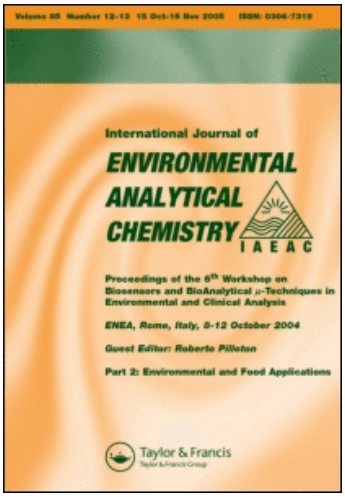


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# Analysis of Hydroxylated Atrazine Degradation Products in Soils

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# ANALYSIS OF HYDROXYLATED ATRAZINE DEGRADATION PRODUCTS IN SOILS

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Hydroxylated atrazine degradation products (HADPs) have been shown to persist in soils and contaminate surface waters throughout the Midwestern United States, yet expedient analytical methods for their determination in soils are lacking. The developed method employs a mixed-mode extractant [3:1 0.5M $KH_2PO_4$, pH 7.5:$CH_3CN$, v/v] designed to disrupt the two primary mechanisms of HADP sorption to soils: cation exchange and hydrophobic interactions. Strong anion exchange solid-phase extraction (SPE) is used for sample clean-up followed by isolation and concentration using strong cation exchange SPE. HADPs were quantitated by LC/MS/MS and LC/UV. Method recoveries were determined by spiking $^{14}$C-HADPs into three soils with lengthy atrazine use histories. Recoveries ranged from 74–81% for $^{14}$C-hydroxyatrazine (HA), 79–88% for $^{14}$C-deethylhydroxyatrazine (DEHA), and 64–77% for $^{14}$C-deisopropylhydroxyatrazine (DIHA). HADP soil concentrations ranged from 66.9–178 μg $kg^{-1}$ for HA, 8.99–40.9 μg $kg^{-1}$ for DEHA, and 5.27–16.2 μg $kg^{-1}$ for DIHA. Utilization of the mixed-mode procedure, in conjunction with existing methodologies for analysis of atrazine and its chlorinated metabolites, enables a more complete and accurate quantitation of all the major stable atrazine residues in soils. HADPs comprised an average of 91% of the total atrazine residues in three agricultural surface soils, with HA the major constituent present in all soils. These data indicate that repeated atrazine use results in HADPs as the predominant atrazine residues in surface soils.

*Keywords:* Atrazine metabolites; Hydroxyatrazine; Deethylhydroxyatrazine; Deisopropylhydroxyatrazine; LC/MS/MS; LC/UV

## INTRODUCTION

Hydroxylated atrazine degradation products (HADPs) are a major class of atrazine metabolites that are persistent in soils and contaminate surface waters throughout the Midwestern United States[1–5]. However, well accepted analytical methods for their quantitation in soils and sediments have been lacking. This has

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resulted in a paucity of field-based concentration data for the HADPs. To date, only two studies have reported field levels of HADPs in soils or sediments[5,6]. However, the study by Muir and Baker[6] used a semi-quantitative method for determination of hydroxyatrazine (HA) and deethylhydroxyatrazine (DEHA) in soil[7]. No reports to date have included deisopropylhydroxyatrazine (DIHA) levels in soils.

Aqueous methanol or acetonitrile have been most commonly used for soil extraction of triazine herbicides and their degradation products[7–12], but supercritical fluid extraction has also been used for recovery of HA from soil[13]. The use of aqueous-organic solvent mixtures assumes that hydrophobic interactions and hydrogen bonding are the dominant sorption mechanisms of the target analyte[14]. Aqueous-organic mixtures often do not quantitatively extract HADPs from soil because of solubility limitations or the inability of these extractants to disrupt other types of sorption mechanisms, such as ion exchange. Soils in which aqueous methanol or acetonitrile results in HADP recoveries of 50% or less indicate that sorption by hydrophobic interactions and hydrogen bonding do not always predominate for these metabolites[15,16].



Extraction of HADPs from soils has also utilized acids or acidified organic solvents in an attempt to increase extraction efficiency. Since HADPs are weak bases with dissociation constants of about $10^{-5}$, the use of acidity was designed to increase the solubility of the HADPs by protonating them. However, reported HA recoveries using acids or acidified organic solvents have varied from 22 to 95%[7,8,17,18]. A considerable portion of this variability can be attributed to the pH-dependent charge of some soil colloids. For example, Goswami and Green[17] reported HA recoveries of >90% from three Hawaiian soils using acidified methanol at pH 2.5. Because these soils were comprised of colloids with pH-dependent charge, HA was effectively extracted due to the absence of negatively charged soil colloids. This same procedure was used for extraction of HA from a Ca-montmorillonite with resulting HA recoveries of only 33%. Goswami and Green[17] concluded that the use of acidified methanol was less suitable for soils with a high permanent negative charge. Lerch et al.[18] substantiated this by showing that recoveries of HADPs from a montmorillonitic alfisol were significantly lower using acidified methanol compared to a mixed-mode extractant [3:1 0.5M $KH_2PO_4$, pH 7.5:$CH_3CN$, v/v]. Despite the extensive work on the triazine herbicides, no generally accepted method for extraction of HADPs from soils has emerged because the mechanisms controlling their sorption were not clearly understood.

Recent studies have shown that HADPs sorb to soils by both cation exchange and hydrophobic interaction mechanisms; this has been referred to as mixed-mode sorption[18]. Thus, methods designed for quantitative recovery of

HADPs in soils must employ an extractant that can disrupt both mechanisms. Using the mixed-mode extractant and exhaustive sequential extraction, Lerch et al.[5], detected HA at levels of 14–640 μg $kg^{-1}$ in field soils and 11 to 96 μg $kg^{-1}$in stream sediments. However, the method used by Lerch et al.[5] was not optimized for quantitation of N-dealkylated HADPs nor did the method employ a clean-up and concentration step to achieve sub-part per billion detection limits. Thus, the primary objective of this research was to develop an optimized method based on the use of the mixed-mode extractant for quantitation of HADPs in soils. A secondary objective was to compare the utility of LC/UV to that of LC/MS/MS for routine analyses.

## EXPERIMENTAL

### Chemicals and standards

Analytical standards of HA (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine), DEHA (2-hydroxy-4-amino-6-isopropylamino-*s*-triazine), DIHA (2-hydroxy-4-ethylamino-6-amino-*s*-triazine), hydroxyterbutylazine (2-hydroxy-4-ethylamino-6-tertbutylamino-*s*-triazine; HT), and deethylhydroxyterbutylazine (2-hydroxy-4-amino-6-tertbutylamino-*s*-triazine; DEHT) were ≥95% pure (Crescent Chemical, Hauppauge, NY, USA). Separate stock solutions containing 10 mg $L^{-1}$ of each HADP were made in 0.1 M HCl, except for DEHT which was in 50% $CH_3CN$ [1:1 $CH_3CN$:$H_2O$, v/v]. Working standards were a mixture of the HADPs plus either HT or DEHT prepared in the 40% $CH_3OH$ [2:3 $CH_3OH$:$H_2O$, v/v] at concentrations of 5 to 5000 μg $L^{-1}$. For working standards of 1000 μg $L^{-1}$ or greater, the acidity from the stock solutions was neutralized with an appropriate amount of NaOH before bringing to volume with 40% $CH_3OH$. All solvents, $KH_2PO_4$, and $H_3PO_4$ used for soil extractions or analyses were HPLC grade. $KH_2PO_4$ solutions were adjusted to pH 7.5 using reagent grade NaOH (50% or 75% w/v solutions). The HCl used for HA stock solutions was reagent grade.

### Mixed-mode extraction procedure

An overview of the procedure is shown in Figure 1. The mixed-mode extractant is 3:1 0.5 M $KH_2PO_4$, pH 7.5: $CH_3CN$ (v/v). The extractant is heated to 70°C before addition to the samples. Soil samples, 25.0 g dry weight, were extracted three times each with 50 mL of mixed-mode extractant in 250-mL Teflon centri-

fuge tubes at 70°C using an orbital shaker at 400 rpm. Preliminary experiments were conducted to determine the optimum extraction temperature based on recovery of HA from the IA soil (see soil descriptions below) at temperatures of 25, 40, 55, and 70°C. This experiment showed a linear increase in HA concentration with increasing temperature, but higher temperatures were not investigated because of the possibility of atrazine hydrolysis at temperatures exceeding 75°C[19]. Soils spiked with $^{14}$C-atrazine were subjected to the entire mixed-mode procedure to check for possible atrazine hydrolysis. No $^{14}$C-HADPs were detected, indicating that atrazine hydrolysis was negligible.

The sequence of shaking times was 1, 2, and 0.5 h. Preliminary experiments were used to determine the optimal shaking time of 2 h, while the other two shaking times were arbitrary. After each extraction, samples were cooled for 10–15 minutes at –20°C to allow the Teflon tubes to harden, centrifuged for 30 min. at 3500 rpm and 10°C, and the supernatant decanted into a 250-mL graduated cylinder. The three supernatants were combined, and the total extract volume recorded. $CH_3CN$ was evaporated using a Savant concentrator at 60°C and ≈665 Pa (Savant Instruments, Farmingdale, NY, USA).



Sample clean-up was achieved with 20g quaternary amine anion exchange (SAX) solid-phase extraction (SPE) cartridges (Varian, Harbor City, CA, USA). The SAX cartridges were conditioned by rinsing with 30 mL of $CH_3OH$ followed by 60 mL of HPLC grade $H_2O$. Sample solution was passed through the cartridge at flow rates of 5–10 mL min$^{-1}$. SAX SPE allows HADPs to pass through with minimal retention while sorbing dissolved organic acids that may interfere with analysis. Therefore, the breakthrough solution from the SAX step must be collected. Although HADP retention to the SAX SPE was typically very low, some retention was observed, particularly for HA. Therefore, following sample throughput, the SAX columns were rinsed with 10 mL of 80% $CH_3OH$ [4:1 $CH_3OH$:$H_2O$, v/v] to disrupt any hydrogen bonding between the HADPs and the silanol groups followed by an additional rinse of 10 mL of HPLC grade $H_2O$ in order to achieve quantitative recovery. These rinsing solutions were collected in the same container as the sample (breakthrough) solution. Preliminary experiments showed that rinsing with more than 10 mL of 80% $CH_3OH$ will decrease HADP recovery because the additional methanol causes greater breakthrough from the cation exchange SPE step (described below). After the SAX step, the samples were acidified to pH ≈ 2.5 with 4 mL of concentrated $H_3PO_4$ (amount will vary depending upon extract volume). Acidification is necessary to protonate the HADPs in order to isolate them by cation exchange SPE. Isolation and concentration of the HADPs was achieved with 2 g propylbenzenesulfonic acid cation exchange (SCX) SPE cartridges (Varian, Harbor City, CA). SCX cartridges were conditioned sequentially with 24 mL of $CH_3OH$, 24 mL of $H_2O$,

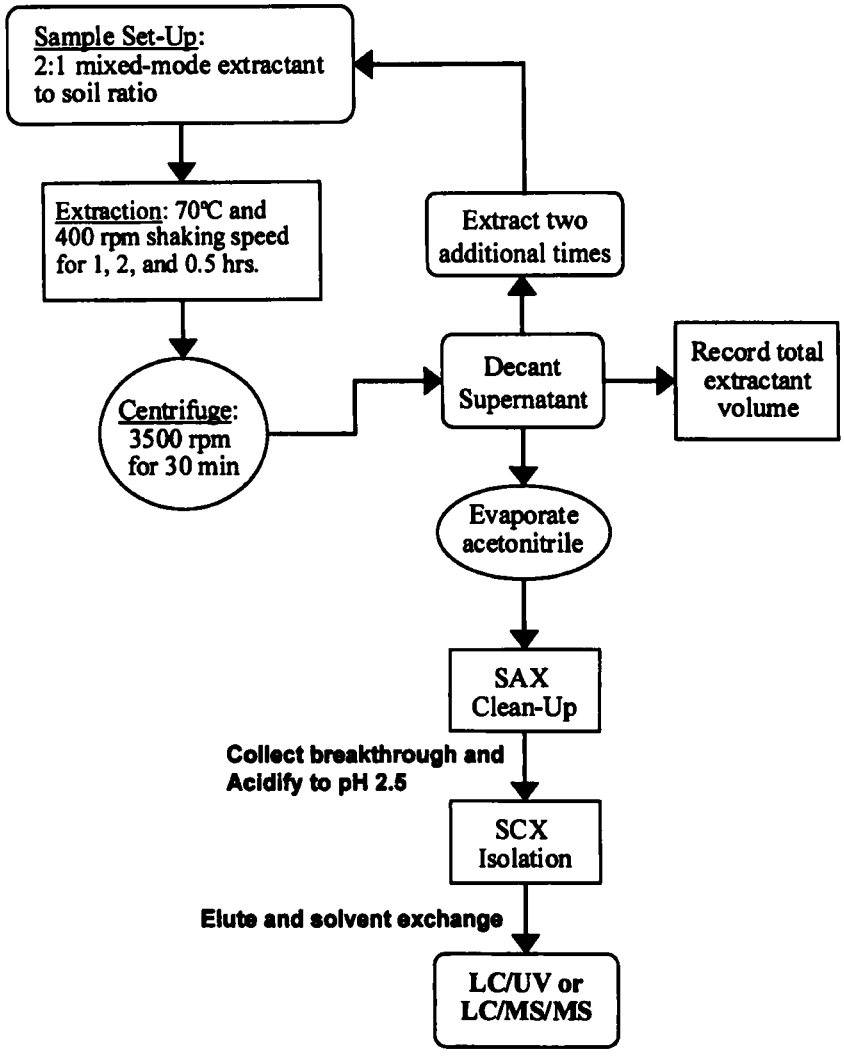


FIGURE 1 Procedural scheme for the mixed-mode extraction of HADPs from soil

and 24 mL of 0.05 M $KH_2PO_4$, pH 2.5. Sample solutions were passed through the SCX cartridges at flow rates of 3–5 mL $min^{-1}$. HADPs were recovered from the SCX SPE by elution with 10 mL of 8:1:1 $CH_3OH$: $NH_4OH$: $H_2O$ at a flow rate of ≈1 mL $min^{-1}$. The eluant was evaporated under a stream of ultra pure $N_2$ in a water bath at 45–50°C. Samples were reconstituted with 1.0 mL of 40% $CH_3OH$, sonicated for 5 minutes, vortex mixed for about 30 seconds, and filtered

through stacked 0.45 μm nylon and 0.2 μm Anotop (Whatman International, Maidstone, UK) syringe filters.

## LC/UV analyses

LC/UV analyses were conducted using a deactivated $C_8$ column (LC-8-DB; Supleco, Inc., Bellefonte, PA) with a binary pump system, as described by Lerch and Donald[20]. Mobile phases were: A, 5 mM $KH_2PO_4$, pH 7.0–7.5; and B, $CH_3OH$. For DEHA and DIHA, an isocratic method of 86% A: 14% B at a flow rate of 1 mL $min^{-1}$ was used with a sample injection volume of 25–40 μL and detection at 210 nm. Retention times were 4.5 min for DIHA and 7.8 min for DEHA. For HA, an isocratic method of 55% A: 45% B at a flow rate of 1.25 mL $min^{-1}$ was used with a sample injection volume of 40 μL and detection at 220 nm. HA retention time was 7.0 min. For DEHT, a gradient step was added to the method used for DEHA and DIHA by increasing mobile phase B from 14% to 30% at 8.0 min and holding at 30%B for 11 min, then cycling back and equilibrating at 14%B. Retention time for DEHT was 16.5 min. For HT, a gradient step was added to the HA method by increasing B from 45% to 50% at 7.0 min. and holding 50%B for 6 min., then cycling back and equilibrating at 45%B. HT retention time was 11.8 min. Nominal HPLC detection limits were 10 μg $L^{-1}$ for DIHA and DEHA and 5 μg $L^{-1}$ for HA. On a soil weight basis, detection limits were approximately 0.5 μg $kg^{-1}$ for DEHA and DIHA and 0.25 μg $kg^{-1}$for HA.



## LC/MS/MS analysis

A PE Sciex API-365 LC/MS/MS system (Toronto, Canada) with Shimadzu LC-10$AT_{vp}$ HPLC system (Columbia, MD) was used for HADP quantitation. A turbo ion spray ionization technique was employed as the interface. Positive ion detection for the compounds were used in multi-reaction monitoring (MRM) mode for quantitation, using the parent→product ion pairs listed in Table I. A spherisorb SCX HPLC column (150 × 2.1 mm, 5 μm) was employed for the separation. Mobile phase A was 9:1 $H_2O$:$CH_3OH$ with 1% HOAc and 5 mM $NH_4OAc$, and mobile phase B was 1:9 $H_2O$:$CH_3OH$ with 0.1% HOAc and 25 mM $NH_4OAc$. Mobile phase programming was 40% B for 4.5 min., then a rapid linear gradient from 40% B to 90% B in 0.5 min. Flow rate was 0.3 mL $min^{-1}$. Sample injection volume was 20 μL. LC/MS/MS quantitation by MRM mode provides high specificity for compound identification because it has three points of identification: 1) retention time; 2) mass screening for molecular ion in the first quadrupole; and 3) quantitation of a diagnostic daughter ion in the third quadrupole. Limits of quantitation (LOQ) were 0.05 μg $kg^{-1}$ for all HADPs.

TABLE I Multi-reaction monitoring (MRM) set-up for quantitation of HADPs by LC/MS/MS

| *Compound* | *Molecular ion → Daughter ion* |
|---|---|
| Hydroxyatrazine (HA) | 198 $[M + H]^+ \rightarrow$ 156 $[M - C_3H_7 + 2H]^+$ |
| Deethylhydroxyatrazine (DEHA) | 170 $[M + H]^+ \rightarrow$ 128 $[M - C_3H_7 + 2H]^+$ |
| Deisopropylhydroxyatrazine (DIHA) | 156 $[M + H]^+ \rightarrow$ 86 $[M - C_2H_5 - NH - HCN + 2H]^+$ |

## Recovery determinations

HADP recoveries using the mixed-mode extraction procedure were determined using $^{14}$C-HADPs. Duplicate 25-g (dry weight) soil samples for each HADP were spiked with 1667 Bq of $^{14}$C-HA, $^{14}$C-DEHA, or $^{14}$C-DIHA. Final concentrations of each HADP were 30 μg kg$^{-1}$ of $^{14}$C-HA, 50 μg kg$^{-1}$ of $^{14}$C-DEHA, and 44 μg kg$^{-1}$ of $^{14}$C-DIHA. Spiked soils and a blank for each soil type were incubated at 22–25°C for 72 h in 250-mL Teflon screw cap bottles. Alkali traps consisting of 10 mL of 2M NaOH were placed in all samples to trap $^{14}CO_2$. Average C mineralization was 6.9% of the added $^{14}$C-HADPs. In order to reflect more realistic recoveries than that obtained by extraction of freshly spiked samples, the incubation time was chosen to enhance HADP sorption yet maintain acceptably low degradation. A mass balance for each $^{14}$C-HADP was obtained by liquid scintillation counting before and after each step in the mixed-mode extraction procedure. Reported HADP recoveries were corrected for mineralization and $^{14}$C removed for liquid scintillation counting.

Recovery of the surrogate compounds, HT and DEHT, was determined by spiking 25 g (dry weight) soil samples to a level of 25 μg kg$^{-1}$. Spike samples were incubated at 22–25°C in 250-mL Teflon screw cap bottles for 24 hours. Spikes were performed in duplicate for each soil and the surrogates were evaluated separately.

## Soils

Soils were collected from 0–15 cm depth at three Midwestern US locations at varying times from 1995 to 1997 (Table II). The Iowa site (IA) has been in continuous corn since 1964, and atrazine has been applied 14 times since 1972. The Kansas site (KS) has been in a corn-soybean rotation, and atrazine has been applied 15 times since 1972. The Ohio site (OH) has been in a corn-soybean rotation since 1991. Atrazine has been applied at the OH site 4 times since 1987. A complete description of the soil classification, atrazine use, and cropping history of the soils was described by Lerch et al.[5] Soils were stored refrigerated

(2–4°C) at field moisture content (15.5 to 22.2%, wet weight basis) until analyses were performed.

TABLE II Soil characterization data

| *Sample Location* | *Site Designation* | *pH* | *CEC*[a] | *Organic Matter* | *Sand* | *Silt* | *Clay* | *Texture*[b] |
|---|---|---|---|---|---|---|---|---|
| | | | *(meq/100g)* | *%* | *%* | *%* | *%* | |
| Iowa | IA | 6.3 | 13.2 | 3.1 | 20 | 50 | 30 | SiCL |
| Kansas | KS | 6.4 | 10.8 | 1.7 | 30 | 52 | 18 | SiL |
| Ohio | OH | 6.3 | 13.2 | 3.2 | 12 | 46 | 42 | SiC |

a. Cation exchange capacity.
b. Texture: SiC = silty clay; SiL = silt loam; SiCL = silty clay loam.



## RESULTS AND DISCUSSION

### Recovery of $^{14}$C-HADPs by mixed-mode extraction

The mixed-mode extraction resulted in acceptable recoveries of $^{14}$C-HADPs for the three soils studied (Table III). Average recoveries were 77.9% for HA, 83.5% for DEHA, and 71.2% for DIHA. HADP recovery was very consistent with relative variation (i.e., range/mean) of 6% or less for all compounds and soils, except DIHA recovery from the OH soil. Losses were primarily due to unextractable residues, retention on SAX, or breakthrough from the SCX. Unextractable residues were greatest for the IA soil because of its high shrink-swell capacity, resulting in greater levels of entrained solution than the other two soils. As much as 20% of the added extractant was entrained within the IA soil whereas less than 10% was entrained within the OH and KS soils. Retention on SAX was a significant loss pathway for HA and DIHA, particularly for the KS soil. Despite rinsing of the SAX cartridge with 80% $CH_3OH$ and $H_2O$, some H-bonding or sample entrapment in the void volume of the SAX cartridge still occurred. Losses due to SCX breakthrough were largely a function of compound polarity, with the extent of breakthrough in the order: DIHA > DEHA > HA. Since all three compounds are in cationic form for the SCX step, the differences in breakthrough can be attributed to the strength of secondary hydrophobic interactions between the triazine ring and the SCX benzene ring. Thus, HA is the most non-polar and would be expected to have the strongest hydrophobic interactions with the SCX SPE resulting in lower breakthrough. This is consistent with a previous study of HADP breakthrough from SCX SPE[20].

TABLE III Mass balance of $^{14}$C-HADPs for the mixed-mode extraction procedure

| *Compound* | *Soil* | *Unextractable* | *Retention on SAX* | *Retention on SCX* | *SCX Breakthrough* | *Recovered in Final Extract* | *Total* |
|---|---|---|---|---|---|---|---|
| | | % of $^{14}$C Applied | | | | | |
| HA | IA | 18.3 ± 2.05[a] | 0.00 | 1.26 ± 2.51 | 0.73 ± 0.09 | 80.9 ± 2.56 | 101 ± 2.18 |
| | KS | 10.6 ± 7.95 | 8.28 ± 0.71 | 3.64 ± 6.79 | 4.56 ± 0.39 | 73.8 ± 0.78 | 101 ± 0.06 |
| | OH | 7.06 ± 0.16 | 2.99 ± 2.66 | 3.86 ± 2.66 | 4.12 ± 0.30 | 79.0 ± 4.83 | 97.0 ± 4.56 |
| | Mean | 12.0 | 3.76 | 2.92 | 3.14 | 77.9 | 99.7 |
| DEHA | IA | 14.7 ± 3.26 | 0.00 | 0.00 | 1.26 ± 0.07 | 83.0 ± 1.78 | 99.0 ± 4.97 |
| | KS | 7.29 ± 1.00 | 0.00 | 3.20 ± 0.79 | 11.0 ± 3.41 | 79.2 ± 2.89 | 101 ± 0.32 |
| | OH | 1.19 ± 0.62 | 1.10 ± 1.23 | 3.15 ± 6.29 | 1.81 ± 0.59 | 88.4 ± 5.31 | 95.7 ± 0.27 |
| | Mean | 7.73 | 0.37 | 2.12 | 4.69 | 83.5 | 98.6 |
| DIHA | IA | 16.7 ± 2.06 | 0.00 | 1.12 ± 2.24 | 3.95 ± 1.07 | 76.5 ± 0.96 | 98.3 ± 2.20 |
| | KS | 5.79 ± 3.09 | 9.42 ± 1.81 | 0.00 | 20.3 ± 2.33 | 64.1 ± 2.18 | 99.6 ± 1.43 |
| | OH | 6.14 ± 1.55 | 0.00 | 4.66 ± 2.47 | 13.8 ± 8.34 | 72.9 ± 11.5 | 97.5 ± 4.09 |
| | Mean | 9.54 | 3.14 | 1.93 | 12.7 | 71.2 | 98.5 |

a. Mean ± range (n=2).

### Cation exchange (SCX) Solid-Phase Extraction

Losses of HADPs by retention to the SCX SPE were low for all three HADPs indicating that 8:1:1 $CH_3OH$: $NH_4OH$:$H_2O$ was an effective eluant (Table III). Four factors are critical in the choice for the SCX eluant: 1) the counter ion must be at high enough concentration to saturate the exchange sites to insure quantitative desorption of HADPs; 2) the solution pH must be greater than 7.2 in order to completely deprotonate the HADPs upon entering the solution phase; 3) the eluant should contain a polar organic solvent to aid in solubility of the HADPs; and 4) the eluant should be volatile enough to facilitate evaporation. The SCX SPE isolation and elution step can be characterized by the following reactions:

(1) $K^+X^- + HADP^+ \rightleftharpoons HADP^+X^- + K^+$
where $X^-$ = SCX bonded phase and
$HADP^+$ = protonated HA, DEHA, or DIHA.

(2) $HADP^+X^- + NH_4^+ \rightleftharpoons NH_4^+X^- + HADP^+$

(3) $HADP^+ \rightleftharpoons HADP + H^+$

Reaction (1) shows the isolation of HADPs via the exchange reaction between the SCX, which is saturated with $K^+$ following conditioning, and a HADP, which is protonated by the addition of acid to the sample solution following the SAX step. Reaction (2) shows the initial elution step via the exchange reaction between the $NH_4^+$ in the eluant and the protonated HADP. Reaction (3) represents deprotonation of the HADPs due to the high pH (>7.2) of the eluant. Reaction (3) is critical to quantitative recovery of the HADPs since, by Le Chatelier's principle, removal of the protonated HADP drives reaction (2) to completion.

### Evaluation of potential surrogates

Two surrogate compounds were evaluated as a means of correcting for sample-to-sample variation in HADP recovery when using the method for routine analyses. The ideal surrogate should be a structural analogue of the HADPs and should not routinely be released into the environment. Since terbutylazine is not a registered herbicide in the United States, the hydroxylated terbutylazine degradation products, HT and DEHT, meet both criteria. HT recoveries from soil were much lower and more variable than HADP recoveries with an average of 52.8% for the three soils (Table IV). The average DEHT recovery of 66.8% was an improvement over HT, but its recoveries were still lower than the HADPs for two of the three soils. For the KS soil, DEHT recovery was 74.6% compared to

HADP recoveries of 72.4% as a group. However, the limited usefulness of DEHT as a surrogate was particularly evident by its low recoveries from the OH soil. Overall, the lower and more variable recoveries of HT and DEHT from soil preclude us from recommending either compound as a useful surrogate for the determination of HADPs.

TABLE IV Recovery of hydroxytertbutylazine (HT) and deethylhydroxytertbutylazine (DEHT) by mixed-mode extraction

| *Soil* | *HT* | *DEHT* |
|---|---|---|
| | *% Recovery*[a] | |
| IA | 55.1 ± 6.05 | 72.0 ± 11.6 |
| KS | 56.9 ± 12.1 | 74.6 ± 13.3 |
| OH | 46.6 ± 32.4 | 53.8 ± 0.79 |
| Mean | 52.8 | 66.8 |

a. Mean ± range (n=2).



Lower recoveries of the surrogates compared to the HADPs were possibly a result of their greater hydrophobic interactions with soil. Based on its greater retention to a $C_8$ HPLC column, HT is more non-polar than any of the HADPs resulting in hydrophobic interactions with soil organic matter as a more dominant mechanism for sorption than cation exchange. The polarity of DEHT, based on $C_8$ retention, is intermediate to that of the HADPs as a group; hence, it is a better structural analogue than HT and presumably its sorption mechanisms and recovery from soil should have been similar to the HADPs. The presence of the tertbutyl group may, however, result in greater sorption of DEHT via hydrophobic interactions than the HADPs. Thus, the low $CH_3CN$ content of the mixed-mode extractant was insufficient to disrupt the more extensive hydrophobic interactions of these surrogate compounds with soil. The other possibility for low recoveries of HT and DEHT is that their dissociation constants are significantly higher because of the tertbutyl group, resulting in lower recoveries from the SCX SPE. Weber[21]showed that increasingly non-polar alkyl groups at the 4 or 6 positions of the triazine ring increases the basicity of the molecule. However, recovery of HT and DEHT from SCX SPE was >95% using standard solutions with approximately the same solution matrix, in terms of ionic strength and pH, as actual samples. Therefore, the dissociation constants of HT and DEHT must be similar to the HADPs, and apparently, more extensive hydrophobic interactions were the main reason for their lower recoveries.

## Analysis of HADPs in soils

HADPs were detected at significant levels in all three soils (Table V). Concentrations were consistently in the order: HA > DEHA > DIHA. Full scan daughter ion spectra, obtained using LC/MS/MS, qualitatively confirmed the presence of all three compounds (Figure 2). All spectra closely matched reference standards, as well as LC/MS/MS daughter ion spectra reported for HADPs in other environmental matrices[2,5,22]. Although the presence of HA had previously been confirmed for these soils[5], the spectra for DEHA and DIHA represent the first definitive confirmation of these compounds in soils. Muir and Baker[6,7] determined HA and DEHA in atrazine-treated soils by derivatization followed by analysis using gas chromatography with flame ionization detection. HA and DEHA levels reported by Muir and Baker[6]were typically greater than those reported here due to the higher atrazine application rates in use at that time and the shorter time between atrazine application and sampling.



TABLE V Concentration of HADPs in soils and comparison of LC/UV to LC/MS/MS

| *Soil* | *LC/MS/MS* | | | *LC/UV vs. LC/MS/MS* | | |
|---|---|---|---|---|---|---|
| | *HA* | *DEHA* | *DIHA* | *HA* | *DEHA* | *DIHA* |
| | | $\mu g\ kg^{-1}$ | | | % Difference[a] | |
| IA | 162 | 40.9 | 16.2 | −11.7 | % −13.4 | −11.7 |
| KS | 66.9 | 8.99 | 5.27 | −3.9 | −41.3 | 0.0 |
| OH | 178 | 21.1 | 11.5 | −2.8 | 2.8 | 30.4 |
| | LC/UV | | | | | |
| IA | 181 | 46.4 | 18.1 | | | |
| KS | 69.5 | 12.7 | 5.27 | | | |
| OH | 183 | 20.5 | 8.0 | | | |

a. Expressed relative to LC/MS/MS; % difference = $[(C_{ms} - C_{uv})/C_{ms}]^*100$ where $C_{ms}$ = concentration determined by LC/MS/MS and $C_{uv}$ = concentration determined by LC/UV.

Comparison of HADP quantitation by LC/UV and LC/MS/MS showed generally good agreement, particularly for HA (Table V). Co-elution during LC/UV analysis can lead to a high bias in the quantitation, which was apparently the case for DEHA in the KS soil. In addition, LC/UV analysis of DIHA is complicated by its poor retention to $C_8$ which results in elution on the shoulder of the solvent peak. This can lead to inaccurate integration such as the low quantitation of DIHA in the OH soil. One of the main advantages of LC/MS/MS is that it

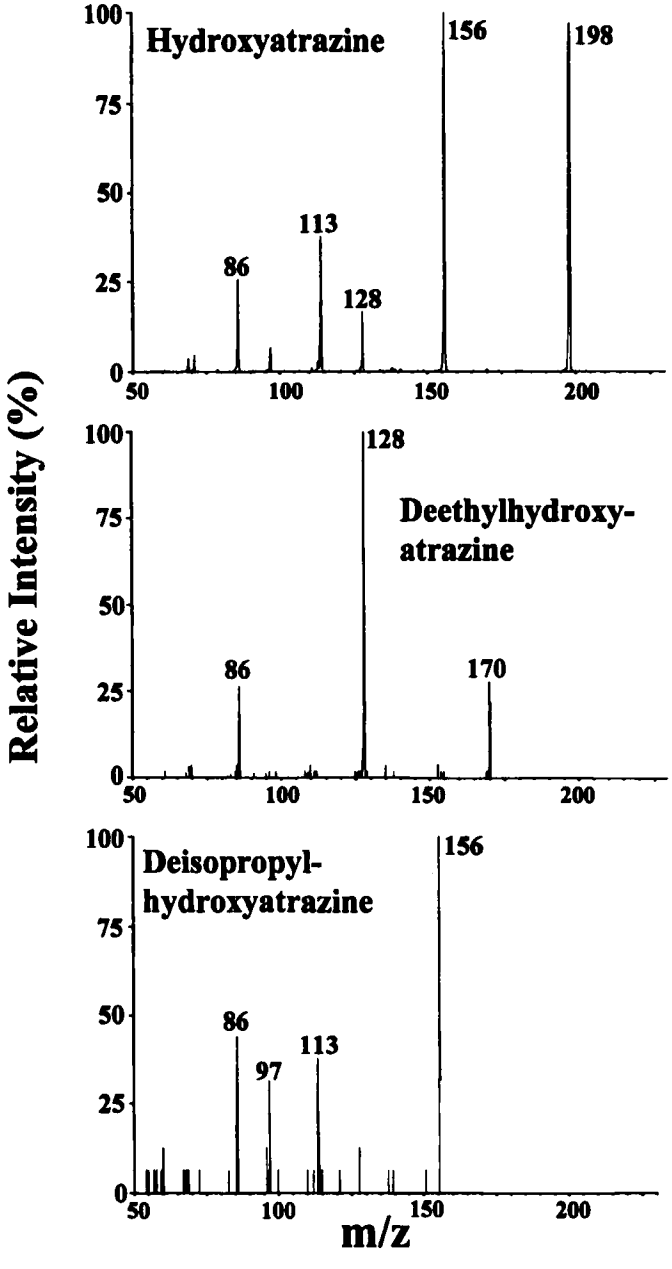


FIGURE 2 LC/MS/MS full scan daughter ion spectra of HADPs isolated from the OH soil. Labeled peaks represent diagnostic daughter ions. Base peak intensities: 326,000 cps for HA; 15,050 cps for DEHA; and 3,200 cps for DIHA

excludes many interfering compounds, as can be seen in the MRM sample chromatograms (Figure 3). Using MRM, the sensitivity of LC/MS/MS is 5 to 10 times greater than LC/UV as well. However, even with MRM, adequate separation of the HADPs is required for accurate quantitation because of the similarity in their daughter ion spectra. For example, the m/z 86 daughter ion chromatogram used for quantiation of DIHA illustrates the need to separate DIHA from HA (Figure 3). The high levels of HA in the sample resulted in greater intensity of the m/z 86 daughter ion (the larger unshaded peak at scan time 352) than DIHA. Hence, co-elution of HA and DIHA can result in severe overestimation of DIHA concentrations. While LC/MS/MS is clearly the superior analytical tool for quantitation and definitive confirmation, its cost is often prohibitive for routine use. Based on the results presented here, LC/UV provides an acceptable alternative for routine analyses of HADPs in soils.



## Total atrazine residues in soils

Using the data reported for atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIA) levels in these soils[5] combined with the HADP levels reported here, a more complete picture of the distribution of atrazine and its stable metabolites in soils can now be determined. Degradation studies reporting bound atrazine residues based on soil extraction by aqueous methanol or acetonitrile most likely have HADPs which can be recovered by mixed-mode extraction[18]. Hence, previous studies have tended to underestimate HADPs and overestimate bound residues. The average proportion of atrazine and its metabolites show that HADPs are the predominant form of atrazine residues remaining in these soils, collectively accounting for 91% of the total residues (Figure 4). HA was the major metabolite present, but even the proportions of DEHA and DIHA were greater than or comparable to atrazine and the chlorinated metabolites. Muir and Baker[6] also reported that HA and DEHA were the major atrazine residues in soils 12 months after atrazine application. These data indicate that HADPs predominate in soils compared to atrazine and its chlorinated metabolites due to a combination of the following factors: 1) more extensive formation leads to higher concentrations; 2) greater sorption of HADPs to soil leads to less off-site hydrologic transport; and 3) less extensive degradation, or conversely greater thermodynamic stability, results in greater persistence. Each of these factors are supported by many other studies[2,3,5,6,23–27]. Since these soils represent a broad geographic distribution, varied atrazine inputs, and varying times between atrazine application and sample collection, the similarity in distribution of atrazine residues, based on the relatively low standard deviations, indicates that HADPs will comprise the majority of atrazine residues in soils receiving repeated atrazine applications.

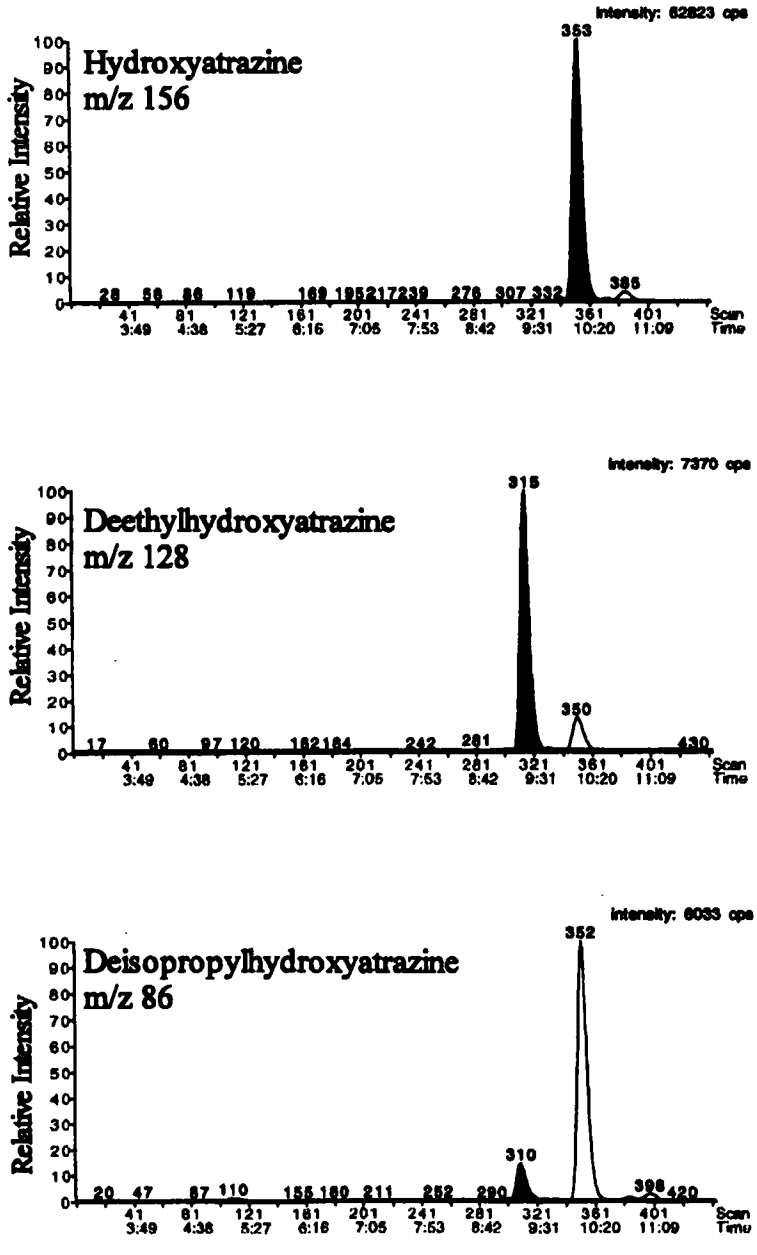


FIGURE 3 Multi-reaction monitoring (MRM) chromatograms of the IA soil extract. Mass to charge ratios (m/z) correspond to the daughter ion used for quantitation. Shaded areas correspond to the indicated HADP

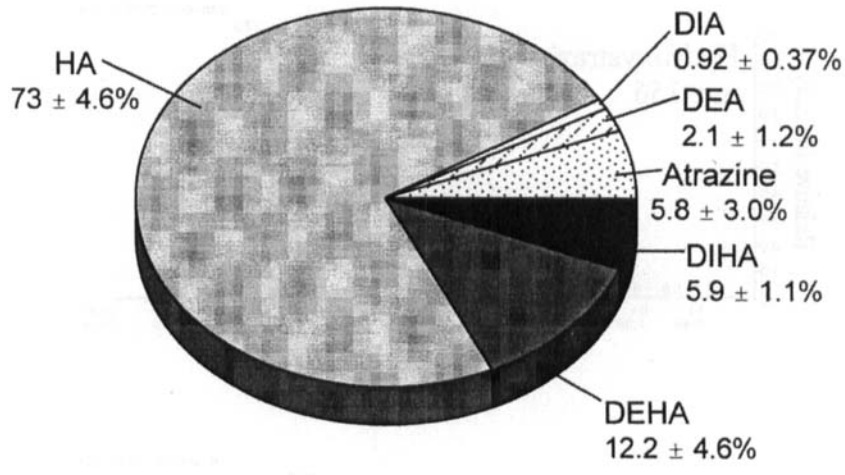


FIGURE 4 Average proportion of atrazine and its degradation products in three agricultural soils. Values represent mean ± standard deviation



## SUMMARY AND CONCLUSIONS

The mixed-mode extraction procedure was used successfully for the quantitation of HADPs in three agricultural soils. The method provides acceptable recovery for HADP quantitation, is amenable to both LC/UV and LC/MS/MS analyses, and is sensitive to sub-ppb levels. The results presented here also represent the first definitively confirmed levels of DEHA and DIHA in soils. HT and DEHT were poorly recovered by mixed-mode extraction due, most likely, to their greater hydrophobic interactions with soil organic matter. Utilization of the mixed-mode procedure, in conjunction with existing methodologies for analysis of atrazine and its chlorinated metabolites, enables a more complete and accurate quantitation of all the major stable atrazine residues in soils. HADPs comprised an average of 91% of the total atrazine residues in three agricultural surface soils, with HA the major constituent present in all soils. These data indicate that repeated atrazine use results in HADPs as the predominant atrazine residues in surface soils.

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