Analysis of Hydroxylated Atrazine Degradation Products in Water Using Solid-Phase Extraction and High-Performance Liquid Chromatography

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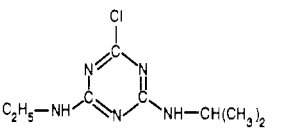
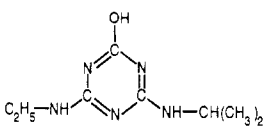
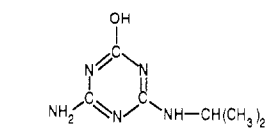
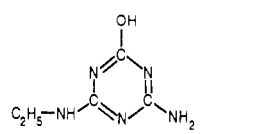
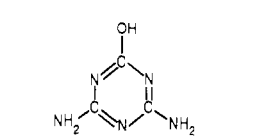
The fate of hydroxylated atrazine degradation products (HADPs) has not been studied extensively in aquatic environments because there are no published quantitative analytical methods that do not use radioisotopes. This paper presents a new method that has been developed to analyze these degradation products in water. Three HADPs, hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA), were extracted, concentrated, and purified from spiked (1 or 5 ppb) laboratory water and stream water samples (0.25 L) using SCX (propylbenzenesulfonic acid) cation-exchange, solid-phase extraction columns. They were then separated and quantified by high-performance liquid chromatography using a deactivated, reversed-phase octyl (C_8) column with UV detection at 220 nm. The limit of quantitation was 0.13 ppb for HA and 0.40 ppb for the two N-dealkylated HADPs. Recoveries of 5 ppb HADP spikes from stream water containing 5.6–13.5 ppm of dissolved organic C averaged 89.1 ± 6.0% (mean ± standard deviation) for HA, 87.1 ± 3.7% for DEHA, and 90.4 ± 4.2% for DIHA.

INTRODUCTION

Atrazine is one of the most commonly used herbicides in the United States (National Agricultural Statistics Service, 1993). Consequently, there are many published methods for the analysis of atrazine and its chlorinated degradation products in water (Chau and Lee, 1982; Lee and Stoker, 1986; Di Corcia et al., 1987; Huang and Pignatello, 1990; Nash, 1990; Steinheimer and Ondrus, 1990; Thurman et al., 1990). However, no methodology exists for routine analysis of hydroxylated atrazine degradation products (HADPs) (Figure 1) in water which do not use radioisotopes. This is surprising because hydroxyatrazine (HA) is a major abiotic degradation product of atrazine in water (Esser et al., 1975; Hapeman-Somich, 1991; Durand et al., 1991) and soil (Best and Weber, 1974; Burkhard and Guth, 1981; Ghadari et al., 1984; Winkelmann and Klaine, 1991). Lack of routine nonradioactive methods for quantitative analysis of HADPs has limited research on the fate of atrazine in surface waters (Kolpin and Kalkhoff, 1993).

Hydroxyatrazine strongly sorbs to soil organic matter (Brouwer et al., 1990), slowly leaches through soil (Schiavon, 1988), and persists longer in the soil than either atrazine or chlorinated atrazine degradation products (Winkelmann and Klaine, 1991). Consequently, contamination of surface water by HA was thought to be improbable. However, HA has been detected in stream and reservoir water (Adams and Randtke, 1992). Kolpin and Kalkhoff (1993) suggested that HA had formed abiotically via photolysis in an Iowa stream, although this remains to be proven. Atrazine can inhibit growth and photosynthesis of green algae and cyanobacteria, but HA was found to be nontoxic to these same aquatic microorganisms (Stratton, 1984). Therefore, the formation of HA and N-dealkylated HADPs may be an important path for atrazine detoxification, at least for aquatic photosynthetic species, but the overall impact of these compounds on aquatic ecosystems is currently unknown.

The chemical properties of HADPs are distinct from those of atrazine. They are less water soluble (except at low pH) (Shiu et al., 1990) and stronger bases than atrazine (Figure 1) (Vermuellen et al., 1982). In addition, HADPs are tautomeric compounds with the ketone and enol isomers in equilibrium. Therefore, HADPs possess two stable configurations that contribute to the difficulties in isolating and analyzing these compounds from environmental samples (T. R. Steinheimer, personal communi-

Compound Name	Chemical Structure	pk_a*
Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine		1.71
Hydroxyatrazine (HA), 2-hydroxy-4-(ethylamino)-6-(isopropylamino)-s-triazine		5.15
Deethylhydroxyatrazine (DEHA), 2-hydroxy-4-amino-6-(isopropylamino)-s-triazine		4.57
Deisopropylhydroxyatrazine (DIHA), 2-hydroxy-4-(ethylamino)-6-amino-s-triazine		4.65
Didealkylhydroxyatrazine (DDHA), Ammeline, 2-hydroxy-4,6-amino-s-triazine		

*Vermeulen et al., 1982

Figure 1. Chemical structures and dissociation constants (pK_a) of atrazine and hydroxylated atrazine degradation products (HADPs).

cation). The HADPs must be derivatized for analysis by gas chromatography (Muir and Baker, 1978); thus, high-performance liquid chromatography (HPLC) is better suited for their analysis. HADPs in solid form are essentially insoluble in organic solvents (e.g., methanol, acetonitrile, ethyl acetate, and hexane) compared to atrazine (Budavari et al., 1989). Because of their chemical properties, strategies for quantitative analysis of HADPs in water are necessarily different from those for atrazine.

Solid-phase extraction (SPE) is commonly used to extract and purify herbicides from water (Di Corcia et al., 1987; Bogus et al., 1990; Steinheimer and Ondrus, 1990; Thurman et al., 1990; Johnson et al., 1991; Adams and Randtke, 1992). Solid-phase extraction of herbicides is faster, less polluting, and more easily automated than older liquid–liquid extraction methods. Steinheimer and Ondrus (1990) reported HA recoveries of 88–97% for spiked stream water samples (1.6–4 ppb) using cylcohexyl silica bonded SPE for extraction. Adams and Randtke (1992) successfully used octadecyl (C_{18}) SPE for HA extraction but did not report recoveries. These investigators used CH_3OH to elute atrazine degradation products from SPE, which may cause coelution problems for waters containing high levels of dissolved organic carbon (>5 ppm). SPE has been used to only a limited extent for HA extraction from water, and no published SPE methods exist for extraction and purification of N-dealkylated HADPs. Therefore, the objective of this research was to develop and evaluate the performance of a routine method for extraction, separation, and quantitation of hydroxylated atrazine degradation products in water using SPE and HPLC.

MATERIALS AND METHODS

Standard Materials. All materials were of the highest purity available. Hydroxyatrazine (HA) [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], deethylhydroxyatrazine (DEHA) [2-hydroxy-4-amino-6-(isopropylamino)-*s*-triazine], deisopropylhydroxyatrazine (DIHA) [2-hydroxy-4-(ethylamino)-6-amino-*s*-triazine], and didealkylhydroxyatrazine (DDHA or ammeline) [2-hydroxy-4,6-amino-*s*-triazine] were 94–99% pure and were provided by Ciba-Geigy Corp. (Greensboro, NC). All stock standard solutions (10 and 100 ppm) were prepared in 0.1 M HCl. Standard solutions were prepared in 0.5 M KH_2PO_4 (pH 7.5)/CH_3CN (75/25 v/v) at concentrations ranging from 15 to 1000 ppb. Acetonitrile (EM Science Omni Solv, Gibbstown, NJ) and KH_2PO_4 (Fisher Scientific, Pittsburgh, PA) were of HPLC grade and HCl was of reagent grade. The HCl and KH_2PO_4 solutions were filtered through 0.2-μm filters before addition of HADPs. All KH_2PO_4 solutions were adjusted to pH 7.5 using reagent grade NaOH (75% w/v solution) or to pH 2.5 with reagent grade concentrated H_3PO_4. HPLC grade heptanesulfonic acid was in the sodium salt form dissolved in CH_3OH at approximately 0.33 M (Kodak Chemical Co., Rochester, NY).

Evaluation of Various SPE Bonded Phases. SPE columns (500 mg in 2.8-mL polypropylene reservoirs; Varian, Harbor City, CA) of six nonpolar SPE bonded phases (ethyl, C_2; octyl, C_8; octadecyl, C_{18}; cyclohexyl, CH; phenyl, PH; and endcapped cyanopropyl, CN) and a cation-exchange (propylbenzenesulfonic acid, SCX) bonded phase were initially evaluated for breakthrough (i.e., lack of retention) of HADPs. Triplicate samples containing 100 ppb of HADPs (HA, DEHA, DIHA, and DDHA) in 25 mL of HPLC grade H_2O for the nonpolar bonded phases or 25 mL of 0.05 M KH_2PO_4, pH 2.5, for the SCX bonded phase were used to evaluate breakthrough. Nonpolar SPE cartridges were conditioned as described by Thurman et al. (1990), and the SCX cartridges were conditioned as described below. Samples were passed through the SPE cartridges at 3–5 mL min^{-1}, and the last 5–10 mL was collected in glass test tubes. For the SPE breakthrough study, a 30-μL portion of the collected samples was injected for HPLC analysis.

SCX SPE Procedure. SCX bonded phase SPE extraction was performed as follows: (1) SCX columns were conditioned sequentially with 7–9 mL each of CH_3CN, H_2O, and 0.05 M KH_2PO_4 at pH 2.5. This step was repeated from the beginning if the sorbent dried. (2) Water samples (250 mL) (except where noted) were adjusted to pH 2.5 with 1 M KH_2PO_4 (pH 2.5) and passed through the columns immediately after conditioning. (3) The columns were then washed with 15–20 mL of H_2O and air-dried for at least 30 min, but no more than 1 h, by pulling a 25–40 cmHg vacuum through them. (4) Retained HADPs were eluted with 2 mL of 0.5 M KH_2PO_4 (pH 7.5)/CH_3CN (75/25 v/v). The CH_3CN concentration of the eluent was found to be critical to HA recovery, whereas recovery of the N-dealkylated HADPs depended primarily upon KH_2PO_4 concentration. Flow rates through the SPE columns were 3–8 mL min^{-1} except during the elution step during which the rate was 2–3 mL min^{-1}. Precipitated Ca^{2+} and Mg^{2+} phosphates were removed from the SCX column eluate by filtration through 0.2-μm Anotop 25 syringe filters (Alltech Associates, Inc., Deerfield, IL). Hydrolysis of atrazine or its chlorinated degradation products by acidification of the sample (in step 2 above) was negligible (≤2.2% hydrolysis for atrazine and its chlorinated dealkylated degradation products after 18 h of incubation at pH 2.5). Step 2 was usually completed within 1 h, and most SPE extractions lasted 50–75 min.

High-Performance Liquid Chromatography. A Beckman Model 338 HPLC system (Beckman Instruments, Inc., San Ramon, CA) was used. The system consisted of two Model 110B pumps operated at a 1 mL min^{-1} flow rate, a Model 507 autosampler with a column oven at 30 °C and a 100-μL sample loop, and a Model 166 variable-wavelength UV detector set to 220 nm. A deactivated octyl (C_8) reversed-phase column (LC-8-DB, 5 μm, Supelco, Inc., Bellefonte, PA) with dimensions of 250 mm by 4.6 mm (i.d.) was used. Eluates (i.e., 0.5 M KH_2PO_4/CH_3CN, 75/25 v/v) from the SCX procedure were used for all HPLC separations.

Two different isocratic HPLC methods were used for HA and the N-dealkylated HADPs because their partitioning behavior differs significantly. For HA, the mobile phase was 40% aqueous CH_3OH and the sample injection volume was 40 μL (HA retention time was 13.0 min). For DEHA, DIHA, and DDHA the mobile phase was 15% aqueous CH_3OH and the sample injection volume was 10 μL (DDHA retention time was 3.3 min, DIHA retention time was 5.1 min, and DEHA retention time was 9.0 min). Quantitation of DDHA for the concentration and volume study (discussed below) was accomplished by ion-pair HPLC using a mobile phase of CH_3CN (7.5%) and 25 mM KH_2PO_4 with 5 mM heptanesulfonic acid (92.5%) and a Beckman octadecyl column with dimensions of 250 mm by 4.6 (i.d.) mm. The sample injection volume was 20 μL, and DDHA retention time was 7.0 min.

The limits of detection and quantitation were determined by evaluating the signal-to-noise ratio (SNR) of baseline sections for each isocratic HPLC method used. For both methods, the SNR was about 2×10^{-5} absorbance unit for standard solutions and processed stream water samples. The limits of detection and quantitation were set at 3 and 10 times the SNR, respectively. For HA, the limit of detection was 0.04 ppb and the limit of quantitation was 0.13 ppb for 250-mL water samples. For DEHA and DIHA, the limit of detection was 0.12 ppb and the limit of quantitation was 0.40 ppb for 250-mL water samples. HPLC regression lines of UV absorbance vs HADP concentration used for quantitation were linear over the concentration range of 25–1000 ppb for all HADPs, and coefficients of determination (r^2) were at least 0.99. To relate the standard curve to a water basis, concentrations should be divided by 125 because 250-mL H_2O samples were concentrated to 2 mL by the SPE procedure.

Effect of Sample Volume and Concentration on Recovery of Hydroxylated Atrazine Degradation Products from SCX. The effect of concentration and sample volume on percent HADP recovery from SCX columns was evaluated in an experiment using a factorial design of three concentrations (0.2, 1, and 10 ppb) and five volumes (100, 250, 500, 1000, and 2000 mL). Various concentrations of HADPs were prepared in 0.05 M KH_2PO_4 at pH 2.5. Each HADP concentration by volume treatment was duplicated. The SPE procedure was as previously described except that sample volume was a variable in this experiment.

Stream Water Sampling and Handling. Stream water from Goodwater Creek was sampled at a monitoring site equipped with a V-notch weir used to measure stream flow for the entire

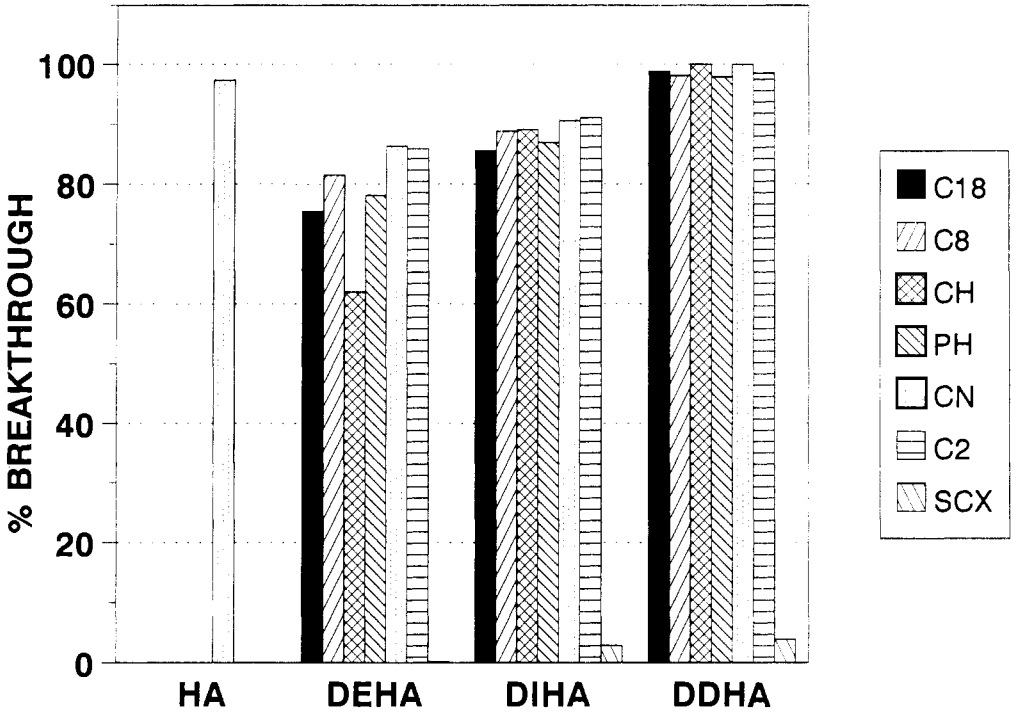


Figure 2. Breakthrough of hydroxylated atrazine degradation products (HADPs) from various solid-phase extraction (SPE) bonded phases. For nonpolar bonded phases, samples were 25 mL of 100 ppb HADPs in HPLC H_2O; for SCX bonded phase, samples were 25 mL of 100 ppb HADPs in 0.05 M KH_2PO_4, pH 2.5.

7250-ha Goodwater Creek watershed in Audrain County, MO (latitude, 92° 03′ W; longitude, 39° 18′ N). Approximately 72% of the Goodwater Creek watershed was cropped in 1991, and 19% of this was planted to corn (about 992 ha) (S. Rikoon and R. J. Vickers, personal communication, University of Missouri Department of Rural Sociology). Five 1-L grab samples were collected in amber glass jars with Teflon-lined screw caps on a weekly basis or as permitted by stream flow between June and December 1992. Collection bottles were cleaned by rinsing in deionized water and acetone, followed by heating at 300 °C for 8 h. All samples were filtered through 0.45-μm nylon filters (Whatman, Hillsboro, OR) to remove suspended sediment before being combined and mixed to provide a uniform sample for each sample day. Adsorption of the HADPs by the nylon filters was negligible. Comparison of HADP recoveries from filtered and unfiltered spiked synthetic samples (5 ppb) showed no significant difference. Field stream water samples were stored at 2–4 °C for no more than 10 days before analysis. Storage of synthetic water samples spiked to 5 ppb for 16 days showed no significant reduction in HADP recovery compared to unstored samples.

Laboratory duplicate subsamples of the composited stream water samples (for selected dates) were spiked with HA, DEHA, and DIHA at either 1 or 5 ppb to evaluate the accuracy and precision of the method by determination of analyte recovery. In addition, triplicate subsamples of untreated, composited water samples were analyzed for the presence of dissolved HADPs. The filtered sediment was not analyzed for sorbed HADPs. On the basis of HPLC retention times, the presence of dissolved HADPs has been tentatively confirmed in untreated field samples. Concentrations of HADPs in untreated samples were highest from June to September 1992, with maximum levels of 5.7 ppb for HA, 1.9 ppb for DEHA, and 0.72 ppb for DIHA. Confirmation of HADPs in the untreated samples by HPLC/mass spectrometry using thermospray and particle beam interfaces is pending. Percent spike recoveries were calculated for each HADP (i.e., HA, DEHA, or, DIHA); the calculation was corrected for the background HADP concentration using the equation

$$\% \text{ recovery} = \{([HADP_t] - [HADP_{ut}])/[HADP_{sp}]\} \times 100$$

where $[HADP_t]$ is the total dissolved HADP concentration (ppb) in spiked stream water sample (i.e., $[HADP_{ut}] + [HADP_{sp}]$), $[HADP_{ut}]$ is the apparent HADP concentration (ppb) of untreated stream water sample, and $[HADP_{sp}]$ is the spiked HADP concentration (i.e. 1–5 ppb).

Table 1. Chemical Properties of Surface Water from Goodwater Creek, MO, June–December 1992

property	
pH	7.9 ± 0.2[a]
dissolved organic C, ppm	7.8 ± 2.4
NO_3-N, ppm	0.82 ± 0.65
NH_4-N, ppm	0.19 ± 0.15
total PO_4-P, ppm	0.21 ± 0.08
Ca, ppm	28.7 ± 10.5
Mg, ppm	7.4 ± 3.4
total alkalinity,[b] ppm	105 ± 54

[a] Mean ± standard deviation reported for all data. [b] $CaCO_3$ equivalent.

Selected stream water samples were analyzed for dissolved organic C (DOC), total alkalinity, soluble Ca and Mg, and total soluble PO_4-P by standard methods 531B, 232B, 350-CaB, 350-MgB, and 450-P, respectively (Franson et al., 1989) (Table 1). After sample acidification with H_2SO_4, NO_3-N and NH_4-N were measured according to automated colorimetric methods using a Traacs 80 system (Bran and Luebbe, Elmsford, NY). For NO_3-N, the standard Cd reduction method (Franson et al., 1989) was modified by using hydrazine sulfate in the presence of Cu to reduce NO_3^- to NO_2^- (Industrial Method 782-86T, Bran and Luebbe). For NH_4-N, the indophenol blue method was used in the presence of disodium ethylenediaminetetraacetic acid (Franson et al., 1989). Water pH was determined with a combination pH electrode after the samples were allowed to reach ambient temperature (22–25 °C).

RESULTS AND DISCUSSION

Evaluation of Various SPE Bonded Phases. All nonpolar bonded SPE phases, except CN, quantitatively extracted HA from water, resulting in <5% breakthrough (Figure 2). However, N-dealkylated HADPs showed breakthroughs of 62–100% from nonpolar SPE phases, and breakthrough increased with increasing analyte polarity. Average breakthroughs of N-dealkylated HADPs

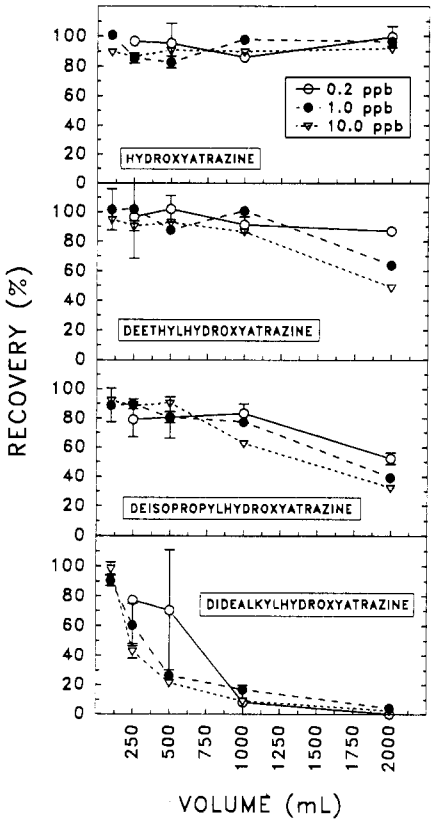


Figure 3. Recovery of hydroxylated atrazine degradation products (HADPs) in standard solutions from cation-exchange (SCX) solid-phase extraction (SPE) columns as a function of sample volume and analyte concentration. Standard solutions were 0.05 M KH_2PO_4 at pH 2.5. Error bars denote one standard deviation about the mean.

from nonpolar SPE were as follows: DEHA, 78.2%; DIHA, 88.7%; DDHA, 98.9%. The CN bonded phase did not adequately retain any of the four analytes, with breakthroughs of 86.3–100%. In contrast, the SCX bonded phase showed <5% breakthrough for all four HADPs. Overall, the retention mechanisms of HADPs to the SCX bonded phase (see discussion below) resulted in significantly lower breakthrough compared to any of the nonpolar SPE phases. In addition, the acidified water samples used with SCX shift the tautomeric equilibrium to the enol form of the HADPs. Thus, the SCX procedure negates any potential complications associated with the isolation of both tautomers from solution. On the basis of these results, the SCX phase was evaluated further for quantitative extraction of HADPs from water.

Effect of Sample Volume and Concentration on Recovery of Hydroxylated Atrazine Degradation Products from SCX. Recovery of all HADPs, except HA, from SCX SPE depended on water sample volume over the range 100–2000 mL (Figure 3). Recovery was independent of concentration for all HADPs over the concentration range of 0.2–10 ppb. Mean percent recoveries at 250 mL, the sample volume chosen for stream water samples, were as follows (mean ± standard deviation): HA, 89.5 ± 5.9%; DEHA, 96.3 ± 13.7%; DIHA, 85.7 ± 7.7%; DDHA, 60.3 ± 16.7%. The volume dependence of HADP recovery increased with increasing analyte polarity. On the basis of retention time in reversed phase HPLC columns, retention to nonpolar SPE phases, and degree of N-dealkylation, HADP polarity was observed to be (from least to most polar) HA < DEHA < DIHA < DDHA. As polarity increased, breakthrough occurred at progressively smaller water sample volumes. Because of the low recovery of DDHA by SCX and the need to use large water sample volumes to attain low detection limits, DDHA was not analyzed in subsequent work involving stream water samples. Statistical analysis of HADP recovery on SCX columns showed that sample volumes of up to 1000 mL could be used for all HADPs, except DDHA, without significant breakthrough.

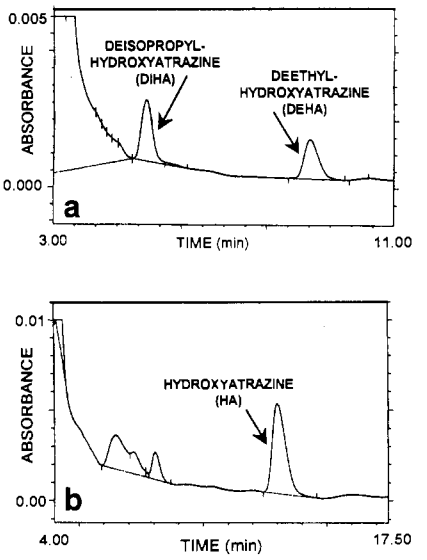


Figure 4. Chromatograms obtained from cation-exchange (SCX) solid-phase extraction (SPE) cleanup of 250-mL surface water samples originally spiked to 5 ppb: (a) deethylhydroxyatrazine (DEHA) and deisopropylhydroxyatrazine (DIHA); (b) hydroxyatrazine (HA).

Two mechanisms seem to be responsible for HADP retention by SCX, as suggested by knowledge of HADP polarity and the effect of sample volume on HADP recovery (Figure 3). First, all analytes were retained chiefly by cation exchange. Because the dissociation constants of the HADPs (Figure 1) were above the adjusted pH of the water samples, they were positively charged, and because the dissociation constant of the SCX bonded phase, $pK_a < 1$, was below the adjusted pH of the water sample, the SCX was negatively charged. Second, hydrophobic interactions between the triazine ring and the SCX bonded phase benzene ring may explain the differences in recovery as a function of increasing sample volume between different HADPs. As the analyte polarity increases, the hydrophobic interaction would likely decrease, resulting in lower retention and recoveries for more polar HADPs, particularly at higher sample volumes. This was supported by comparison of recoveries between the 100- and 2000-mL treatments (Figure 3). Recoveries, averaged over concentration, for the 100- and 2000-mL treatments, respectively, were as follows: HA, 95.3 and 95.9%; DEHA, 98.3 and 66.6%; DIHA, 90.5 and 41.4%; DDHA, 94.4 and 2.2%. Hydrophobic interactions (in conjunction with cation exchange) have been shown to be responsible for selective retention of organic cations on the SCX bonded phase (Simpson, 1992), but the mechanisms responsible for HADP retention to SCX require further research.

Effectiveness of SCX as a Cleanup Technique. Stream water samples used in this study contained substantial amounts of dissolved organic C, averaging 7.8 ppm with a range of 5.6–13.5 ppm (Table 1). Nevertheless, the chromatograms of spiked stream water samples show that the SCX-HPLC method provided excellent cleanup and separation of HADPs from stream water (Figure 4). Also, no significant coelution problems were observed in the chromatograms over the 6-month sampling period.

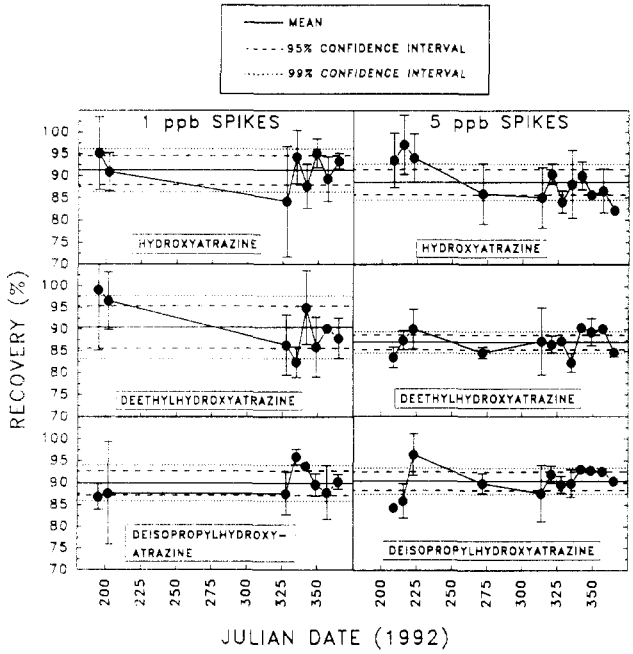


Figure 5. Quality control charts for evaluation of accuracy, precision, and reproducibility of spike recovery measurements for hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA) over time. Means (●) ± standard deviation (bars) are presented in addition to the grand mean (—) and 95% (——) and 99% (- - -) confidence intervals.

Potential coeluting compounds could include the hydroxylated degradation products of other triazine herbicides such as simazine, cyanazine, and propazine. SCX cation-exchange phase has advantages over previously used nonpolar bonded phases (Bogus et al., 1990; Nash, 1990; Steinheimer and Ondrus, 1990; Thurman et al., 1990) for sample cleanup. Because of the hydrophobic nature of organic matter, its adsorption to nonpolar SPE bonded phases is likely to be greater than to SCX. Thus, HADP recovery using nonpolar SPE may be lower due to competition by organic matter for hydrophobic adsorption sites (Johnson et al., 1991), particularly for stream water with high levels of dissolved organic C.

Recovery of HADPs from Stream Water. The accuracy, precision, and reproducibility of the method are summarized in quality control charts from spike recovery determinations of HADPs from stream water over time (Figure 5). Quality control charts provide a statistical basis to evaluate analytical method performance over time and help in quickly identifying problems that may develop (Taylor, 1987).

Spike recovery at 1 ppb showed that the SCX method performed consistently for all three analytes over 24 weeks from June to December 1992 (Figure 5). No more than two observations of any analyte fell outside the 99% confidence interval during this period, and the standard deviation of recovery measurements for individual sample days (shown by standard deviation bars in Figure 5) were generally less than 10%. Hydroxyatrazine and DEHA showed typical patterns in which the early observations vary about the mean more than later observations. This pattern illustrates that precision improved as the skills required to perform the procedure were mastered. In addition, analysis of variance of the mean percent recoveries over time showed that no two daily means ($n = 2$ per sample day) differed significantly from each other ($P > 0.37$) for any of the sampling times. Mean recoveries of all 1 ppb spikes were as follows (mean ± standard deviation): HA, 91.5 ± 6.3%; DEHA, 91.3 ± 8.6%; DIHA, 89.6 ± 5.6%.

Spike recovery at 5 ppb also showed that the SCX method performed well over time (Figure 5). The first three observations for each HADP were generally outside the 99% confidence interval, but, thereafter, most observations were within the 95% confidence interval. Thus, precision at 5 ppb also increased with time as was observed for the 1 ppb spikes. Confidence intervals and standard deviations for individual sample days were smaller for all three HADPs at 5 ppb than for 1 ppb spikes, indicating greater precision of the method at the higher concentration. Analysis of variance of mean recoveries over time showed that the mean recovery for DIHA on Julian date 223 was significantly greater ($P = 0.04$) than the recoveries on Julian dates 209 and 216. For HA and DEHA, none of the individual observations were significantly different from each other. Mean recoveries of all 5 ppb spikes were as follows (mean ± standard deviation): HA, 89.1 ± 6.0%; DEHA, 87.1 ± 3.7%; DIHA, 90.4 ± 4.2%.

On the basis of comparisons with nonpolar SPE bonded phases, the SCX SPE showed superior retention of all four HADP analytes. Recovery of HADPs from SCX SPE depended on sample volume for the N-dealkylated HADPs and was independent of sample concentration for all four HADPs. Volume dependence of HADP recovery was related to analyte polarity. Recoveries of HA, DEHA, and DIHA from spiked stream water, containing high levels of dissolved organic C, were shown to be near 90% for all three analytes for 1 and 5 ppb spikes. Quality control charts of recovery determinations from spiked stream water showed the method was reproducible, precise, and accurate over a 6-month period. The precision of the method increased with time for both 1 and 5 ppb spikes, and measured recovery of 5 ppb spikes was consistently more precise than that of 1 ppb spikes. For the first time, a validated method is available for routine analysis of HADPs dissolved in water at levels of quantitation as low as 0.1 ppb. This method removes one of the major limitations to the investigation of atrazine fate in the environment by facilitating field research and monitoring efforts directed at the occurrence of HADPs in water.

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Registry No. Supplied by Author: Atrazine, 1912-24-9; hydroxyatrazine, 2163-68-0; deethylhydroxyatrazine, 19988-24-0; deisopropylhydroxyatrazine, 7313-54-4; didealkylhydroxyatrazine, 645-92-1.

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