Analytical Method for the Determination of Atrazine and Its Dealkylated Chlorotriazine Metabolites in Water Using Gas Chromatography/Mass Selective Detection

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A multiresidue method is reported for the determination of atrazine and its dealkylated chlorotriazine metabolites in water. Water samples are buffered to pH 10 and partitioned in ethyl acetate. Final analysis is accomplished using gas chromatography/mass selective detection (GC/MSD) in the selected ion monitoring (SIM) mode. The limit of detection (LOD) is 0.050 ng and the limit of quantification (LOQ) is 0.10 ppb for 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 2-amino-4-chloro-6-(isopropylamino)-*s*-triazine (G-30033), 2-amino-4-chloro-6-(ethylamino)-*s*-triazine (G-28279), and 2,4-diamino-6-chloro-*s*-triazine (G-28273). The mean procedural recoveries were 90, 92, 98, and 85% and the standard deviations were 12, 13, 16, and 20% for atrazine, G-30033, G-28279, and G-28273, respectively (n = 30). The study was conducted under U.S. EPA FIFRA Good Laboratory Practice Guidelines 40 CFR 160 for method validation. The reported procedure accounts for residues of G-28273 in water that are not included in EPA Method 507.

Keywords: *Ground water; surface water; atrazine; dealkylated chlorotriazine metabolites; didealkylated chlorotriazine; gas chromatography/mass selective detection; Good Laboratory Practices (GLP)*

INTRODUCTION

Atrazine is a restricted-use herbicide manufactured by several agricultural chemical companies and sold under various trademarks. It is most often used in corn, sorghum, and sugar cane production for the control of annual broadleaf and grass weeds and may be applied pre- or postemergence. It metabolizes in plants and animals (Esser et al., 1975; Shimabukuro et al., 1971) and undergoes environmental degradation via physical, chemical, or biological transformation processes to form dealkylated chlorotriazine metabolites. The rate of atrazine degradation via hydrolysis is a function of the soil organic matter content, pH, temperature, incorporation depth, etc. (Armstrong et al., 1967; Adams and Thurman, 1991; Winkelmann and Klaine, 1991). Residence time and population can significantly affect the microbially mediated atrazine degradation rate (Kaufman and Kearney, 1970). The structures, codes, and chemical names of atrazine and its dealkylated chlorotriazine metabolites (G-30033, G-28279, and G-28273) are shown in Figure 1. Other triazine compounds may also metabolize and/or degrade to form dealkylated chlorotriazine metabolites (e.g., simazine can transform to G-28279 and G-28273 but not G-30033), so the presence of these compounds in the environment is not unique to atrazine. Degradation of atrazine and its dealkylated chlorotriazine metabolites can lead to the formation of hydroxytriazines, but these do not appear to have any toxicological significance.

Studies on the occurrence and fate of atrazine and its dealkylated chlorotriazine degradates in ground and surface waters have prompted the need for analytical methods that are sensitive and selective for the detection of these analytes. Many sample preparation procedures and instrumental methods have been published for the analysis of atrazine and other herbicides in water (Di Corcia and Marchetti, 1992; Vassilakis et al., 1998; Hogenboom et al., 1998; Pocurull et al., 1998). A few methods have been published that address the analysis of water for atrazine, G-28379, and G-30033 (Durand and Barcelo, 1990; Dupas et al., 1996; Spliid and Koppen, 1996; Sabik and Jeannot, 1998; Ferrari et al.,

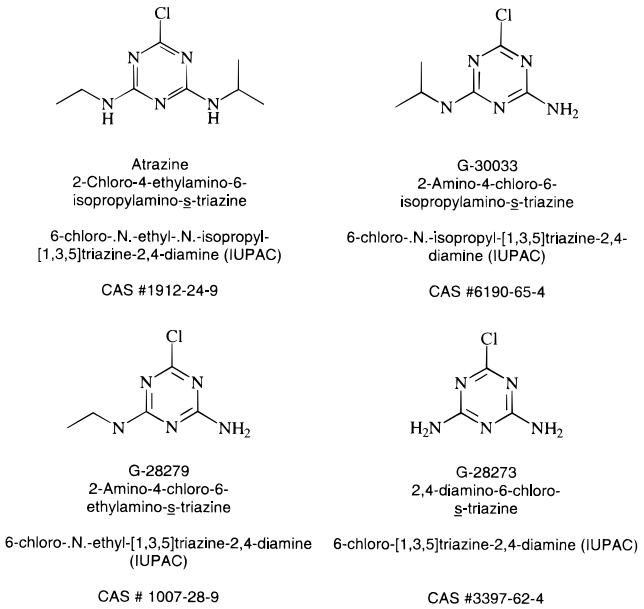

Figure 1. Structures and chemical names of atrazine and its chloro dealkylated metabolites.

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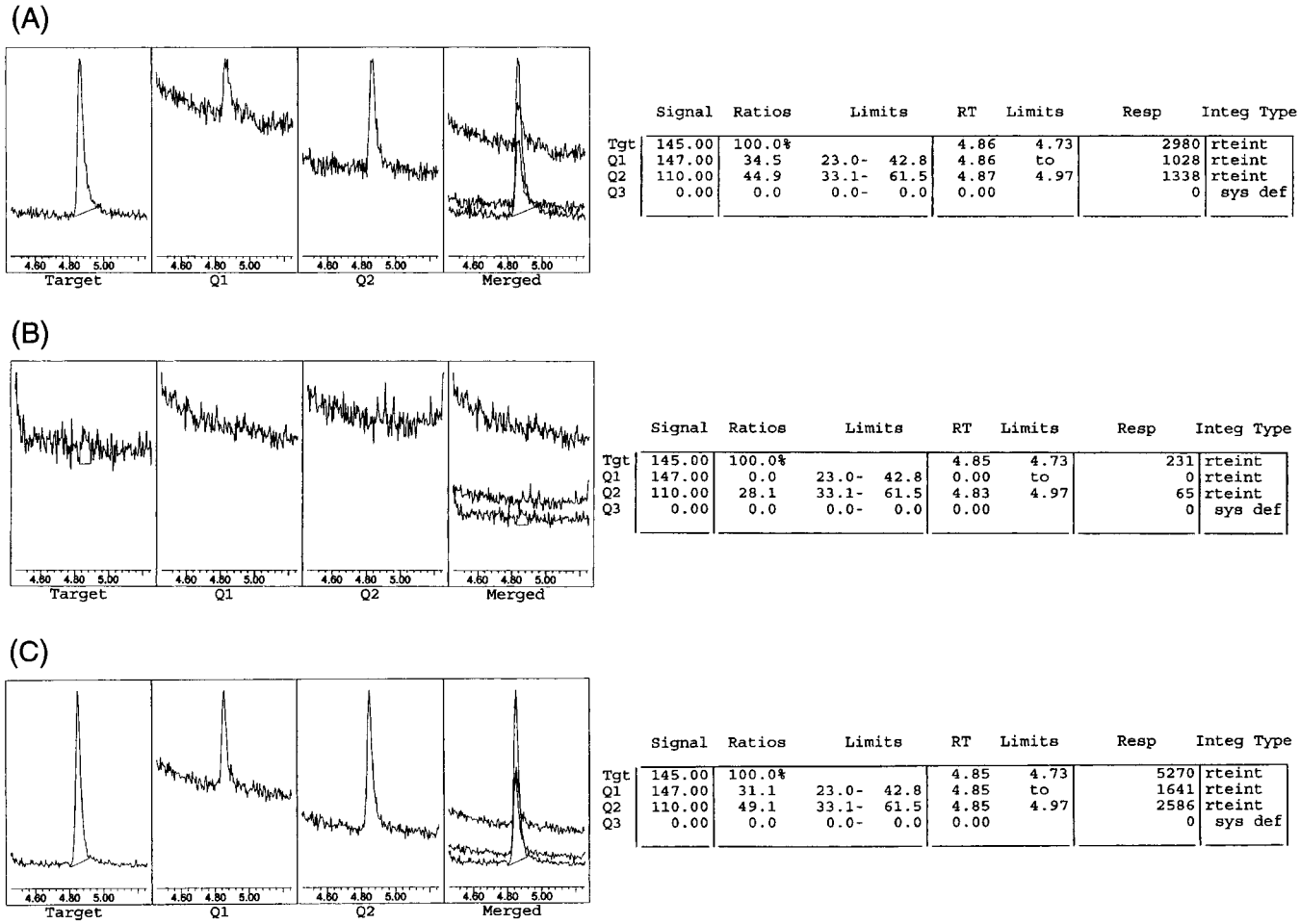


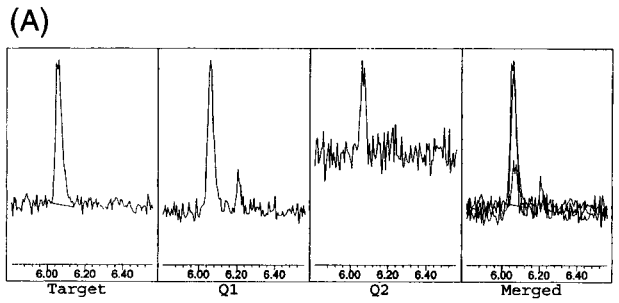
Figure 2. Representative SIM chromatograms for G-28273: (A) 0.050 ng injected standard; (B) control; (C) 0.10 ppb procedural recovery sample.

1998), and in some cases the methods also included the analysis of hydroxyatrazine and/or hydroxydealkylated chlorotriazine degradates (Lerch and Donald, 1994; Berg et al., 1994; Rollag et al., 1996; Cai et al., 1996; Ferrer et al., 1997; Hernandez et al., 1997; Loos et al., 1999). A few papers address solely the analysis of the hydroxy degradates of atrazine (Cai et al., 1994; Lerch et al., 1995; Stutz et al., 1998). These analytical procedures generally rely on either on- or off-line solid phase extraction (SPE) for sample preparation and usually gas chromatography (GC) or liquid chromatography (LC) interfaced with mass spectrometry for the final analysis. However, these studies do not include methodology for G-28273, the didealkylated metabolite of atrazine. Nor does U.S. EPA Method 507 include this analyte. One procedure was reported for the preparation of water samples for G-28273 analysis using graphitized carbon black as extraction sorbent, but it was developed solely for the analysis of this single metabolite (Cai et al., 1995). Immunoassay techniques have been reported that address the analysis of atrazine and G-28273 (Muldoon and Nelson, 1994; Muldoon et al., 1994; Del Valle et al., 1996) as well as the analysis of multiple analytes (Wortberg et al., 1996). However, the number of analytes that can be simultaneously monitored is limited, and positive detections are still dependent on mass spectral confirmation. Optimal mass spectrometer operating conditions for the analysis of atrazine and its three dealkylated chlorotriazine metabolites have been described for GC (Loos et al., 1999; Thurman et al., 1990; Meyer et al., 1993; Frassanito et al., 1996) and LC interfaced instrumentation (Spliid and Koppen, 1996; Sabik and Jeannot, 1998; Cai et al., 1996; Ferrer et al., 1997; Abian et al., 1993).

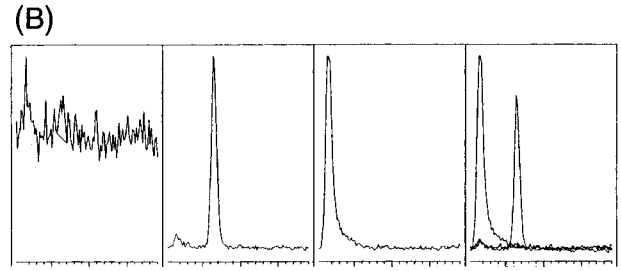
Thus, a Good Laboratory Practices (GLP) validated analytical method acceptable to the EPA that included the analysis of G-28273 in addition to atrazine, G-30033, and G-28279 was needed to support various ground and surface water monitoring studies (Balu, 1992; Balu et al., 1998; Cheung et al., 2000). In this study, classical liquid/liquid partitioning was employed for ground (well), surface (lake), and laboratory (deionized) water sample preparation followed by analysis using gas chromatography/mass selective detection (GC/MSD) in the selected ion monitoring (SIM) mode. The method was validated under GLP guidelines and is applicable to the analysis of several parent herbicides and metabolites including all three of the dealkylated chlorotriazine metabolites of atrazine.

EXPERIMENTAL PROCEDURES

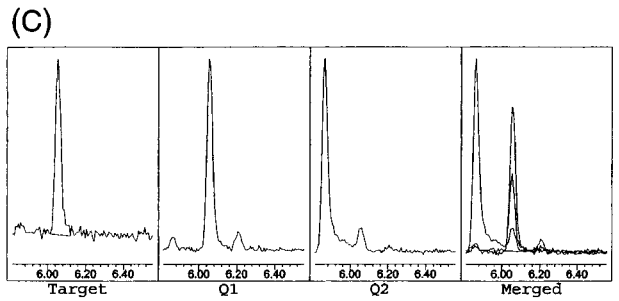
Solvents and Reagents. HPLC grade solvents methanol (A452-4), ethyl acetate (E189-4), and dichloromethane (D143-4), Optima grade acetone (A929-4), ACS grade sodium chloride (S640-3), sodium bicarbonate (S631-3), and sodium hydroxide (S318-100) were all obtained from Fisher Scientific Co. Anhydrous sodium sulfate (8024) was obtained from Mallinckrodt, and dichlorodimethylsilane (D6, 082-6) was obtained from Aldrich.



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	157.95	100.0%		6.06	5.91	1270	rteint
Q1	172.95	98.0	71.3- 132.3	6.06	to	1245	rteint
Q2	175.00	25.5	22.8- 42.4	6.06	6.21	324	rteint
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	157.95	100.0%		6.06	5.91	126	rteint
Q1	172.95	4236.5	71.3- 132.3	6.06	to	5338	rteint
Q2	175.00	0.0	22.8- 42.4	0.00	6.21	0	rteint
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	157.95	100.0%		6.05	5.91	2989	rteint
Q1	172.95	210.2	71.3- 132.3	6.05	to	6284	rteint
Q2	175.00	0.0	22.8- 42.4	0.00	6.21	0	rteint
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def

Figure 3. Representative SIM chromatograms for G-28279: (A) 0.050 ng injected standard; (B) control; (C) 0.10 ppb procedural recovery sample.

Preparation of Solutions. The pH 10 buffer solution was prepared by mixing 500 mL of 0.050 M $NaHCO_3$ with 107 mL of 0.010 M NaOH in a 1-L volumetric flask and diluting to the mark with deionized water (Dean, 1979). A 5% dichlorodimethylsilane solution was prepared by mixing 200 mL of dichlorodimethylsilane with 3.8 L of dichloromethane. (Please note and adhere to the recommended safety guidelines for handling this silanizing material.)

Standards. Analytical standards of atrazine (98.7%), G-30033 (99%), G-28279 (98%), and G-28273 (97%) were obtained from the Analytical and Product Chemistry Department, Novartis Crop Protection, Greensboro, NC. Stock solutions of each standard were prepared by weighing 10.0 mg of atrazine and 5.0 mg of G-30033, G-28279, and G-28273 (all corrected for percent purity) into each of four 100-mL volumetric flasks. Each was then diluted to the mark with methanol. A 5.0 μg/mL mixed standard was prepared and serially diluted in acetone to produce calibration standards of 0.025, 0.050, 0.10, 0.20, 0.50, and 1.0 μg/mL. Injections of 2.0 μL of each standard produced a calibration range of 0.050−2.0 ng.

Selected mixed analytical standards can be used to fortify control water samples for procedural recovery purposes. For example, the addition of 1.0 mL of the 0.050 μg/mL analytical standard to a 500-mL water sample results in analyte concentrations of 0.10 ppb [the limit of quantitation (LOQ) of the method]. The analysis of control and fortified control water samples provides procedural recovery and method performance data. The volume of standard used to fortify samples should not exceed 2 mL.

Sample Storage. Water samples to be analyzed for residues of atrazine and its dealkylated chlorotriazine metabolites should be stored under refrigerator conditions (4 °C) until analyzed. The results of a storage stability study indicate that these compounds are stable in water under refrigerator conditions for at least 2 years (Cheung and Yokley, 1995).

Sample Preparation. A 500-mL water sample (measured in a volumetric flask) is transferred to a 1-L Erlenmeyer flask to which is added 60 g of NaCl (prerinsed with two 50-mL portions of ethyl acetate), 30 mL of pH 10 $NaHCO_3$ buffer, and 2−5 drops of 10% NaOH solution. The sample is shaken to dissolve the NaCl and then transferred to a 1-L separatory funnel. The sample is partitioned four times using 75 mL of ethyl acetate for each partitioning step. The four ethyl acetate fractions are pooled and dried through 50−100 g of granular anhydrous sodium sulfate (prerinsed with two 50-mL portions of ethyl acetate), which is supported in a carbon filter tube by a plug of glass wool. The dried ethyl acetate fraction is collected in a silanized 500-mL round-bottom flask (note that the use of silanized glassware at this point in the method is critical to obtaining acceptable recoveries for the more polar analytes, e.g., G-28273). The sample is reduced in volume to ∼4 mL using a rotary evaporator and a water bath temperature of 30−35 °C. The sample is then transferred to a 50-mL concentration tube using two 2−4-mL portions of methanol to rinse the 500-mL round-bottom flask. The solvent reduction step is continued using a gentle stream of dry nitrogen gas. Methanol and then acetone are used successively to azetrophe water. The sample is reduced to just dryness and reconstituted in 1.0 mL of acetone for GC/MSD analysis. Dissolution of the residue is enhanced by using a vortex mixer for 10−15 s.

Instrumentation. Analyses were performed using either a Hewlett-Packard model 5890 series II gas chromatograph interfaced (capillary direct) to a 5972 mass selective detector (GC/MSD) or an Agilent 6890 series GC/5973 MSD, both operated in the selected ion monitoring (SIM) mode. The MSD transfer lines were maintained at 280 °C, and tuning was

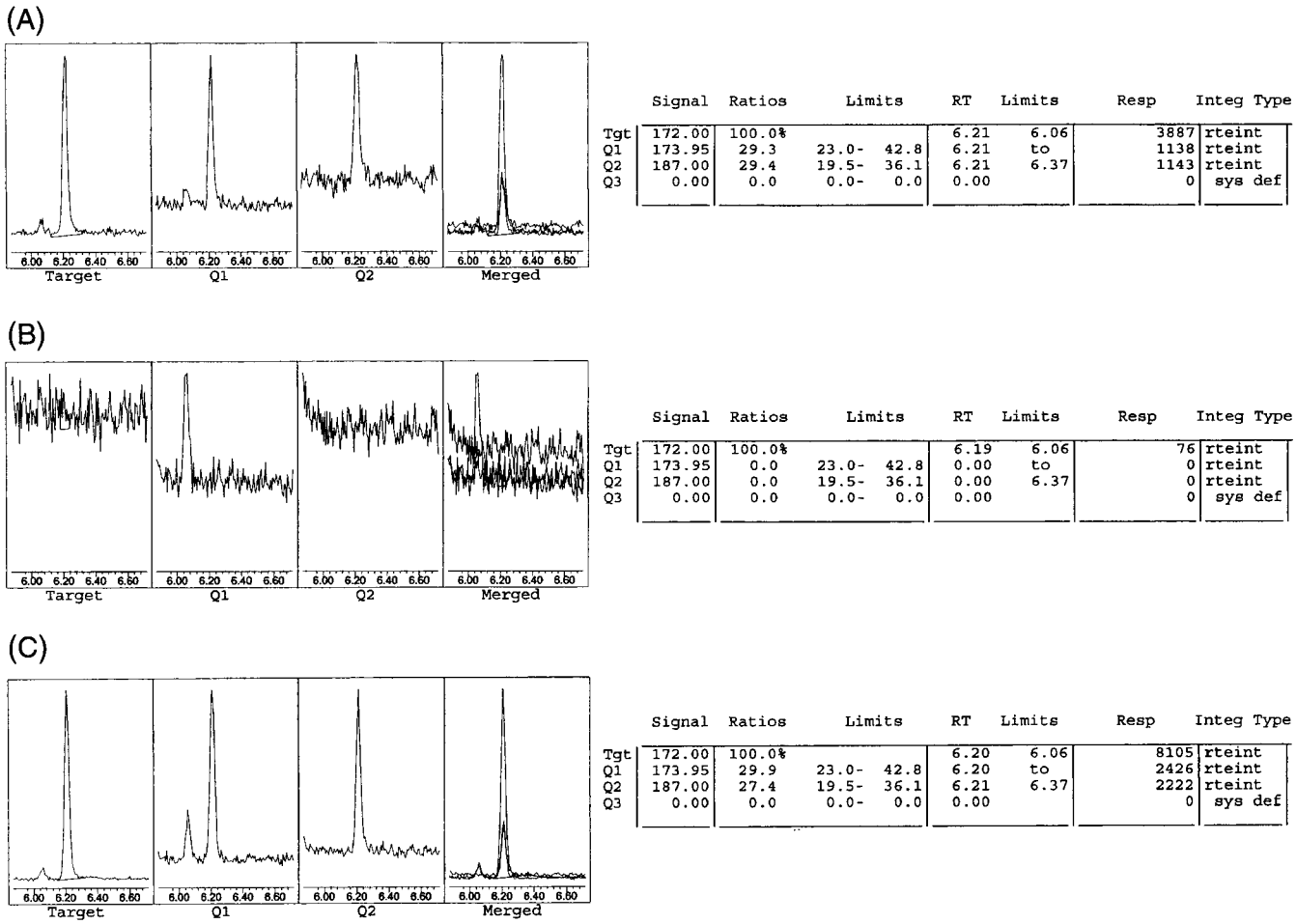


Figure 4. Representative SIM chromatograms for G-30033: (A) 0.050 ng injected standard; (B) control; (C) 0.10 ppb procedural recovery sample.

Table 1. Temperature Program Used during the GC/MSD Analyses

parameter	value
oven, initial temp	100 °C
oven, initial time	0 min
injector temp	225 °C
ramp 1 rate	40 °C/min
final temp	170 °C
final time	0 min
ramp 2 rate	3 °C/min
final temp	210 °C
final time	0 min
ramp 3 rate	40 °C/min
final temp	260 °C
final time	2 min
injector temp	225 °C
MSD temp	280 °C
column head pressure (0 time)	12 psi

performed on a daily basis with perfluorotributylamine (PFTBA) to ensure accurate mass calibration. The GCs were equipped for splitless injection, and Hewlett-Packard HP-5 or J&W DB-5.625 capillary columns (0.25 mm i.d. × 30 m, 0.25-μm film thickness) were employed for the separation. Electronic pressure programming (EPP) was utilized in conjunction with the temperature program detailed in Table 1.

The ions of interest for each analyte, shown in Table 2, were chosen after inspection of the full-scan mass spectra obtained via electron ionization at 70 eV. In general, the most abundant ion was assigned as the target ion for quantification purposes to maximize sensitivity. The qualifier ions were generally the ions of next highest abundance. For G-28273 and G-30033, the isotopic M + 2 ion for chlorine was included among the confirmation ions. The ions shown in Table 2 can be interchanged for target and/or qualifier ion purposes if interferences are encountered for the selected target ion in field samples.

Table 2. Retention Time (t_R), Target and Qualifier (Q) Ions, and Qualifier/Target Ion Ratios Used for the GC/MSD Analyses

analyte	t_R[a] (min)	target ion (m/z)	Q ion 1 (m/z)	Q ion 2 (m/z)	Q1/target ion ± 20% acceptance range[b]
G-28273	4.9	145	147	110	23.0−42.8
G-28279	6.1	158	173	145	71.3−132.3
G-30033	6.2	172	174	187	23.0−42.8
atrazine	7.6	200	215	173	37.0−68.8

[a] This will vary according to column length and other operating parameters. [b] This confirmation ratio will vary slightly from analytical set to analytical set.

Sample Analysis. Each analytical method validation set consisted of six analytical standards of various concentrations, a blank (acetone), a control, and controls fortified with the four analytes at the 0.10−100 ppb concentration level for procedural recovery purposes. Additional standards were dispersed throughout the run as a means of checking the stability of the system for variances in MSD sensitivity and/or column performance. Each analytical set contained a minimum of two and terminated with one of these "stability check" or "quality control" standards (Jenke, 1996a−c).

The various water samples used in this study were obtained from a private residence in Forsyth County, NC, using well water (ground water), Lake Higgins, Guilford County, NC (surface water), and PicoPure deionized water from our laboratory.

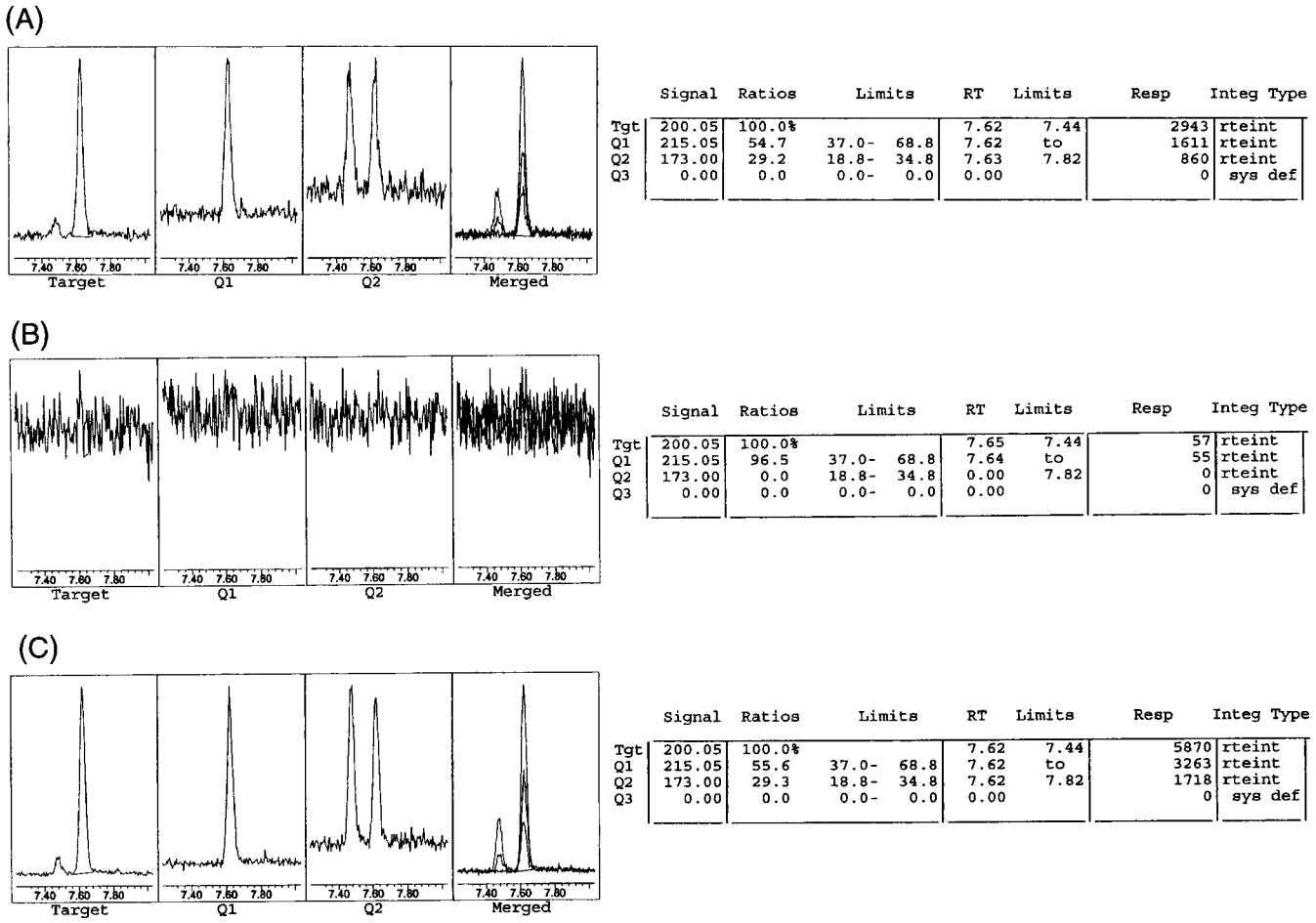


Figure 5. Representative SIM chromatograms for atrazine: (A) 0.050 ng injected standard; (B) control; (C) 0.10 ppb procedural recovery sample.

RESULTS AND DISCUSSION

GC/MSD Analyses. Representative SIM chromatograms of a 0.050-ng injected standard, control, 0.10 ppb procedural recovery samples for G-28273, G-28279, G-30033, and atrazine in ground water are shown in Figures 2, 3, 4, and 5, respectively. The nanograms injected and respective responses for the target ions for each analyte were used for construction of the calibration plots. A quadratic curve more closely fit the standard responses than a linear regression plot. The degree of curve fit was simply determined by creating quadratic and linear regression plots (plotting nanograms injected versus response) for the same set of standards. Then, each individual standard peak response was compared to both the quadratic and linear regression calibration plots to generate "nanograms found" values (i.e., as if the standard were an unknown). In general, the "nanograms found" values generated using the quadratic fit provided a closer match to the known nanograms injected values for each standard at each concentration level than did the use of linear regression. This phenomenon may be due to instrumental injection bias. Even though the quadratic provided a better overall fit, the linear regression correlation coefficients were still typically >0.99.

The limits of analyte confirmation were established by arbitrarily calculating ±20% of the Q1/target ion ratio as measured for an analytical standard, and this range is shown in Table 2 for each analyte. The chance of obtaining Q1/target ion ratios outside the arbitrarily chosen acceptable range of ±20% increases when samples containing coeluting matrix components are injected and when sample components at the sub-parts per billion concentration level are analyzed. In this work, consistent analyte confirmation (i.e., Q1/target ion ratios within ±20%) was obtained for recovery samples at and above the LOQ of the method. Occasionally, the Chemstation software used failed to recognize and integrate a qualifier ion peak, but it was still clearly present in the individual ion and merged ion chromatograms at the correct ratio (e.g., the Q2 ion shown in Figure 3C).

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 *m*/*z* ratios of the target ions and the same retention times were occasionally detected in the control samples, but the qualifier ions were absent and the concentrations were always $\ll 0.10$ ppb. The control chromatograms shown in Figures 2B−5B were scaled differently from the standards (A) and procedural recovery samples (C) depicted in Figures 2−5 as demonstrated by inspection of the signal/noise ratio. The Chemstation software automatically scales each ion chromatogram to the largest peak in that time window.

A summary of the procedural recovery data obtained during the method validation is shown in Table 3. Ground (well) and surface (lake) waters were used in the validation because these represent the sample types

Table 3. Summary of Procedural Recovery Data Obtained for Fortified Ground Water, Surface Water, and Deionized Water

		% recovery (SD)			
water	*n*	atrazine	G-30033	G-28279	G-28273
ground	12[a]	91 (8.7)	94 (7.6)	104 (18)	76 (12)
surface	12	96 (10)	98 (12)	100 (13)	80 (14)
deionized	6[b]	74 (8.0)	75 (7.3)	81 (4.5)	114 (16)
range[c]		64−115	64−120	73−134	59−134
all	30	90 (12)	92 (13)	98 (16)	85 (20)

[a] Fortification levels: 0.10 (7), 0.50 (1), 1.0 (3), and 100 (1) ppb. [b] Fortification levels: 0.10 (4) and 1.0 (2) ppb. [c] Range for all three water sample types.

most often included in water monitoring or survey studies. Deionized water was also used in the validation because this is representative of the water samples typically used for control and procedural recovery purposes. The recoveries for the deionized water samples were ~15−20% lower than the recoveries obtained for ground and surface waters except for G-28273, for which the overall recovery was ~20% higher. This may be an artifact of the method validation and/or the limited number of deionized water samples ($n = 6$) used in the study. This trend was not observed after using the method to generate hundreds of recovery values in the analysis of thousands of water samples in support of various Novartis studies. In one study alone, using deionized water for control and procedural recovery purposes, the mean recoveries were 98% for atrazine ($n = 233$), 108% for G-30033 ($n = 224$), 116% for G-28279 ($n = 224$), and 95% for G-28273 ($n = 221$). These mean recoveries more closely resemble those obtained in ground and surface waters than those obtained in deionized water during the method validation.

The mean procedural recoveries obtained during the method validation are all in the acceptable range of 70−120%, even though 4 of the 30 individual recovery values in this study deviated outside this range (i.e., 64, 64, 134, and 134%). Note that in a method validation study conducted under GLP, all acquired recovery data must be reported unless there is a known reason for discarding a sample result (e.g., the sample was spilled during preparation for analysis). Therefore, individual procedural recoveries will be occasionally outside the 70−120% range. On the basis of these results, the method described in this paper is valid, accurate, and reliable for the determination of atrazine and its dealkylated chlorotriazine metabolites in water at an LOQ of 0.10 ppb. It is likely that the LOQ of the method could be lowered to 0.050 ppb or less using the sample preparation procedure described in this paper, but we have not yet performed a GLP method validation at lower concentration levels for verification. A lower method LOQ is justifiable solely on the basis of the signal/noise characteristics of the GC/MSD instrumentation used to perform the final fraction analyses.

It should be noted that the analysis of surface water samples requires more frequent GC/MSD maintenance than does the analysis of ground water samples. The method described in this paper relies on classical liquid/liquid partitioning. Its strength is the ability to extract and analyze for the polar triazine metabolite G-28273. Its weakness is the ability to extract at least some portion of almost every organic compound present in the water sample. Surface water samples frequently contain motor oil, polycyclic aromatic hydrocarbons, solvents, and numerous other organic components at concentration levels significantly higher than the parts per billion level of herbicides analyzed using the method described herein. Although these coextractives are not likely detected using SIM GC/MSD, their presence can adversely affect analyte t_R and peak shape as well as decrease the useful lifetime of the capillary GC column. Some of the sample matrix components irreversibly adsorb to the bonded stationary phase in the first meter or so of the capillary column. We found it necessary to perform daily injector maintenance (replacement of washer, gold seal, insert, and thorough cleaning with methanol and acetone) and to clip ~1 m from the injector end of the capillary GC column every 2−3 days to maintain good peak shape and sensitivity (especially for G-28273).

Conclusions. The results presented in this paper demonstrate that the method presented herein and validated according to FIFRA GLP 40 CFR Part 160 standards is valid, accurate, and precise for the determination of atrazine and its dealkylated chlorotriazine metabolites in water. The method LOQ is 0.10 ppb (defined as the lowest recovery value evaluated during this study), and the limit of detection (LOD) is 0.050 ng injected (defined as the lowest concentration standard injected and used to construct the calibration plot). The sample preparation portion of this method is more labor intensive and requires the use of larger volumes of organic solvents (and the associated disposal costs) than procedures that rely on SPE. However, the primary value of this method for water monitoring studies is the ability to quantitatively analyze for G-28273. The method is also applicable to the analysis of numerous other herbicides and metabolites detected in ground and surface waters but not presented here (e.g., metolachlor, metalaxyl, prometon, simazine, prometryn, ametryn, GS-11354, GS-11355, GS-26831, and others).

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