HPLC Determination of Atrazine and Principal Degradates in Agricultural Soils and Associated Surface and Ground Water

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A method for the quantitative estimation of atrazine and its principal degradation products in agricultural soils and associated surface and ground water is described. Bonded-phase extraction through cyclohexyl cartridges is used to isolate two principal degradates of atrazine from both surface and ground water samples. Soil is first extracted with organic-free water followed by dilute hydrochloric acid under heated conditions in a microwave extraction system. Reversed-phase HPLC employing an acetonitrile–water gradient is used for separation of analytes on a stabilized C_{18} analytical column. Analytes are detected by monitoring multiple wavelengths using a photodiode array detector. Identification is by retention time and molecular absorption spectra matching against reference standards. Terbuthylazine serves as a surrogate analyte. Method validation involved determination of percentage recovery for all analytes from fortified soil material and from spiked water. Amendment levels ranged from 0.10 to 10.0 μg/L for water and from 100 to 10 000 μg/kg for soils. Nominal limit of detection is 0.4 μg/kL for water and 40 μg/kg for soils.

INTRODUCTION

Atrazine is one of the most widely used herbicides in agriculture in the United States. In 1982, the estimated total domestic usage exceeded 34 million kg (Gianessi and Puffer, 1991; Gianessi et al., 1985). Atrazine continues to be used for pre- and postemergent control of both grasses and broadleaf weeds in corn, wheat, sorghum, and many other crops throughout the Midwest and across the nation. Typical application rates for atrazine on corn have been 2.2–4.4 kg ha^{-1} of active ingredient depending upon soil properties, nature of crop, atmospheric conditions, and/or type of irrigation program (*Crop Protection Chemicals Reference*, 1990). In 1990, maximum application rates were reduced to 3.3 kg ha^{-1}. Since that time, maximum label-recommended application rates have been reduced further. For the 1993 growing season, rates are 2.7 kg ha^{-1} pre-emergence on highly erodible land with >30% residue cover and 2.2 kg ha^{-1} for highly erodible land with <30% residue cover. Rates for non-highly erodible land are currently at 2.7 kg ha^{-1}. In areas requiring a postemergence application, total atrazine may not exceed 2.7 kg ha^{-1} per calender year (Ciba-Geigy Corp., 1993). Currently, many Iowa farmers are using <1.7 kg ha^{-1}. During 1990, atrazine usage in Iowa exceeded 5.8 million lb.

During the past 15 years, much emphasis was placed on chromatographic methods for the determination of triazine herbicides. Several techniques using either packed or capillary column gas chromatography with a nitrogen–phosphorus selective detector (NPD) have been reported for residue determination of herbicides in water. The NPD is especially appropriate for the determination of triazine herbicides, all of which contain 5 mol of nitrogen/mol (35% nitrogen by weight). Most of these methods are for multiresidue determinations and used for detecting many triazines in a single extract (Pressley and Longbottom, 1982; Bradway and Moseman, 1982; Popl et al., 1983; Steinheimer and Brooks, 1984; Lee and Stokker, 1986). Each of these methods uses either liquid- or solid-phase extraction. Researchers using a more recent method describe the use of bonded-phase adsorbents together with high-resolution gas chromatography/mass spectrometry (GC/MS) for semiquantitative estimation of 21 pesticides (including 8 triazines) in ground water (Bagnati et al., 1988). Solvent extraction followed by GC/MS employing an ion-trap detector is applied to lower Mississippi River samples for a series of herbicides including several atrazine metabolites (Pereira et al., 1990). An enzyme-linked immunosorbent assay (ELISA) was compared with a GC/MS technique for determination of triazines, including atrazine metabolites, in water samples (Thurman et al., 1990). A novel approach using pressure devices to extract atrazine and dealkylated hydroxyatrazine congeners from plant tissues also has been reported (Nelson and Khan, 1989). High-performance liquid chromatography (HPLC) also is successful for separation of mixtures of triazine herbicides and other crop protection agents. Most commonly, detectors of choice are the spectrophotometer or the mass spectrometer. Many methods use reversed-phase mode of separation by employing methanol–water or acetonitrile–water as mobile phase (Paschal et al., 1978; Dufek and Pacakova, 1979; Subach, 1981; Binner, 1981; Beilstein et al., 1981; Lawrence, 1981; Williamson and Evans, 1981; Parker et al., 1982; Pacakova et al., 1988). Separation of dealkylatrazines and hydroxyatrazine by reversed-phase HPLC is described (Vermeulen et al., 1982). The simultaneous determination of 22 pesticides in both surface and ground water by HPLC using photodiode array detection has been reported (Reupert and Ploger, 1988). A detection limit of 50 μg/L is claimed for seven herbicides in 1-L samples. Reversed-phase HPLC separation of organic bases similar to atrazine degradates, specifically azaarenes and their hydroxy and methoxyl analogs, has been reported by Steinheimer and Ondrus (1986, 1990a,b). Recently, the determination of atrazine, hydroxyatrazine, dealkylatrazines, and other pesticides in water by bonded-phase extraction together with a radial-compression cartridge separation and photodiode array detection has been described (Steinheimer and Ondrus, 1990a,b; Schlett, 1990). HPLC is also applied to the determination of atrazine residues in soils. This method has been compared with a colorimetric method (Vickrey et al., 1980) and with a capillary column gas chromatographic method (Xu et al., 1986) for the parent herbicide and selected metabolites.

Paired-ion HPLC has recently been reported for atrazine and hydroxyatrazine determination in soils (Wenheng et al., 1991).

Rapid and inexpensive immunochemical assays for pesticide residues in environmental samples are a developing new technique for water-quality studies. A recent paper describes the first successful application of monoclonal antibody based ELISA determination of hydroxyatrazine and atrazine in soil and water (Schlaeppi et al., 1989).

This paper describes a rapid analytical method for simultaneous determination of atrazine, deethylatrazine, and deisopropylatrazine in agricultural soil and in associated surface and ground water. It incorporates bonded-phase extraction on reversed-phase sorbents. It is designed to serve as a tool for support of field-research conducted under the Iowa Management System Evaluation Area (MSEA) Program, a federal interagency/state cooperative study of Best Management Practices (BMPs) and Water Quality in the Midwest. BMPs are defined in the context of combinations of tillage, crop rotation or sequencing, and agrochemical usage. One objective is to characterize the biogeochemical fate of atrazine applied to croplands in the Midwest for weed control and to understand water-quality impacts by monitoring the distribution and movement of degradates.

MATERIALS AND METHODS

Reagents. Methanol and acetonitrile of pesticide residue quality and of HPLC optical purity were used. Millipore Milli-Q, or equivalent, organic-free water, free of ultraviolet absorbing compounds, as determined by blank-gradient HPLC, was used where water is required in stock standard solutions, mobile phases, and solutions for soil extraction. Purity certified crystalline standards were obtained from the Pesticide Repository, U.S. EPA (Research Triangle Park, NC), from Promochem, Ltd. (St. Albans, England), or from Crescent Chemical Co. (Hauppage, NY). All standard reference materials assayed at 96% or greater and were used without further purification. Triazine stock solutions having concentrations of 100 mg/L were prepared in methanol in glass-stoppered actinic volumetric glassware and stored in a freezer at −16 °C. Hydroxyatrazine stock solution (100 mg/L) was prepared in water by dropwise addition of reagent grade HCl and stored in a refrigerator at 4 °C. Standards were prepared by serial dilution of stock solutions with methanol or acetonitrile in glass-stoppered volumetric flasks. Stock solutions were added to water and soil samples to evaluate recovery efficiency and method sensitivity.

Apparatus. Samples were extracted using solid-phase extraction cartridges containing cyclohexyl-modified silica sorbent (Analytichem International, Harbor City, CA) on a VAC ELUT SPS-24 (Analytichem International) 24-port vacuum manifold. Soil–water mixtures were heated using a Model 2100, 950-W laboratory microwave extraction system (CEM Corp., Mathews, NC) configured with a 12-position carousel. This extractor is capable of both temperature- and pressure-controlled operation in open and sealed vessels. Supercritical fluid extraction of soil was performed using a Model 7680A SFE Module (Hewlett-Packard Co., Avondale, PA) with 7-mL stainless steel thimble tubes.

Equipment. HPLC analyses were performed on a Model 1090M Series II liquid chromatograph (Hewlett-Packard Co.) capable of ternary gradient separations and equipped with an autoinjection system, a thermostatically controlled column compartment, and a photodiode array detector. Instrumental system control was maintained through a Model 9000 Series ChemStation running LC-Pascal software (Rev. 5.22e). A built-in six-port column switching valve permitted two-column operation. Soil and water extracts were analyzed together in a single sequence, each on a column dedicated to that sample matrix.

Chromatography. Instrumental chromatographic parameters involved both ternary system pumping functions and flow programming. A LiChrospher 100 RP-18, 5 μm, 125 × 4 mm (H-P p/n 799250D-564) column was used. Photodiode array data were acquired at 220, 235, and 254nm, with a 4-nm bandwidth, and against a reference signal at 380 nm with a 40-nm bandwidth. Detector sensitivity was set at 20 mAU full scale. Spectral data were acquired between 200 and 400 nm with a sampling frequency of 640 ms. Injection volume was 25 μL. Channel A, acetonitrile; B, methanol; C, water. Gradient separation conditions:

time, min	channel A	channel B	channel C
0.10 (solvent)	10.0%	00.0%	90.0%
6.00 (solvent)	25.0%	00.0%	75.0%
21.00 (solvent)	65.0%	00.0%	35.0%
23.00 (flow)	1.50 mL/min		
23.00 (solvent)	100.0%	00.0%	00.0%
25.00 (solvent)	00.0%	100.0%	00.0%
27.00 (flow)	0.50 mL/min		
27.00 (solvent)	25.0%	00.0%	75.0%
30.00 (solvent)	10.0%	00.0%	90.0%

Table I. Selected Properties of the Loam (Nashua) and Silty Loam (Treynor) Soils Used for Recovery Studies

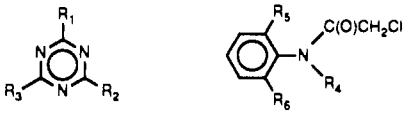
soil property	Nashua site	Treynor site
total organic carbon,[a] %	4.48	1.81
cation-exchange capacity,[b] mequiv/100 g	36.1	32.3
pH[c]	6.7	6.1
sand,[d] %	42.5	10.0
silt,[d] %	47.5	77.5
clay,[d] %	10.0	12.5

[a] Methods of Chemical Analysis of Water and Wastes, U.S. EPA, Method 415.1, 1979. [b] Methods of Soil Analysis, Agronomy No. 9, Part 2, Method 57-3, 1965; Sodium Saturation Method. [c] Recommended Chemical Soil Test Procedures for the North Central Region, Section 3, 1988. [d] Methods of Soil Analysis, Method 43-5.

Procedure for Soil Extraction. Twenty-five milliliters of organic-free water was added to 10.0 g (field-moisture corrected)) of soil material contained in a 250-mL centrifuge bottle. The bottles are rotated on the carousel of a microwave oven while the samples are heated to 95–98 °C. The mixtures are centrifuged at 2300 rpm for 3 min, and the supernate is decanted into a 250-mL centrifuge bottle. The extraction is repeated once with organic-free water. Three additional extractions are done using 0.35 N HCl, combining the extracts in a separate centrifuge bottle, and the pH is adjusted to 7.0 using dropwise addition of either dilute (1:1) ammonium hydroxide or 0.2 N NaOH. The water extracts are combined with the neutralized acid extracts and centrifuged at 2300 rpm to remove precipitated metal hydroxides. Sample extracts are further clarified by filtration of the decantate through a Whatman No. 40 filter paper. The centrifuge bottle and filtration apparatus were rinsed with organic-free water. Bonded-phase extraction and elution are carried out on each soil extract (total volume of filtrate was about 125 mL) using the cartridge chemistry described in the procedure for water samples.

Soil Spiking Technique. Surface soil grab samples, collected in 1988, were taken from corn and soybean fields representative of agricultural land use at two locations within the Iowa MSEA program. Samples were collected from the shallow oxidized glacial till deposits of northeastern Iowa located at the Northeast Research Center, near Nashua, and from the deep loess deposits of silty loam found at the Deep Loess Research Station in southwestern Iowa, near Council Bluffs. Selected properties are given in Table I. All sampled fields at both locations have been under continuous corn production for many years. The soil samples were thoroughly mixed to ensure homogeneity. Stones, twigs, and plant residue were removed. Samples were not wet or dry sieved. All soils used for recovery studies were amended with atrazine congeners by weighing 10.0-g portions of well-mixed material into a 250-mL centrifuge bottle. An appropriate volume of spiking solution, 50–500 μL of methanolic solutions at 200 ppm concentration, was diluted into 25 mL of organic-free water, and the mixture was added to the sample bottle. This was stirred magnetically for several hours and evaporated to dryness overnight uncovered in a fume hood. These air-dried soils were used directly.

Table II. Structures, Names, and Abbreviations for Atrazine, Hydroxyatrazine, Two Dealkylatrazines, and Related Compounds, along with Alachlor and Metolachlor



	R_1	R_2	R_3
deethyldeisopropylatrazine (DEDIA)	chloro	amino	amino
deisopropylatrazine (DIA)	chloro	ethylamino	amino
deethylatrazine (DEA)	chloro	amino	isopropylamino
hydroxyatrazine (HA)	hydroxy	ethylamino	isopropylamino
atrazine (A)	chloro	ethylamino	isopropylamino
terbuthylazine (TBA)	chloro	ethylamino	*tert*-butylamino
cyanazine (CYA)	chloro	ethylamino	2-methylpropionitrilamino

	R_4	R_5	R_6
alachlor (ALCLR)	methoxymethyl	ethyl	ethyl
metolachlor (MTLCLR)	2-methoxy-1-methylethyl	methyl	ethyl

Procedure for Water Samples. Water samples were filtered through 40 mm diameter organic-binder-free glass-microfiber depth filters in an all-glass vacuum filtration apparatus. Using a glass capillary digital micropipettor, 2.5–5.0 μL of a methanol solution of the surrogate compound, 100 mg/L TBA, was added to the sample. Volumes used for extraction ranged from 0.1 to 2.0 L. Larger volumes provide a greater level of enrichment but required longer extraction times and resulted in lower recoveries for certain analytes. Generally, 0.25–0.50 L was found to provide a reasonable level of enrichment with minimal loss during extraction. Before extraction, each water sample was adjusted to pH 7.0–7.5 by dropwise addition of dilute aqueous ammonia or phosphoric acid, as needed. Analytichem BondElut cartridges, 500 mg, of cyclohexyl specificity were activated by delivering not less than 12 mL (3 column volumes) of methanol followed by 12 mL (3 column volumes) of water through each cartridge with a glass syringe. The activated cartridges were positioned on the multiport vacuum manifold and fitted with adapters connected to glass 250-mL reservoirs. Using an applied vacuum of about 0.015 kPa, water samples were drawn through the cartridges at a nominal flow rate of about 20 mL/min. Following extraction, the cartridges were placed in 15-mL graduated centrifuge tubes and centrifuged for 1 min at 1500 rpm to remove residual water. Cartridges were then eluted by air displacement of 2.0 mL of acetonitrile (delivered from a volumetric pipet) into a clean, dry centrifuge tube. The cartridge is placed in the tube and centrifuged at 1500 rpm to ensure quantitative recovery of eluant from the cartridge. The total volume was vortexed, transferred to an autosampler vial, and sealed for instrumental analysis.

RESULTS AND DISCUSSION

Although atrazine has been studied extensively, the details of its many pathways to complete degradation and mineralization are not well understood. However, there is a general agreement that the first steps in major routes of degradation are biotic N-dealkylation and abiotic hydrolytic dechlorination (Somasundaram and Coats, 1991; Grover, 1989; Grover and Cessna, 1991; Kearney and Kaufman, 1969). These processes may occur simultaneously and perhaps competitively, depending upon the local soil environment. Chemical hydrolysis may proceed much more rapidly than metabolism, especially in acidic soils. Stabilization of these reactive intermediates through bioconjugation may also occur. Although the abiotic hydrolysis of field-applied atrazine occurs most rapidly, the N-dealkylation reactions are probably more important for water-quality studies because they occur in the soil where they are promoted by microorganisms and because these dealkylated congeners are more water soluble than either atrazine or hydroxyatrazine. Water solubility measurements in distilled water at room temperature show the following: deisopropylatrazine, 650 ppm; hydroxyatrazine, 7 ppm; deethylatrazine, 340 ppm; atrazine, 33 ppm; terbuthylazine, 8 ppm (personal communication from M. G. Ondrus).

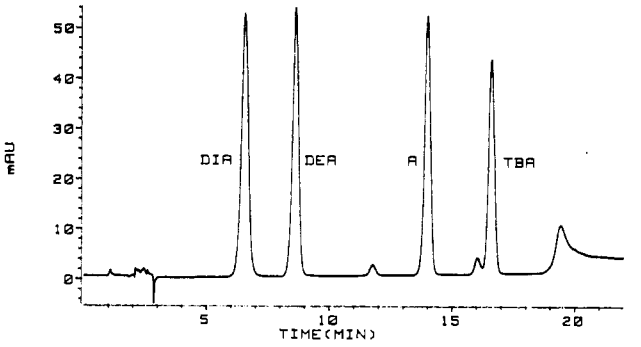


Figure 1. Chromatogram of DIA, DEA, A, and TBA, on a LiChrospher 100 RP-18 (5 μm) column (125 × 4 mm) using an unmodified acetonitrile–water gradient. Photodiode array detector was operated at 220 nm with sensitivity set at 20 mAU full scale (156-ng injection).

Table II shows structures, names, and abbreviations for atrazine, hydroxyatrazine, the two dealkylatrazine derivatives, and related compounds, along with alachlor and metolachlor. Terbuthylazine (TBA) serves as surrogate analyte because its chemical behavior mimics that for atrazine, it is inexpensive and commercially available at high purity, and it is well-resolved chromatographically from all other triazine peaks. TBA is not registered for agricultural purposes in the United States; therefore, its occurrence as a water contaminant is unlikely. The LC separation conditions were chosen so that all primary analytes and surrogate are resolved from each other and to the baseline within a 20-min analytical run cycle. The additional 10-min recycle time is necessary to flush the most polar of the chemical artifacts from the column; this is particularly important for sample sequences encompassing different types of soil. As shown in Figure 1, an end-capped *n*-octadecyl column under acetonitrile–water mobile phase provides a separation with excellent peak symmetry and without the necessity of ionic amendments to the solvent, such as dilute acids or bases, buffers, or counterions required for paired-ion separations. The use of these ionic agents on reversed-phase systems often diminishes the life of an analytical column and prevents its uses in other separation modes, while causing possible corrosion to stainless steel hardware components. The chromatogram in Figure 1 represents the response of 156 ng of each compound on column and was recorded at 220 nm at a photodiode array sensitivity of 20 mAU full scale. Signal-to-noise ratios for all analytes exceed 100.

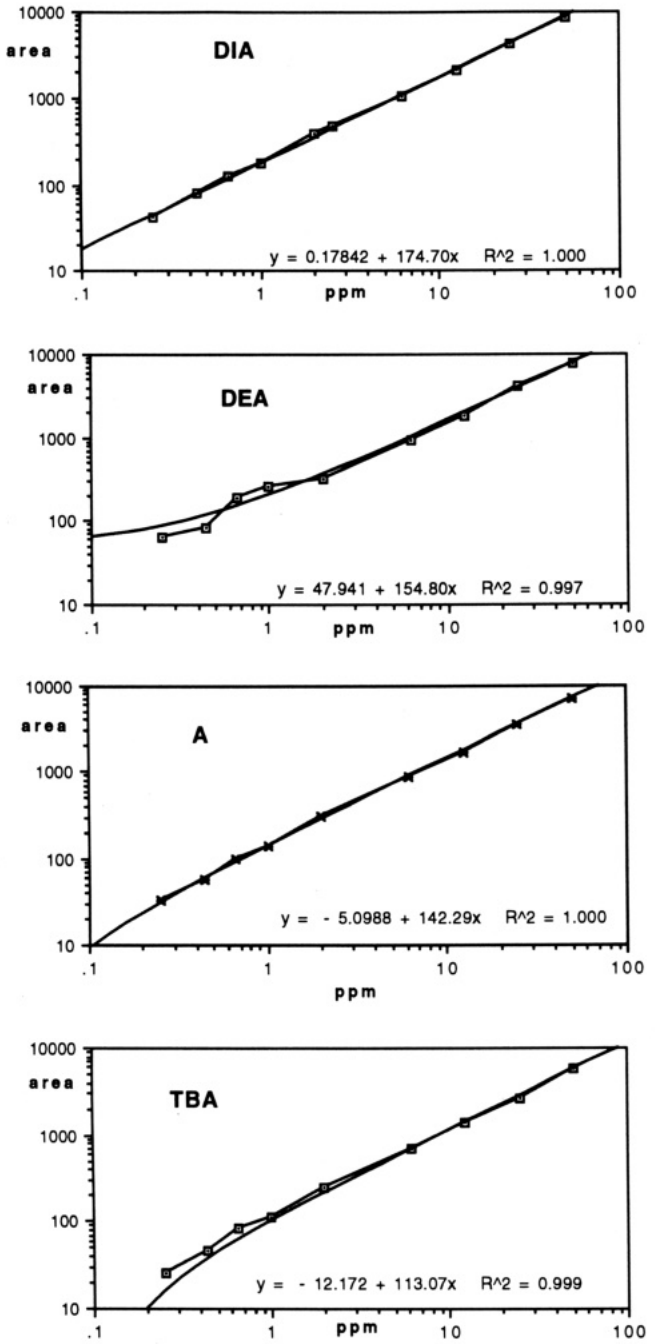


Figure 2. Multipoint calibration plots used for quantitation of DIA, DEA, A, and TBA.

Figure 2 illustrates typical nine-point calibration plots obtained for each analyte over a concentration range 6.25–1250.0 ng injected. These extended standard curves are necessary to accommodate the range of concentrations normally encountered in both soil and water samples. The instrumental software permits a peak-by-peak statistical evaluation for each peak of the chromatogram to establish the repeatability of instrument performance on repetitive injections before a long sequence or for assessing compliance within specified regulatory guidelines. Using this feature, the following variances are observed for this analytical separation: retention time, RSD <0.1%; area, RSD <10%; height, RSD <15%; width, RSD <10%. Pacakova et al. (1988) has studied the reversed-phase HPLC behavior of a series of triazines and their hydroxyl analogs in terms of mobile-phase composition and pH and concluded that the use of photometric detectors is preferred over electrochemical devices for low-level residue

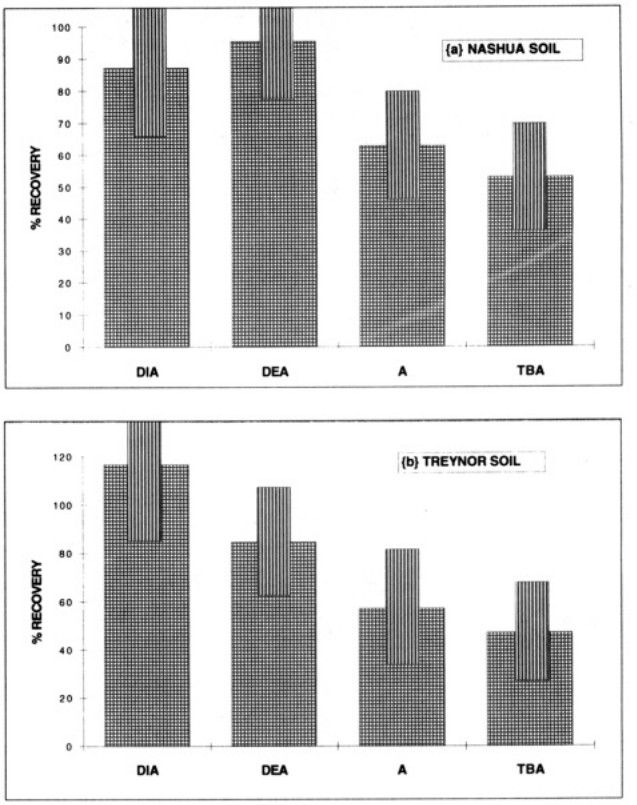


Figure 3. Recovery of analytes plus surrogate from soil amended at 1–2 ppm. Error bars are standard deviations from the mean of quadruplicate determinations. (a) Nashua; (b) Treynor.

measurements. Weed-control agents, other than atrazine, are finding increasing usage in the corn and soybean rotations practiced by most Iowa farmers. Because of similarity of properties, several of these are likely to occur in water or sorbed to soil together with their corresponding degradates. Were they present in extracts, these chemicals would be distinguished on the basis of their retention time together with the respective UV absorption spectrum. Table III lists these properties for the primary analytes, several of their hydroxy analogs, and other herbicides and degradates with a probability of co-occurrence in agricultural field samples. For the most part, herbicides likely to be coapplied with atrazine do not break down to derivatives that would interfere with instrumental analysis.

The soil extraction procedure involves removal of atrazine and terbuthylazine by extraction with water heated to boiling point in a 950-W laboratory microwave digestor. DIA and DEA are removed by heating with dilute hydrochloric acid, which produces the protonated species, the corresponding triazinium ion, which is partitioned into the aqueous phase. In addition, extraction with aqueous solvents alone, as an alternative to the more commonly used methanol–water or acetonitrile–water mixtures, appears to improve the partitioning process with respect to amounts of coextracted soil organic matter. This method seems to yield cleaner extracts and simpler chromatograms. For DIA and DEA, dilute acid may also catalyze hydrolysis of covalent bonds responsible for the chemisorption of these analytes to the complex soil organic matter. Furthermore, given the amphoteric and tautomeric nature of hydroxyatrazine, the acid extraction may release hydroxy analogs bound by other types of interactions by shifting the position of the tautomeric equilibrium. During method development, a series of experiments were conducted to determine the optimum concentration of dilute HCl required for maximum recovery of A and TBA commensurate with minimization

Table III. Retention Times Measured for Primary Analytes, Other Products of Initial Soil Degradation Processes, Most Probable Co-occurring HPLC Interferences, and UV Absorption Data for All Compounds

	R_t	UV, λ_{max}	intensity
atrazine-derived chemical			
deethyldeisopropylhydroxyatrazine	3.27	218, 258	high, low
deethyl,deisopropylatrazine	3.45	202, 230	high, medium
deisopropylhydroxyatrazine	5.15	210, 235	high, low
deethylhydroxyatrazine	6.15	210, 235	high, low
deisopropylatrazine	6.58	224, 262	high, low
hydroxyatrazine	7.65	204	high
deethylatrazine	8.65	224, 268	high, low
atrazine	14.00	218, 262	high, low
terbutylazine	16.63	224, 268	high, low
potential HPLC interferences			
diketometribuzin	8.72	202, 260	high, high
metribuzin	10.85	212, 228, 295	high, high, high
deaminodiketometribuzin	11.15	212, 228, 295	high, high, high
cyanazine	11.44	218	high
simazine	11.40	222, 268	high, low
carbofuran	12.32	210, 226, 278	high, low, low
alachlor	17.65	224, 268	high, low
metolachlor	18.65	224, 268	high, low

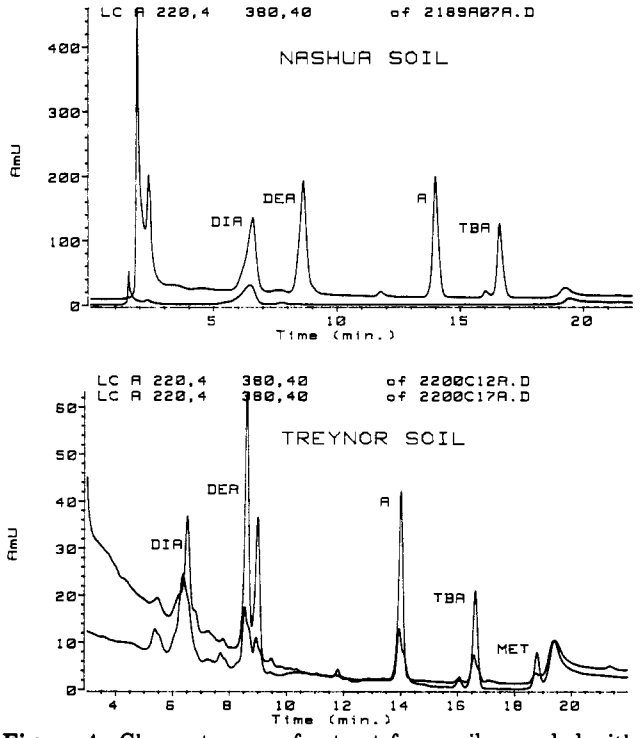


Figure 4. Chromatogram of extract from soil amended with analytes plus surrogate 1–2 ppm. (a) Nashua; (b) Treynor.

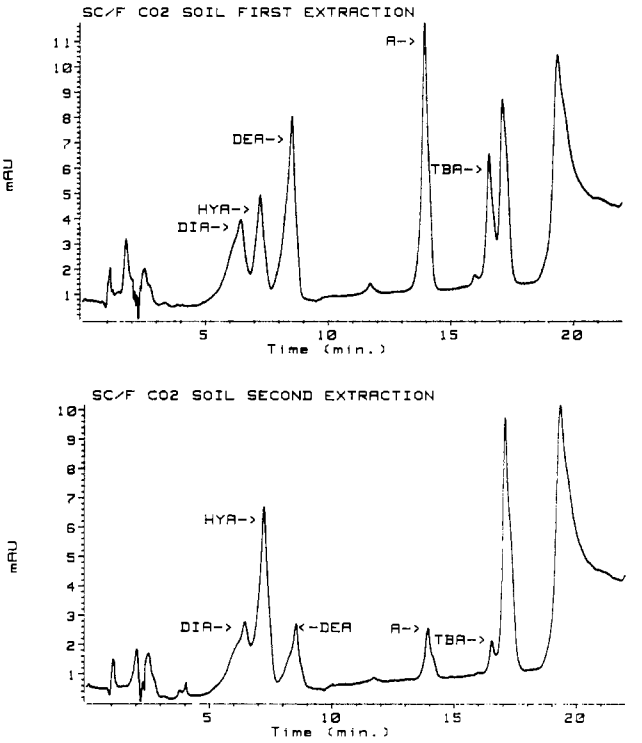


Figure 5. Chromatogram from extract of Nashua soil. Spike: 5 ppm each of DIA, DEA, A, and TBA. Sample: 10.0 g of soil amended with 10% water. (a) First extraction: CO_2 density, 0.3 g/mL at 121 bar; flow rate, 1.0 mL/min; T = 80 °C; equilibration time, 2 min; run time, 5 min. (b) Second extraction: CO_2 density, 0.9 g/mL at 350 bar; flow rate, 4.0 mL/min; T, 50 °C; run time, 20 min; other conditions unchanged.

of hydroxy analog formation. Extraction of Nashua soil with supercritical carbon dioxide (SFE) was also investigated with emphasis on recovery for DIA and DEA. Amendment of a 10.0-g sample with 1 mL of water resulted in measurable recovery for both metabolites, along with A and TBA.

Figure 3 plots typical recoveries obtained for each soil type when spiked within the range 1–20 ppm. DIA and DEA mean recoveries over the concentration range are 85–95% for the Nashua soil and 115–85% for the Treynor soil, respectively. A and TBA means are somewhat lower—65–55% for the Nashua soil and 55–50% for the Treynor soil, respectively. Error bars, showing the standard deviations from the mean of 25 replicate determinations, are approximately 30–40% for each analyte. Uncertainties of measurement associated with pesticide analysis of a soil matrix are much greater than those normally identified with a water matrix. Physical complexity, heterogeneous nature, and spatial and temporal variability of soil combine to yield larger variance. Both metabolites are extracted more efficiently from soil with dilute HCl than are the corresponding parent compounds. This is a direct result of their increased Brønsted–Lowry basicity associated with the primary amino group conjugated with an azaarene ring system. Recovery experiments with two tandem cartridges of identical surface chemistry show no evidence of first-cartridge breakthrough for either A or TBA, indicating that use of the 2.0-mL elution volume may be insufficient for full recovery. Studies are underway to assess the effect of a combination of larger elution volume, together with additional elutions of smaller volumes, for increased total recovery. In addition, other studies (personal communication from M. G. Ondrus) using only parent compounds as spiked analytes do not support the suggestion that the less basic A and TBA are more

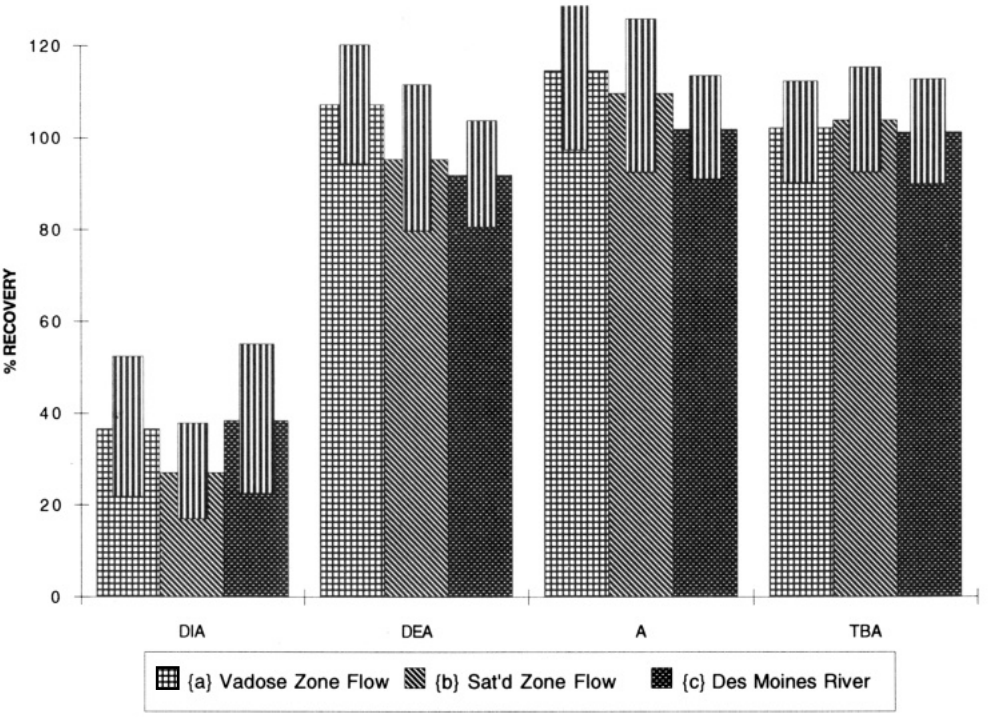


Figure 6. Recovery of analytes plus surrogate from water samples amended at 1 ppb. (a) Unsaturated-zone ground water beneath a 40-ha watershed under ridge-till continuous corn at Treynor. (b) Saturated-zone ground water from watershed at Treynor. (c) Des Moines River (June 1992).

susceptible to acid hydrolysis, leading to their respective hydroxy analogs, than are DIA and DEA. Figure 4 shows typical chromatograms obtained from the aqueous acid system as extractant for both Nashua and Treynor soils. Prominence of the analyte peaks, relative to the soil matrix, is apparent and illustrates the effectiveness of the sample preparation chemistry in reference to maximizing analyte recovery while minimizing the chromatographic effects of coextracted organic matter. As shown by the second trace in Figure 4, small amounts of additional analyte, especially the metabolites, are recovered when a stacked cartridge configuration is used for extraction. Supercritical carbon dioxide extraction of the Nashua soil, amended with 5 ppm each of DIA, DEA, A, and TBA, furnished the chromatograms in Figure 5. Two extractions were performed, each at a different density of CO_2. While all four spike analytes were recovered from the analyte trap, the chromatogram complexity is slightly greater, indicating larger amounts of coextracted organic matter or more rapid formation of hydroxy analogs than seen with the aqueous extractants. Additional experiments, which compare optimized extraction protocols directly, along with LC/MS characterization of each peak in the chromatogram, should resolve these issues.

Agricultural water samples are filtered and processed by solid-phase extraction on BondElut cartridges. Earlier studies demonstrated that the cyclohexyl packing material is the bonded phase best suited for removal of atrazine and its degradation products from water. On the basis of the complexity of the chromatogram, the cyclohexyl cartridges are superior to *n*-octadecyl cartridges for discrimination between low molecular weight analytes and high molecular weight organic matter in the extraction process. Other surface chemistry specificities that were evaluated include *n*-octadecyl, *n*-octyl, phenyl, cyano, amino, and aliphatic diol. For determinations that focus on the most water soluble analytes, such as deisopropylatrazine, a larger capacity cyclohexyl cartridge is suggested. For most natural water samples with dissolved organic carbon levels <5 mg/L, the use of a test sample volume of 0.25–0.5 L is recommended. An elution volume of 2.0 mL produces acceptable recoveries for all analytes sorbed onto the packing. Extraction of a 0.250-L water sample followed by elution with 2.0 mL of methanol produces an enrichment factor of 125. This condition meets the processing requirements for all surface and ground water samples analyzed to date. Similarly, extraction of 10-g soil sample followed by ellution with 2.0 mL produces an enrichment factor of 5. Although greater enrichment factors can be achieved with larger extraction volumes or larger soil weights, the larger sample size is accompanied by potential problems such as longer extraction time, lower recovery percentage, and increased concentrations of potentially interfering compounds. The plots in Figure 6 summarize the results of spikes on a representative Iowa agricultural drainage surface water and on both vadose-zone and saturated-zone ground water. Across the concentration range 1–20 ppb, mean recoveries for DEA, A, and TBA in all matrices fell within 115–90% of theoretical values, with an RSD of <25%. Compared to the other compounds, recovery of DIA in all matrices was greatly reduced. This has been reported by others (Thurman et al., 1990) and probably results from its lower distribution coefficient, a manifestation of greater water solubility. Triazines and their degradates encompass a broad range of water solubilities such that complete and quantitative recovery for each from a single pass through a procedure is not practical. As a result, optimum test sample weight is compromised. Water samples having volumes from 100 to 1000 mL and soil weights from 1 to 10 g are relatively easy to work with. A good compromise between length of extraction and sensitivity of the method is found with 250-mL water samples and 10-g soil samples.

During 1988 and 1989, well-water samples were obtained from two midwestern cornbelt locations. One site was an atrazine spill which occurred during a mixing operation in west-central Wisconsin; the other was an atrazine-contaminated potable supply well in the southern part of the state. Each sample was split, with one portion analyzed according to this method and the other analyzed inde-

pendently in another laboratory using the traditional solvent extraction–gas chromatographic approach. Comparison of results for DIA, DEA, and A showed excellent agreement within expected variances associated with multilaboratory, multioperator, multimethod collaboration (Steinheimer and Ondrus, 1990a,b). The results show that the bonded-phase extraction HPLC method is capable of generating water-quality data for atrazine metabolites equivalent to that produced from classical GC approaches.

CONCLUSION

Atrazine and metabolites are determined in both agricultural soils and associated water by a common protocol. The method will support the herbicide–degradate analysis requirements of the five-state MSEA program. It lays the groundwork for other important advances for LC techniques including HPLC with particle beam mass spectrometry as detector and the determination, underivatized, of non-gas-chromatographable hydroxy analogs of DIA, DEA, DEDIA, and other triazines. Removal of polar, ionic, and hydrophilic herbicide analytes from soil by admixture only with aqueous extractants is a viable approach for emerging new weed control agents such as sulfonylureas and imidazolinones.

ABBREVIATIONS USED

A, atrazine, 2-chloro-4-(ethylamino)-6-[(1-methylethyl)-amino]-*sym*-1,3,5-triazine; HA, hydroxyatrazine, 2-hydroxy-4-(ethylamino)-6-[(1-methylethyl)amino]-*sym*-1,3,5-triazine; DEA, deethylatrazine, 2-chloro-4-amino-6-[(1-methylethyl)amino]-*sym*-1,3,5-triazine; DIA, deisopropylatrazine, 2-chloro-4-amino-6-(ethylamino)-*sym*-1,3,5-triazine; TBA, terbuthylazine, 2-chloro-4-(ethylamino)-6-[(2-methyl-2-propyl)amino]-*sym*-1,3,5-triazine.

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Registry No. Supplied by Author: Alachlor, 15972-60-8; atrazine, 1912-24-9; carbofuran, 1563-66-2; cyanazine, 21725-46-2; deaminodiketometribuzin, 52236-30-3; deethylatrazine, 6190-65-4; deethyldeisopropylatrazine, 3397-62-4; deethylhydroxyatrazine, 19988-24-0; deisopropylatrazine, 1007-28-9; deisopropylhydroxyatrazine, 7313-54-4; diketometribuzin, 56507-37-0; hydroxyatrazine, 2163-68-0; metolachlor, 51218-45-2; metribuzin, 21987-64-9; simazine, 122-34-9; terbuthylazine, 5915-41-3.