentation maintenance than the analysis of ground or DI water samples, especially with regard to daily cleaning of the injection port, replacement of the insert, gold seal, and washer, column clipping, and installation of a new DB-5 capillary column after only a few sets of sample analyses. This appeared to be due to irreversible adsorption of coextracted organic compounds inside the injection port and to the stationary phase of the column. This adversely affected the t_R and peak shape of, primarily, DDA. In this work, one DB-Wax capillary column was used throughout the method development process (>30 injected sets of samples), and a second column was used throughout the method validation (7 sets of samples) without any deleterious effects on DDA. This increased column life appears to be due to two reasons: (1) the nonpolar coextractives are more likely to remain in the gas phase and pass through the DB-Wax column with minimal partitioning into the stationary phase and (2) the quantity of nonpolar coextractives in the final fraction from SPE sample preparation is greatly reduced compared to the quantity contained in the final fraction from liquid/liquid partitioning sample preparation. Thus, contamination of the injection port and capillary column is reduced.

It is evident in other publications (*7*−*10*) that the LLMV is higher in ground water than in surface or DI water for some of the analytes and, in particular, DDA. We have long recognized the adverse effects of water hardness on the procedural recovery of the more polar analytes, especially when using ion exchange to retain the desired analytes. The use of a 500-mg MCX SPE cartridge provides enough capacity to quantitatively retain field realistic levels of DDA in ground water containing at least a water hardness of 120 mg/L (ppm). However, this capacity can be easily exceeded if base is used during the initial pH adjustment of a sample prior to loading of the SPE cartridges. These added cations compete with the analytes for the available ion exchange sites, and this allows some of the analyte(s) to pass through the MCX cartridge unretained. For quality control purposes, one should always perform a procedural recovery verification experiment using a representative portion of the water sample to be analyzed.

The potential for using solely the MCX SPE cartridge for sample preparation was investigated in this work due to the dual reverse phase and cation exchange mixed-mode functionality of the resin. However, atrazine and, to a lesser extent, DEA and DIA, were especially difficult to quantitatively and reproducibly remove from the cartridge, leading to inconsistent

recoveries. In this work using a C-18 cartridge on top of the MCX cartridge, all of the atrazine was retained on the C-18, DEA was primarily retained on the C-18 (~90%), DIA was retained on the C-18 (~30%) and the MCX (~70%), and all of the DDA was retained on the MCX cartridge. Use of a C-18 cartridge alone was not possible because DDA could not be retained.

In some of our earlier work, we also investigated the use of graphitized carbon SPE cartridges for water sample preparation. In our experience, the volume of elution solvent required to quantitatively remove all of the desired analytes was excessive. However, we did not try reversing the cartridge, followed by "back-elution" of the analytes as reported by Di Corcia et al. (*8*).

Our first objective was to develop a method utilizing GC-MSD because this technique is available in most laboratories. However, it must be noted that among the MSDs in our laboratory, the Agilent model 5973 MSDs have much more sensitivity than the model 5972 MSDs, presumably due to an improved design in the electron multiplier. Thus, it may be necessary to utilize a 5973 MSD to obtain sensitivity comparable to that reported in this work. Our next goal is to validate the method when the final fractions are reconstituted in liquid chromatography (LC) mobile phase instead of acetone and analyzed by LC−electrospray ionization mass spectrometry/mass spectrometry (LC-ESI/MS/MS). This will enhance the selectivity and sensitivity of the method for these selected analytes.

The use of SPE for sample preparation requires much less organic solvent than the liquid/liquid partitioning (LLE) procedure reported previously (*19*), thus reducing the cost associated with the purchase and disposal of relatively large volumes of solvent. The time required for sample preparation of a set of samples using SPE is half that required using LLE, thus increasing overall productivity.

Conclusions. The results presented herein demonstrate the accuracy and precision of this FIFRA GLP guideline 40 CFR Part 160 validated analytical method and its validity for the analysis of atrazine and its dealkylated chlorotriazine metabolites in water at a LLMV of 0.10 μg/L (ppb). The method is less costly and less labor intensive than previously reported methods and/or includes the capability to also quantify DDA. Although the data are not presented here, the method was also successfully validated for the analysis of ground, surface, and DI water for simazine, terbuthylazine, prometryn, ametryn, metribuzin, metalaxyl, and metolachlor.

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